COVER

Same as registration brochure but says Final Program & Abstract Book.

ACKNOWLEDGEMENTS

The Organising Committee is grateful to the following, who at the time of printing, have given their support in many different ways:

(Logos for all companies)

Armed Forces Institute of Regenerative Medicine

Australian Regenerative Medicine Institute

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TERMIS AP 2010 SECRETARIAT

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WELCOME

On behalf of the organising committee as chairpersons of this forthcoming meeting we wish to welcome you to the inaugural TERMIS event in Australia.

This is an unique opportunity for all those involved in science and research to come together with medicine, dentistry, veterinary surgery and allied health disciplines to promote the worldwide advancement of tissue engineering and regenerative medicine; in particular the integration of current laboratory technologies and clinical needs in our diverse mutlicutural region.

We offer our delegates innovative topics and stimulating clinical debates which will be led by a balance of international and national speakers and the opportunity for industry to contribute. The meeting will pay particular interest to expectations, directions and goals for the progress of Tissue Engineering and Regenerative Medicine by scientists and clinicians over the next half decade.

We look forward to your participation and welcoming you to Sydney in spectacular spring time Australia.

Geoff McKellar
Meeting Chair TERMIS AP 2010

Dietmar W. Hutmacher
Scientific Committee Chair TERMIS AP 2010

WELCOME

Welcome to the beautiful city of Sydney, Australia and home of the scientific meeting 2010 for the Asia-Pacific chapter of TERMIS. Geoff McKellar, Ann Collins, and Dietmar Hutmacher have worked extraordinarily hard to organize a scientific program which spans the full spectrum of basic science to clinical translation for regenerative medicine/tissue engineering. The scientific sessions promise to be educational with emphasis upon cutting edge technology, and the social agenda is equally attractive highlighting the best of Sydney.

I am happy to report the "state of health" of TERMIS is excellent. Our membership numbers now approximate 3,000 with the current Asia-Pacific chapter accounting for 1,000 of this total membership. With the registration automatically covering cost of this year's dues, the AP number is certain to rise. The potential strength of the Asia-Pacific chapter is evident in each issue of Tissue Engineering, and by the participation of members from the AP chapter in the European and North American annual meetings. There is a lot yet to be accomplished in the field of Tissue Engineering and Regenerative Medicine and I want to take this opportunity to emphasize two areas that I believe are important in the near-term success of TERMIS: 1) the inclusion of clinicians in our annual meetings and regional workshops, and 2) the collaboration between TE/RM in scientists in the field of developmental biology. By increasing our visibility and outreach to these two groups, it is my firm belief that we will both strengthen the scientific underpinnings of regenerative medicine while simultaneously advancing the clinical translation for which we all hope.

Once again, thank you for your support of the Asia-Pacific chapter meeting.

Sincerely

Steve Badylak
President, TERMIS

Welcome to the 2010 TERMIS-AP Sydney Conference

As the Continental Chair of TERMIS-AP, I personally would like to welcome you to the upcoming meeting, the 2010 TERMIS-AP Conference, in beautiful Sydney, Australia. I also would like to extend my warm invitation, on behalf of the organizing committee, to your colleagues, friends, and family members since this opportunity is not only to discuss scientific matters, but also to establish a network of the people with a regional sense of community. Time has come for us to unite again here in Australia.

TERMIS-AP conferences have been providing an international common ground among scientists and medical professionals alike. They aim at establishing a highly functional infrastructure by integrating the fields of medicine, science, and engineering to help create innovative medical treatments for various health challenges. With the momentum we gained at the 2009 TERMIS World Congress in Korea, we will further continue to take a step forward in the unprecedented field of medicine at the Conference, offering a wide range of topics such as stem cells and cell biology, gene modulation, drug and cell delivery, biomaterials, tissue engineering, and different therapies in a new research field, resulting from a fusion of biomaterials and engineering. These topics are brought to you in the forms of lectures, discussions, and poster presentations to share exciting and latest discoveries in the field. Inspire the next steps in the projects of your research by interacting with the other researchers in the region of AP. You experience the moments with the others through exchanging imaginations and aspirations, the reason why the Conference becomes significant.

With much anticipations and hopes, the field of tissue engineering and regenerative medicine is now ready to take on the central role for developing advanced medical care with cutting-edge medical technology to combat incurable diseases in the new millennium. I assure you that this special occasion will bring you a new opportunity to broaden your horizons and take your research to the next level.

Now join me for the intriguing programs and events that bring us all from the AP region together at the 2010 TERMIS-AP Sydney Conference.

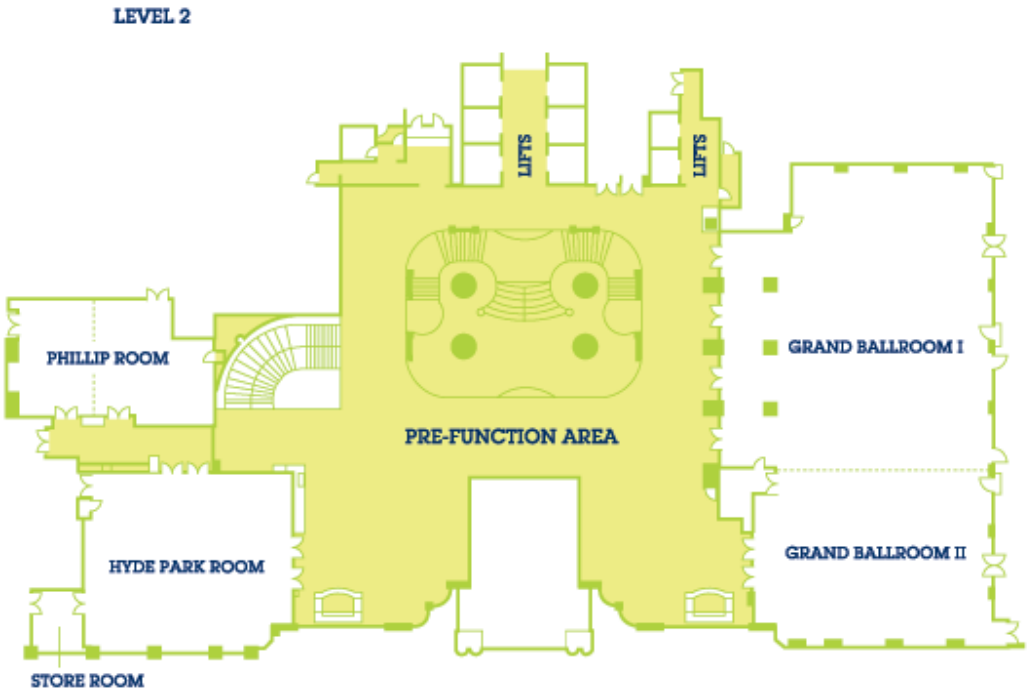
See you all this coming September in Sydney!

Teruo Okano
TERMIS-AP Continental Chair

MEETING VENUE

The TERMIS AP 2010 will be held at:

Sheraton on the Park
161 Elizabeth Street
Sydney NSW 2000 Australia
Tel: +61 2 9286 6000



SOCIAL EVENTS

WELCOME RECEPTION

Date:	Wednesday, 15 September 2010
Venue:	Sheraton on the Park, Sydney
Time:	1800 – 2000 hours
Cost:	Included in full delegates registration fee
Guests and one day delegates:	A$75 per person
Dress Code:	Smart Casula/Lounge Suit

Catch up with colleagues and acquaintances over drinks and canapés at the Sheraton on the Park.

MEETING DINNER

Date:	Thursday, 16 September 2010
Venue:	Sheraton on the Park, Sydney
Time:	1900 – 2300 hours
Cost:	Included in full delegates registration fee
Guests and one day delegates:	A$145 per person
Dress Code:	Smart Casula/Lounge Suit

We hope all delegates and their guests will join us on this occasion to make it a memorable evening of the event. A great evening of food and wine is planned for the Meeting Dinner – with the dinner speaker being Justice Michael Kirby.

NATIONAL SCIENTIFIC COMMITTEE

Chair: **Dietmar W. Hutmacher** ***Co-Chair:*** **Hala Zreiqat**

Clayton Adam
Mark Bartold
Ken Beagley
Arthur Brandwood
Judith Clements
Ann Collins
Justin Cooper-White
Ross Crawford
Tim Dargaville
Paul Dalton
Colin Dunstan
Greg Dusting
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Hans Griesser
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Patrick Warnke
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Jerome Werkmeister
John Whitelock
Tim Woodfield
Mia Woodruff
Yin Xiao

INTERNATIONAL SCIENTIFIC COMMITTEE

AnthonyAtala
Stephen Badylak
Ahnond Bunyaratvej
Yilin Cao
Fulin Chen
Traian Chirila
Georg Duda
Stephen Feinberg
Zigang Ge
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Juergen Groll
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Feng-Huei Lin
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Wei Lu
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David Mooney
Robert Nerem
Michael Nerlich
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Hajime Ohgushi
Teruo Okano
Richard Oreffo
Jung-Keug Park
Graham Parker
Felipe Prosper
Michael Raghunath
Alan Russell
Heinz Redl
Simone Rizzi
Sambit Sahoo
Jan Thorsten Schantz
Chandra Sharma
Tatsuya Shimizu
David Smith
Hsing-Wen Sung
Wojciech Swieszkwski
Yasuhiko Tabata
Martijn Van Griensven
DavidbWilliams
B M T Wing
Mia Woodruff
Jun YaMashita
Masayuki Yamato
Pamela Yelick
James Yoo
X D Zhang
Yufeng Zhang
Zhang Zhiyong

PLENARY SPEAKERS

• Professor James Goh
Head, Division of Bioengineering, Faculty of Engineering, National University of Singapore, Singapore

• Professor Robert Guldberg
Director, Parker H. Petit Institute for Bioengineering and Bioscience (IBB) at the Georgia Institute of Technology, USA

• Professor Silviu Itescu
Chief Scientific Officer, Mesoblast Limited, Australia

• Professor Wayne Morrison
Director, O'Brien Institute, University of Melbourne, Australia

• Professor Teruo Okano
Director, Institute of Advanced Biomedical Engineering and Science at the Tokyo Women's Medical University (TWMU), Japan

• Professor Heinz Redl
Director, Ludwig Boltzmann Institute For Exp. & Clin. Traumatology, Austria

GENERAL INFORMATION

REGISTRATION DESK
The Registration Desk will be located in the Foyer Area on Level 2 of the Sheraton on the Park – opposite the stairs.

Registration hours: 0730 - 2000 hours on Wednesday, 15 September 2010
0815 - 1800 hours on Thursday, 16 September 2010
0815 - 1700 hours on Friday, 17 September 2010

NAME BADGES
All delegates will be given a name badge at registration. For security reasons, we ask that you wear your name badge at all times. This name badge is also the official entrance pass to all conference sessions, exhibition area, teas & lunches each day and inclusive social functions.

MESSAGES
All messages received during the event will be displayed on the message board near the registration desk.

SMOKING POLICY
There will be no smoking in any scientific sessions or in areas where food and beverage are served.

HOTEL ACCOUNTS
All delegates are reminded to pay their hotel account prior to departure from their hotel. Please note that the deposit you have paid on the TERMIS AP 2010 registration form has been credited to your room account, however, each delegate is responsible for the payment of incidentals and any further room costs upon departure from the hotel.

CAR PARKING
Parking is available for delegates underneath the Sheraton on the Park. The current cost of car parking charges to all vehicles (self parking) is as follows:

- 0 - 1 hours - $30
- 1 - 2 hours - $45
- 2 - 3 hours - $55
- 3+ hours - $65 (maximum daily fee)

CREDIT CARDS
Credit cards accepted at the Registration Desk are Mastercard, Visa and Amercian Express – please note that Diners will not be accepted.

DISCLAIMER OF LIABILITY
The TERMIS AP 2010 Organising Committee reserves the right to amend any part of the program or event should it be necessary. The TERMIS AP 2010 Secretariat will not accept liability for damages of any nature sustained by participants or their accompanying persons, or loss of, or damage to, their personal property as a result of TERMIS AP 2010 or related events.

SPEAKER PREPARATION AREA

Speakers are asked to check their audio visual material before presenting. There will be an audio visual preparation area located near the Ballroom on Level 2 of the Sheraton on the Park and we ask that you check-in with these technicians **at least two hours prior to your presentation**. At this time you will be able to provide the technician with a copy of your presentation via USB or CD-rom.

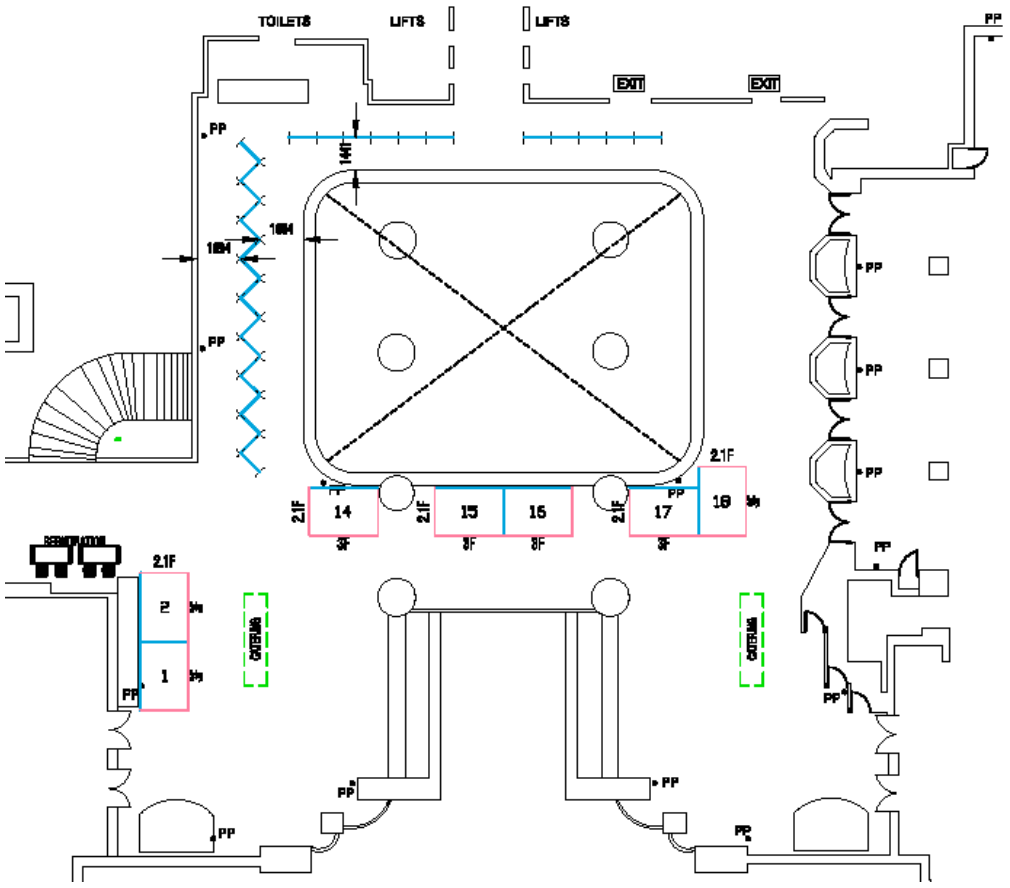
EXHIBITION & POSTER AREA

The Exhibition and Poster Area will be open during the following times:

- 0830 - 2000 hours on Wednesday, 15 September 2010
- 0900 - 1800 hours on Thursday, 16 September 2010
- 0900 - 1700 hours on Friday, 17 September 2010

Morning and afternoon tea refreshments, lunch and the Welcome Reception will be served in this area to enable you to visit all exhibitors whose support to the meeting is invaluable and much appreciated.

Please find below a current floor plan of the area.



We acknowledge with gratitude the support given by these companies and ask that you show your appreciation by visiting all the exhibits.

ORGANISATION	BOOTH
Armed Forces Institute of Regenerative Medicine	Booth 15
BD	Booth 14
Bose Corporation	Booth 2
Laverty Pathology	Booth 18
Life Technologies	Booth 1
Stemcell Technologies Inc	Booth 17
University Co-Operative Bookshop	Booth 16

FINAL PROGRAM

WEDNESDAY, 15 SEPTEMBER 2010

(Grand Ballroom I, Level 2)

0830 - 0900 OPENING CEREMONY: Geoff McKellar, Teruo Okano, Jim Bishop AO & Dietmar Hutmacher

0900 - 0940 PLENARY LECTURE: Wayne Morrison - Importance of Vascularization of Tissue Engineered Construct from a Clinical Point of View

0940 - 1020 PLENARY LECTURE: Robert Guldberg - In Vitro and In Vivo imaging of Vascularization of a Tissue Engineered Construct

1020 - 1100 PLENARY LECTURE: Teruo Okano - Engineering of Vascularization in a Tissue Engineered Construct

1100 - 1130 ***MORNING TEA***

1130 - 1300	**CONCURRENT SESSION 1A: CARTILAGE TE 1** ***(Grand Ballroom II, Level 2)***		**CONCURRENT SESSION 1B: REGENERATIVE MEDICINE ON PANCREAS** ***(Phillip Room, Level 2)***		**CONCURRENT SESSION 1C: OBSTETRICS & GYNAECOLOGY** ***(Hyde Park Room, Level 2)*** ***Sponsored by: CSIRO***		**CONCURRENT SESSION 1D: CARDIAC TISSUE ENGINEERING** ***(Grand Ballroom I, Level 2)***
	Chairs: Travis Klein & Tim Woodfield		*Chair: Feng-Huei Lin*		*Chairs: Anna Rosamilla & Keith McLean*		*Chair: Greg Dusting & Teruo Okano*
1130 - 1200	KEYNOTE: Jos Malda (Abstract 369) - Towards the Engineering of Cartilage Grafts with Zonal Organization	1130 - 1145	Shoichiro Sumi (Abstract 342) - Studies on Poly(vinyl alcohol)-Macro-Encapsulated Islets for Diabetes Mellitus	1130 - 1200	KEYNOTE: Jan Deprest (Abstract 353) - The Use of Xenogenic Implants in Pelvic Floor Surgery	1130 - 1145	OVERVIEW: Greg Dusting
1200 - 1215	Yingying Zhang (Abstract 145) - The Impact of PLGA Scaffold Orientation on Structure and Mechanical Property of Engineered Cartilage	1145 - 1200	Tzong Kuo (Abstract 345) - Establishment of Diabetic Animal Model for Study	1200 - 1215	Hans Pieter Dietz (Abstract 330) - Functional Imaging of the Intact and Damaged Pelvic Floor	1145 - 1215	KEYNOTE: Rod Dilley (Abstract 374) - Supportive Environments for Cardiac Tissue Engineering in Vivo
1215 - 1230	Shuo Yin (Abstract 33) - Chondrogenic Transdifferentiation of Human Dermal Fibroblasts Stimulated with Cartilage Derived Morphogenetic Protein1	1200 - 1215	Ching-Yao Yang (Abstract 359) - Optimization of Islet Transplantation by Preconditioning Strategy	1215 - 1230	Sharon Edwards (Abstract 259) - Scaffolds for the Repair of Pelvic Organ Prolapse	1215 - 1230	Hsing-Wen Sung (Abstract 272) - Injectable Cell Delivery Systems for Myocardial Tissue Engineering: Cell Sheet Fragments, Cell Bodies and Cell Rods
1230 - 1245	Jacqueline Brown (Abstract 221) - Engineering Physeal Tissue Using Human Bone Marrow Derived Mesenchymal Stem Cells	1215 - 1230	Anandwardhan Hardikar (Abstract 408) - Understanding the Role of Non-Coding (nc)RNAs in Regenerative Biology of Endocrine Pancreas	1230 - 1245	Caroline Gargett (Abstract 368) - Potential of Human Endometrial Mesenchymal Stem Cells for Tissue Engineering the Pelvic Floor	1230 - 1235	Dehua Chang (Abstract 123) - Multilayer Cell Sheet Therapy for Myocardical Infarction Model
1245 - 1300	Karsten Schrobback (Abstract 298) - Expression of Cell Surface Markers in Cultured Human Zonal Chondrocytes	1230 - 1245	Kai-Chiang Yang (Abstract 404) - Calcium Phosphate Cement Chamber as Immuno-isolative Device for Bioartifical Pancreas: An In Vivo Study on Diabetic Canine	1245 - 1300	Euan Wallace (Abstract 385) - Clinical Use of Human Amniotic Epithelial Cells in Maternal Fetal Medicine	1235 - 1240	Masaaki Ii (Abstract 130) - Synergistic Effect of Adipose-Derived Stem Cell Therapy and Bone Marrow Progenitor Recruitment in Ischemic Heart
		1245 - 1300	Gilad Amiel (Abstract 247) - Decellularized Porcine-Derived Blood Vessel Matrix Graft for Urethral Replacement in a Rabbit Model			1240 - 1245	Mee-Hae Kim (Abstract 291) - Novel Strategy for Directing the Differentiation of Human Mesenchymal Stem Cells into the Myogenic Lineage on Dendrimer-Immobilized Surface with D-Glucose Display
						1245 - 1250	Daisuke Sasaki (Abstract 285) - Cardiac Differentiation of Mouse ES Cells by Patterning Culture
						1250 - 1255	Payam Akhyari (Abstract 320) - Inhibition of Aortic Valvular Interstitial Cell Degeneration by ECM Modification - Implications for Heart Valve Engineering
						1255 - 1300	Discussion

1300 - 1400 ***LUNCH (Sponsored by: Synthes Australia) & POSTERS***

1400 - 1530	**CONCURRENT SESSION 2A: TENDON / LIGAMENT / MENISCUS** ***(Hyde Park Room, Level 2)***		**CONCURRENT SESSION 2B: BIOMATERIALS_BASED RM** ***(Grand Ballroom II, Level 2)***		**CONCURRENT SESSION 2C: BONE 1** ***(Grand Ballroom I, Level 2)***		**CONCURRENT SESSION 2D: ENABLING TECHNOLOGY FOR TE** ***(Phillip Room, Level 2)***
	Chair: H W Ouyang & Jons Hilborn		*Chair: Masaya Yamamoto & Hsing-Wen Sung*		*Chair: Richard Oreffo & Gilson Khang*		*Chair: Tae Gyun Kwon & Helmut Thissen*
1400 - 1415	Jons Hilborn (Abstract 249) - Engineering of Bone, Cartilage and Tendon Using Cell Free Scaffold	1400 - 1430	KEYNOTE: Ryosuke Kuroda (Abstract 321) - Application for Biomaterials-Based Regenerative Medicine for Orthopaedic Surgery	1400 - 1430	KEYNOTE: Simon Cool (Abstract 230) - Lowering the Efficacious Dose of BMP-2 Using Heparan Glycosaminoglycans	1400 – 1430	KEYNOTE: Sang Jin Lee (Abstract 387) - Biomaterial Induced Stem Cell Recruitment for Insitu Tissue Regeneration
1415 - 1430	Justin Cooper-White (Abstract 386) - Human Knee Meniscal Cells: Characterization of Their Primary Culture and Their Response to Biomimetic Surfaces in 2D and 3D Growth Environments	1430 - 1445	Masaya Yamamoto (Abstracts 380) - Biomaterial Technology to Manipulate Stem Cells for Regenerative Medicine and Therapy	1430 - 1500	KEYNOTE: Molly Stevens (Abstract 406) - New Biomaterials Strategies Bone Engineering	1430 - 1445	Grace Lim (Abstract 313) - Oxygen Generating Matrix for Regenerative Medicine

1430 - 1445	Hamoud Alfaqeh (Abstract 267) - Stem Cell Based Therapy Retards the Progression of Osteoarthritis and Promotes Repair Meniscus Injury of Sheep Model Knee Joint	1445 - 1500	Peter Lelkes (Abstract 183) - Electrospon "Green" Soy Protein-Based Scaffolds for Skin Tissue Engineering and Wound Healing	1500 - 1515	Young-Joon Seol (Abstract 189) - Development of Hydroxyapatite Scaffolds for Bone Tissue Regeneration	1445 - 1500	Matteo Moretti (Abstract 251) - An Automated Perfusion Bioreactor for Online, Real-Time, Non-Invasive Monitoring of pH and pO2 Within Multiple Independent Chambers
1445 - 1500	Jie Li (Abstract 138) - Elongated Morphology and Uniaxial Mechanical Loading Regulate Tenocyte Phenotype	1500 - 1515	Kasuo Ohashi (Abstract 269) - Cell Sheet Engineering Toward Bioengineering of Functional Neo-Islets	1515 - 1520	Claudia Di Bella (Abstract 40) - Rh-OP-1 Associated with Mesenchymal Stem Cells Promote Bone Allograft	1500 - 1515	Drew Titmarsh (Abstract 153) - Diagnostic Microbioreactor Arrays for Multiplexed Microenvironmental Screening of Pluripotent Stem Cell Expansion, Maintenance and Differentiation
1500 - 1515	Yongkang Jiang (Abstract 129) - The Effects of Mechanical Loading on Tissue Engineered Tendon Formation: Structural and Proteomic Analyses	1515 - 1530	Jeyran Shahbazi (Abstract 106) - Laser-Activated Chitosan-Based Bioadhesive for Ocular Tissue Regeneration in Wound Healing Applications	1520 - 1525	Somaieh Kazemnejad (Abstract 51) - Remarkable Osteogenic Differentiation of Human Menstrual Blood Derived Stem Cells in Presence of Human Platelet Releasate	1515 - 1530	Yiwei Wang (Abstract 152) - Efficacy and Safety of Engineered Skin Substitute on Skin Wound Healing
1515 - 1520	Thomas Kok Hiong Teh (Abstract 255) - A Comparative Study of Different Mechanical Conditioning Regimes for the Development of Tissue Engineered Anterior Cruciate Ligament			1525 -1530	ZuFu Lu (Abstract 31) - The Osteoconductivity of Biomaterials is Regulated by BMP2 Autocrine Loop Involving Á2Â1 Integrin and MAPK/ERK Signaling Pathways		
1520 - 1525	Justin Cooper-White (Abstract 264) - Modulation of Gap Junction Expression in Injured Equine Superficial Digital Flexor Tendon (SDFT)						
1525 - 1530	Discussion						
1530 - 1600	***AFTERNOON TEA***						
1600 - 1800	**CONCURRENT SESSION 3A: CARTILAGE TE 2** ***(Grand Ballroom II, Level 2)***		**CONCURRENT SESSION 3B: SILK** ***(Phillip Room, Level 2)***		**CONCURRENT SESSION 3C: MAMMALIAN REGENERATION** ***(Hyde Park Room, Level 2)*** ***Sponsored by: Australian Regenerative Medicine Institute***		**CONCURRENT SESSION 3D: CELL THERAPY FROM BENCH TO BEDSITE** ***(Grand Ballroom I, Level 2)***
	Chairs: Wei Liu & M Ochi		*Chair: Subhas Kundu & Wojciech Chrzanowski*		*Chair: Keith McLean & Jerome Werkmeister*		*Chair: Frank Emmrich & Teruo Okano*
1600 - 1630	KEYNOTE: Guangdong Zhou (Abstract 141) - In Vitro Engineering of Human Ear-Shaped Cartilage Assisted with CAD/CAM Technology	1600 - 1630	KEYNOTE: Antonella Motta (Abstract 238) - Silk Fibroin Source, Molecular Structure and Processing: How do They Affect its Biomedical Behaviour?	1600 - 1630	KEYNOTE: James Godwin (Abstract 388) - Characterizing the Immunological Response to Wounding and Regeneration in the Salamander	1600 - 1630	KEYNOTE: Frank Emmrich (Abstract 409) - Cell Therapy in Stroke
1630 - 1645	Nuno Neves (Abstract 308) - Polycaprolactone Nanofiber Meshes as Scaffolds for Cartilage Tissue Engineering Using a Dynamic Culture System	1630 - 1700	KEYNOTE: Subhas Kundu (Abstract 274) - Silk Fibroin-PVA Hydrogels for Biomedical Applications	1630 - 1645	Tiziano Barberi (Abstract 375) - Directed Differentiation of HESC into Skeletal Muscle: A Potential Source of Muscle Precursor Cells for Therapeutic Applications	1630 - 1700	KEYNOTE: Ali Khademhosseini (Abstract 88) - Microengineered Hydrogels for Stem Cell Bioengineering and Tissue Regeneration
1645 - 1700	Nai-Chen Cheng (Abstract 25) - Different Chondrogenic Pattern of Human and Porcine Chondrocytes Cultured in a Porous Cartilage-derived Matrix	1700 - 1715	Sabine Fuchs (Abstract 325) - Vascularization of Silk Fibroin Based Materials: From Cell-Material Interactions to Prevascularized Tissue Constructs	1645 - 1700	Tania Ahmed (Abstract 109) - Enhanced Wound Repaid Through the Coalescence of Urinary Bladder Matrix and Laser Activated Chitosan Bio-Adhesive	1700 -1730	KEYNOTE: Brian Newsom (Abstract 410) - So You Think You're Ready for the Clinic?
1700 - 1715	Tim Woodfield (Abstract 354) - Mass-Scale Fabrication of Cell Pellets for High Throughput Screening and 3D Assembly of Tissue Engineered Cartilage	1715 - 1730	Rangam Rajkhowa (Abstract 331) - Protein Fibre Particles for Biomedical Applications	1700 - 1715	Kerry Daly (Abstract 143) - Damage Associated Molecular Patterns (DAMPs) and Macrophages in the Host Immune and Remodeling Response to Xenogeneic Biological Scaffolds	1730 - 1800	KEYNOTE: Carsten Werner (Abstract 407) - Multi-Biofunctional Polymer Gels to Aid Regenerative Therapies
1715 - 1730	Illida Mohd Nawi (Abstract 224) - Preliminary Characterisation of a Novel-Designed Mechanical Conditioning Bioreactor to Deliver Biaxial Compression on Chondrocytes Seeded in 3D Agarose Scaffold	1730 - 1745	Hyoung-Joon Jin (Abstract 293) - Human Bone Marrow Stem Cell Response to Silk Fibroin/Carbon Nanotube Composites	1715 - 1730	Marta Fontanilla (Abstract 254) - Evaluation of Growth Factors, Cytokines, Chemokines Secretion and Cellular Migration Induced by Acellular Collagen Scaffolds and Autologous Artificial Connective Tissue		
1730 - 1745	Yu Liu (Abstract 36) - In Vitro Engineering of Human Ear-Shaped Cartilage Assisted with CAD/CAM Technology	1745 - 1800	James Goh (Abstract 144) - Bone - Ligament Tissue Engineering in a Rabbit Model Using BMSC-Seeded Triphasic Silk Scaffolds	1730 - 1745	Koki Hagiwara (Abstract 58) - Promotion of Muscle Repair by Myoblast Transplantation Using the Controlled Delivery System of bFGF		
1745 - 1800	Somaieh Kazemnejad (Abstract 80) - Chondrogenic Differentiation of Menstrual Blood Derived Stem Cells on a Nanofibrous Scaffold			1745 - 1800	Hee Seok Yang (Abstract 170) - Delivery of Growth Factors Contained in Platelet-Rich Plasma Using Heparin-Conjugated Fibrin for Wound Healing		
1800 - 2000	**WELCOME RECEPTION (*Exhibition Area, Level 2*)**						

THURSDAY, 16 SEPTEMBER 2010

	(Grand Ballroom I, Level 2)						
0900 - 0940	PLENARY LECTURE: Heinz Redl - Potential Sources and Application Modes for MSC in Trauma						
0940 - 1020	PLENARY LECTURE: James Goh - Regenerative Medicine: Beyond Cells, Scaffolds and Growth Factors						
1020 - 1100	PLENARY LECTURE: Silviu Itescu - Potential of Adult Stem Cells in the Regeneration of the Cardiovascular System						
1100 - 1130	***MORNING TEA***						
1130 - 1300	**CONCURRENT SESSION 4A: STEM CELLS** ***(Grand Ballroom I, Level 2)***		**CONCURRENT SESSION 4B: SCAFFOLD DESIGN & FUNCTION 1** ***(Grand Ballroom II, Level 2)***		**CONCURRENT SESSION 4C: BIOMATERIALS FOR IN VITRO TISSUE SYNTHESIS** ***(Phillip Room, Level 2)***		**CONCURRENT SESSION 4D: NEURAL TISSUES** ***(Hyde Park Room, Level 2)***
	Chair: Stan Gronthos & Simon Cool		*Chair: Antonella Motta & Daniel Cohn*		*Chair: Takuya Matsumoto & Hyun Joon Kong*		*Chair: David Nisbet & Paul Dalton*
1130 - 1145	OVERVIEW: Stan Gronthos	1130 - 1200	KEYNOTE: Gilson Khang (Abstract 411) - Principles of Scaffold Design and Function	1130 - 1200	KEYNOTE: Hyunjoon Kong (Abstract 339) - Decoupled Control of Rigidity and Permeability of a Cell Encapsulated Hydrogel for Therapeutic Angiogenesis	1130 - 1200	KEYNOTE: Mal Horne (Abstract 378) - Repairing the Brain with Biomaterials
1145 - 1215	KEYNOTE: Peter Ghosh (Abstract 389) Regenration and Repair of Connective Tissues by Mesenchymal Progenitor Cells - New Modalities of Treatment for Disorders of the Musculo-Skeletal System?	1200 - 1215	Andrea O'Connor (Abstract 173) - Delivery of an Anti-Inflammatory Hormone Via Layer-By-Layer Assembly of Biomolecules	1200 - 1215	Jingnan Luo (Abstract 347) - Self-Assembly of Collagen-Mimetic Peptide Amphiphiles into Biofunctional Nanofiber	1200 - 1215	Paul Dalton (Abstract 277) - Implantation of Porous pHEMA into the Spinal Cord: Innate Immunity and Implications for Neural Tissue Engineering
1215 - 1230	Ravenska Wagey (Abstract 57) - Enhanced Immunosuppressive Activity and Chondrogenic Potential of Human Mesenchymal Progenitor Cells Cultured in a Novel Xeno-Free Medium	1215 - 1230	Johnson Chung (Abstract 200) - Drug Eluting Polyurethane Nanocomposite: Release and Preliminary Cell Interactions	1215 - 1230	Koichi Kato (Abstract 365) - Culture Substrates with Immobilized Growth Factors for Use in In Vitro Expansion of Human Neural Progenitor Cells	1215 - 1230	Tianqing Liu (Abstract 78) - A Study for Construction of Three-Dimensional Neural Network In Vitro
1230 - 1235	Justin Cooper-White (Abstract 64) - Effects of Microenvironmental Changes on Human Mesenchymal Stem Cell (hMSC) Behaviour	1230 - 1245	Karl-Heinz Heffels (Abstract 294) - Application of Nanofibre Meshes for Treatment of Diaphragm Hernia	1230 - 1245	Andrew Wan (Abstract 362) - ECM Scaffolds for Three-Dimensional Cell Culture and Tissue Engineering	1230 - 1245	Guei-Sheung Liu (Abstract 334) - Glial Cell Line-Derived Neurotrophic Factor Gene Transfer Exerts Protective Effect in Sciatic Nerve Following Cobstriction-Induced Peripheral Nerve Injury
1235 - 1240	Somaieh Kazemnejad (Abstract 113) - Effect of Lithium Chloride on ß-Catenin Expression, Proliferation and Osteogeneic Differentiation of Human Menstrual Blood Derived Stem Cells	1245 - 1300	Roger Chan (Abstract 333) - A Xenogeneic Scaffold for Vocal Fold Reconstruction: Rheometric Measurements	1245 - 1300	Takuya Matsumoto (Abstract 358) - Scaffold Free 3D Cell Constructs for In Vitro Tissue Synthesis	1245 - 1300	Mitsuhiro Ebara (Abstract 244) - Design of Smart Aliphatic Polyesters with Controlled Parallel-Channel Architectures for Nerve Repair
1240 - 1245	Nuno Neves (Abstract 309) - Chondrogenic Differentiation of Human Bone Marrow Mesenchymal Stem Cells Seeded in 3D Porous Scaffolds in a Flow Perfusion Bioreactor						
1245 - 1250	Jessica Frith (Abstract 112) - Nanoscale Presentation of Cell Adhesion Motifs Via Block-Copolymer Self Assembly: Effect of Ligand Spacing on Mesenchymal Stem Cell Behavour						
1250 - 1255	Yingying Zhang (Abstract 146) - In Vitro Three Dimensional Chondrogenesis of Murine BMSCs Purified by Hypoxia Culturing and MACS with Lineage						
1255 -1300	Zhiyong Zhang (Abstract 165) - Comparison of Different Bioreactor to Culture Fetal Mesenchymal Stem Cells for Bone Tissue Engineering						
1300 - 1400	***LUNCH (Sponsored by: Mesoblast Limited) & POSTERS***						
1300 - 1400	**TERMIS-AP Council Meeting (*Castelreagh Room, Level B1*)**						

1400 - 1530	**CONCURRENT SESSION 5A: STEM CELLS 2** ***(Grand Ballroom I, Level 2)***		**CONCURRENT SESSION 5B: SCAFFOLD DESIGN & FUNCTION 2** ***(Grand Ballroom II, Level 2)***		**CONCURRENT SESSION 5C: CANCER RESEARCH 1** ***(Phillip Room, Level 2)*** ***Sponsored by: Prostate Cancer Foundation of Australia***		**CONCURRENT SESSION 5D: BONE 2** ***(Hyde Park Room, Level 2)***
	Chair: Heinz Redl & Karsten Schrobback		*Chair: Gilson Khang & Carsten Werner*		*Chairs: Pamela Russell & Daniela Loessner*		*Chair: Molly Stevens & Ulrich Noeth*
1400 - 1430	KEYNOTE: Ursula Manuelpillai (Abstract 390) - Human Amnion Epithelial Cell (HAEC) Treatment for Reducing Tissue Inflammation and Fibrosis and Aumenting Regeneration	1400 - 1430	KEYNOTE: Wojciech Swieszkowski (Abstract 405) - Application of Numerical Modelling in the Design and Evaluation of Scaffolds for Tissue Engineering	1400 - 1430	KEYNOTE: Leland Chung (Abstract 392) - Cell Signaling Network Linking Osteomimicry, Oxidative Stress and Cancer Metastases	1400 - 1430	KEYNOTE: Richard Oreffo (Abstract 395) Prospects for Skeletal Regeneration Using Innovative Skeletal Stem Cell Based Approaches - Bridging the Gap
1430 - 1500	KEYNOTE: Gary Brooke (Abstract 391) - Towards Placenta-Derived Stem Cell Treatment for Myocardial Infarction - Early Clinical and Pre-Clinical Experience	1430 - 1445	Wojciech Chrzanowski (Abstract 383) - Glass Filled Polymers for Scaffolds Fabrication - The Modulation of Cell Response by Tailoring Filler Chemistry	1430 - 1445	Nathalie Picollet-D'hahan (Abstract 125) - Polyelectrolyte Multilayer Films Modulate Cellular Markers of Adhesion and Polarity in Normal and Cancerous Prostate Cells	1430 - 1445	Hong Liu (Abstract 4) - Construction of Micro-Nano Two-Level 3-Dimensional Fluorescent Chitosan-Hap Interconnected Networked Bone Scaffold Through a Nano-Crystallon Induced Biomineralization Method
1500 - 1515	Suk Ho Bhang (Abstract 182) - Three-Dimensional Cell Engineering Enhances the Angiogenic Efficacy of Human Umbilical Vein Endothelial Cells	1445 -1500	Zhiyong Zhang (Abstract 270) - The Use of Bio-Functionalized Ultrathin Polycaprolactone Membrane for Development of Tissue Engineered Vascular Grafts	1445 - 1500	Colin Dunstan (Abstract 394) - Calcium and Vitamin D Nutritional Status Impacts on Bone Metastasis in Breast and Prostate Cancer	1445 - 1500	ZuFu Lu (Abstract 32) - Effects of Hydroxyapatite Nanoparticle Shape on Mechanical and Biological Behaviour of Nanocomposite Scaffolds
1515 - 1530	Masaya Yamamoto (Abstract 222) - Fabrication of Jagged1-Immobilized Substrates for the Ex Vivo Expansion of a Cell Population Containing Hematopoietic Stem Cells	1500 - 1515	Yilling, Erin Teo (Abstract 227) - Alignment of Myoblasts Throu Micro-Scaled Topographic Engineering of Ultrathin, Stretched Polycaprolactone Films	1500 - 1515	Verena Reichert (Abstract 300) - Human Primary Osteoblast Matrix as Model to Investigate Interactions of Breast Cancer Cells with the Bone Microenvironment	1500 - 1515	Qizhi Chen (Abstract 18) - A New Sol-Gel Process for Producing NA2O-Containing Bioactive Glass-Ceramics
		1515 - 1530	Yiwei Wang (Abstract 154) - Biomechanical Characterisation of a Micro/Macroporous Polycaprolactone Tissue Integrating Vascular Graft	1515 - 1530	Shirley Sieh (Abstract 281) - Studying Interactions Between Human Prostate Cancer Cells and Osteoblast Using a Novel 3D In Vitro Co-Culture Model	1515 - 1530	Simon Cool (Abstract 202) - Structural Restoration of Rabbit Critical-Sized Ulna Defects Using Novel Heparan Sulfates in Combination with beta-TCP Implants
1530 - 1600	***AFTERNOON TEA***						
1600 - 1800	**CONCURRENT SESSION 6A: STEM CELLS 3 – ADIPOSE** ***(Grand Ballroom I, Level 2)***		**CONCURRENT SESSION 6B: TISSUE ENGINEERING FROM A CLINICAL POINT OF VIEW IN THE ORTHOPAEDIC ARENA** ***(Phillip Room, Level 2)***		**CONCURRENT SESSION 6C: CANCER RESEARCH 2** ***(Hyde Park Room, Level 2)*** ***Sponsored by: Prostate Cancer Foundation of Australia***		**CONCURRENT SESSION 6D: BONE 3** ***(Grand Ballroom II, Level 2)***
	Chair: Ursula Manuelpillai & Heinz Redl		*Chair: Robert Guldberg & Georg Duda*		*Chair: Leland Chung & J Clements*		*Chair: Don Martin & Clive McFarland*
1600 - 1615	Keren Abberton (Abstract 111) - Striking a Balance: A Role for Macrophages in Adipose Tissue Engineering	1600 - 1630	KEYNOTE: Ian Harris (Abstract 286) - The Wide Gap Between Bench and Bedside: Failures in Translating Research into Practice	1600 - 1615	Daniella Loessner (Abstract 30) - Modeling of Cancer Spheroids in Bioengineered 3D Microenvironments	1600 - 1630	KEYNOTE: Nathalie Picollet-D'hahn (Abstract 361) - Taming the Frontiers: Advances in Surface Microenvironments for Regulating Cell and Tissue Growth
1615 - 1630	Akon Higuchi (Abstract 114) - Separation and Differentiation of Adipose-Derived Stem Cells by Membrane Filtration Method	1630 - 1700	KEYNOTE: Ashish Diwan (Abstract 382) - Attack on Back Pain - A Tissue Engineered Response	1615 - 1630	Nigel McMillan (Abstract 366) - Towards RNAi Therapy for Cancer - Solving the Critical Issues	1630 - 1700	KEYNOTE: Donald Martin (Abstract 381) - 3-Dimensional Structures for Measuring Cell Responses
1630 - 1645	Akon Higuchi (Abstract 159) - Preservation of Hematopoietic Stem Cells from Umbilical Cord Blood Stored in a Surface Derivatized with Polymer Nano-Segments	1700 - 1730	KEYNOTE: Georg Duda (Abstract 396) - Chances and Challenges in Bone Regeneration – Learning from Endogenous Regeneration Processes	1630 - 1645	Haiyen Zhau (Abstract 393) - Quantum Dot-Based Probing of Cell Signaling in Prostate Cancer Cells Predicts Patients' Survival	1700 - 1715	M Tarik Arafat (Abstract 134) - Biomimetic Composite Coating on Poly (â-Caprolactone) and Silane Modified Tricalcium Phosphate Scaffolds for Bone Tissue Eingineering
1645 - 1700	Chen Wang (Abstract 98) - Differentiation of Adipose Derived Stem Cells into Contractile Smooth Muscle Cells Induced by TGF-B1 and BMP4	1730 - 1800	KEYNOTE: Ulrich Nöth (Abstract 397) - Regenerative Approaches in Osteochondral, Cartilage and Tendon Repair	1645 - 1700	Roxanne Toivanen (Abstract 398) - Inclusion of Inductive Stromal Cells Enhances the Efficiency of Xenograft Models of Human Localised Prostate Cancer in Mice	1715 - 1730	Gajadhar Bhakta (Abstract 209) - Glycosaminoglycan Hydrogels for Sustained Delivery of Bone Morphogenetic Protein-2
1700 - 1715	Huijie Gu (Abstract 99) - The Effect of Ionic Products from Akermanite Dissolution on Proliferation and Osteogenic Differentiation of Human Adipose-Derived Stem Cells			1700 - 1715	Wan-Chun Yu (Abstract 190) - Purification and Characterization of Cancer Stem Cells in Colon Cancer Cells Treated by Anti-Cancer Drugs	1730 - 1735	Lucie Bacakova (Abstract 94) - Nanofibrous PLGA Scaffolds Loaded with Nanodiamond as Substrates for Adhesion and Growth of Human Bone-Derived Cells
1715 - 1730	Tianqing Liu (Abstract 62) - Cell Cycle Analysis for Human Adipose-Derived Stem Cells at Different Culture Time			1715 - 1730	Parisa Hesami (Abstract 310) - Establishment of a Human Bone Engineered Platform Model to Investigate Prostate Cancer-Bone Interaction in NOD/SCID Mice	1735 - 1740	Arne Berner (Abstract 302) - Benchmarking Autologous Vs Allogenic Mesenchymal Progenitor Cells for the Reconstruction of Critical-Sized Segmental Tibial Bone Defects in a Large Animal Model

1730 - 1745	Lei Cui (Abstract 97) - A Small Diameter Elastic Blood Vessel Wall Prepared Under Pulsatile Conditions from Polyglycolic Acid Mesh and Smooth Muscle Cells Differentiated from Adipose-Derived Stem Cells			1730 - 1800	Discussion (P Russell, E Williams, C Dunstan, L Chung & D Hutmacher)	1740 - 1745	Chao Li (Abstract 157) - Drug Release from a Macroporous 25Hema Hydrogel Under Rheological Stimulations
1745 - 1800	Ganghong Tian (Abstract 101) - Effects of Static Magnetic Field on Viability and Function of Adipose-Derived Stem Cells					1745 - 1750	Chao Li (Abstract 158) - Rheological and Stress Relaxation Properties of Phema and Phema-TIO2 Composite Hydrogels
						1750 - 1755	Sunny-Akogwu Abbah (Abstract 127) - A Multilayered Polymeric-Polyelectrolyte Microcarrier System Potentiated BMP-2 Activities in a Rat Model of Posterolateral Spinal Fusion
						1755 - 1800	Juthamas Ratanavaraporn (Abstract 74) - The Combination of Stromal Cell-Derived Factor-1 and Bone Morphogenetic Protein-2 for Bone Tissue Engineering
1900 - 2300	***MEETING DINNER (Grand Ballroom I, Level 2)***						

FRIDAY, 17 SEPTEMBER 2010

0900 - 1100	**CONCURRENT SESSION 7A: CRANIOFACIAL 1** ***(Grand Ballroom II, Level 2)***		**CONCURRENT SESSION 7B: BIOLOGICAL SCAFFOLDS 1** ***(Grand Ballroom I, Level 2)*** ***Sponsored by: LifeCell - KCI***		**CONCURRENT SESSION 7C: BIOMATERIALS & SURFACES 1** ***(Hyde Park Room, Level 2)***		**CONCURRENT SESSION 7D: GENERAL SESSION** ***(Phillip Room, Level 2)***
	Chair: Stephen Feinberg & Ann Collins		*Chair: Jerome Werkmeister & Sharon Edwards*		*Chair: Nico Voelcker & Mia Woodruff*		*Chair: Clive McFarland & Jons Holborn*
0900 - 0930	KEYNOTE: Robert Hale (Abstract 399) - Regenerative Medicine to Address Conventional Treatment Limitations of Craniomaxillofacial Battle Injuries	0900 - 0930	KEYNOTE: David McQuillan (Abstract 377) - Extracellular Matrix-Directed Regeneration of Soft Tissues	0900 - 0930	KEYNOTE: Helmut Thissen (Abstract 311) - Coatings for the Control of Biointerfacial Interactions	0900 - 0915	Lilia Kuleshova (Abstraxt 288) - Current Progress and Challenges in Cryopreservation of Tissue-Engineered Constructs
0930 - 1000	KEYNOTE: Stephen Feinberg (Abstract 364) - Role of Oral Mucosa in Soft Tissue Engineering in Craniomaxillofacial Surgery	0930 - 0945	Gilad Amiel (Abstract 252) - Bladder Augmentation with a Novel Decellularized Blood Vessel Matrix in a Swine Model	0930 - 0945	Isarawut Prasertsung (Abstract 83) - Enhancement of In Vitro Biocompatibility of Crosslinked Gelatin Films by AC 50Hz Nitrogen Glow Discharge	0915 - 0930	Brooke Farrugia (Abstract 107) - Silicone-Peg Copolymers as a Treatment of Hypertrophic Scars
1000 - 1030	KEYNOTE: Anh Le (Abstract 400) - Immunomodulation of Wound Response	0945 - 1000	Chris Elvin (Abstract 24) - A Rapid Photochemical Crosslinking Method for Fabrication of Covalently Crosslinked Protein-Based Biomaterials	0945 - 1000	Peng-Yuan Wang (Abstract 150) - Preparation of Polydimethylsiloxane (PDMS) Stiffness Gradients for Mesenchymal Stem Cell Culture and Differentiation	0930 - 0945	Shin-Ichi Kanemaru (Abstract 301) - Innovative Regenerative Treatment for the Tympanic Membrane Perforation - Clinical Application
1030 - 1045	Kentaro Doi (Abstract 401) - Facial Reconstruction with Cell-Assisted Lipotransfer (Adipose Progenitor-Enriched Microfat Grafting)	1000 - 1015	YiYi Gong (Abstract 126) - Engineering Cartilage Using Acellular Cartilage Sheet	1000 - 1015	Justin Cooper-White (Abstract 178) - The Effect of Geometrical Constraints on Cell Migration	0945 - 1000	Travis Klein (Abstract 412) - Long-Term Effect of Hydrogel Properties on Human Chondrocyte Behaviour
1045 - 1100	Kieran Rowe (Abstract 169) - The Elastic Properties and Effect of Uniaxial Tension on Adipose Tissue Explants	1015 - 1030	David Sebinger (Abstract 318) - The Capability of Biofunctional Matrices to Influence Embryonic Kidney Development – A Possible Contribution to Treatments Based on Regeneration	1015 - 1030	Megumi Muraoka (Abstract 166) - Behavior of Patterned Endothelial Cells in Three-Dimensional Tissue Engineered by Cell Sheet Technology	1000 - 1015	Qiong Li (Abstract 163) - bFGF Down-Regulate SMA to Antagonize Contraction of BMSC Engineered Cartilage
		1030 - 1045	Jenny Cheng (Abstract 260) - Characterisation of Islet Extracellular Matrix	1030 - 1045	Li-Ying Chen (Abstract 191) - Cultivation of Hematopoietic Stem Cells on the Surface-Modified Materials Having Nano-Segments and Extracellular Matrix Proteins	1015 - 1030	Shui Guan (Abstract 89) - Protocatechuic Acid Combined with BDNF Increase in Production of Rat Dopaminergic Neurons From Cultured Mesencephalic Neural Stem/Progenitor Cells
		1045 - 1100	Shinya Kondo (Abstract 185) - Development of Wound Dressing Composed of Hyaluronic Acid and Collagen Sponge with Epidermal Growth Factor	1045 - 1100	Feriba Dehghani (Abstract 346) - Creation of Porous Chitosan Hydrogel Using Dense Gas CO2	1030 - 1045	Wataru Kamimura (Abstract 219) - Development of Novel Scaffold Material Based on Alginate-Modified Atelocollagen
						1045 - 1100	Koki Hagiwara (Abstract 103) - Aligned Myotubes on Polvinyl Aolcogol Micro Patterned Plates In Vitro
1100 - 1130	***MORNING TEA***						

1130 - 1330	**CONCURRENT SESSION 8A: CRANIOFACIAL 2** ***(Grand Ballroom II, Level 2)***		**CONCURRENT SESSION 8B: BIOLOGICAL SCAFFOLDS 2** ***(Grand Ballroom I, Level 2)***		**CONCURRENT SESSION 8C: SYNTHETIC SCAFFOLDS & HYDROGELS** ***(Hyde Park Room, Level 2)***		**CONCURRENT SESSION 8D: NOVEL THERAPEUTIC TARGETS** ***(Phillip Room, Level 2)***
	Chair: Patrick Warnke & Geoff McKellar		*Chair: Tony Weiss & Nuno Neves*		*Chair: Wojciech Swieszkowski & Paul Dalton*		*Chair: Ted Teng & Hala Zreiqat*
1130 - 1200	KEYNOTE: Pamela Yelick (Abstract 243) - Biologic and Synthetic Scaffolds for Dental Tissue Engineering	1130 - 1200	KEYNOTE: Michael Raghunath (Abstract 384) - Reconstructing the Stem Cell Microenvironment by Delegation - The Liquid and the Solid Way	1130 - 1200	KEYNOTE: Daniel Cohn (Abstract 367) - Engineering Scaffolds for Soft Tissue Regeneration	1130 - 1200	KEYNOTE: Ted Teng (Abstract 402) - Therapeutic Targets for Spinal Cord Injury: Novel Biology Gleaned from Multimodality Studies
1200 - 1215	Ferry Melchels (Abstract 324) - Solid Freeform Fabrication of Hydrogel Structures and Cell Encapsulation	1200 - 1215	James Hudson (Abstract 263) - A Synthetic Bioelastomer with Tunable Modulus for Tissue Engineering Applications	1200 - 1230	KEYNOTE: Murugan Ramalingam (Abstract 271) - Combinatorial Screening of Cell Proliferation on Nanofiber Gradient Libraries	1200 - 1230	KEYNOTE: Hala Zreiqat (Abstract 371) - Resorting to Nature for Inspiration to Engineer Scaffolds for Repair and Regeneration of Skeletal Tissue
1215 - 1230	Cheryl Rahman (Abstract 323) - Injectable Scaffold for Bone Tissue Engineering Applications	1215 - 1230	Veronica Glattauer (Abstract 283) - Eamination of Extracellular Matrix Compositions of Tissue and Cell-Derived Basement Membranes	1230 - 1245	Qizhi Chen (Abstract 17) - A New Family of Elastomeric Biocomposites with a Potential of Wide Applications in Tissue Engineering	1230 - 1245	David Nisbet (Abstract 322) - Brain Response Post Implantation of Electrospun Scaffolds Functionalised with Brain Derived Neurotrophic Factor
1230 - 1245	Joel Boerckel (Abstract 328) - Mechanical Loading Enhances Segmental Bone Defect Repair by Altering Bone Formation and Distribution	1230 - 1245	Takahiro Kakegawa (Abstract 70) - Cell Detachment Along with Electrical Cleavage of a Zwitterionic Oligopeptide Layer	1245 - 1300	Lian Cen (Abstract 11) - Incorporation of Tripolyphosphate Nanoparticles into Fibrous Poly(lactide-co-glycolide) Scaffolds for Tissue Engineering	1245 - 1300	Tetsuji Yamaoko (Abstract 117) - Liver Targetted siRNA Delivery Using Pullulan-PEI Conjugates
1245 - 1300	Kenichi Harimoto (Abstract 236) - Cell-ADHESIVE Advantage of Osteoblasts on Titanium in the Model of In Vitro Osseointegration	1245 - 1300	Naatasha Isahak (Abstract 262) - Neutrophil Activation Induced by Silk Sericin	1300 - 1315	Naoto Mochizuki (Abstract 61) - Fabrication of Thick Cell Sheet Via Electrochemical Reations on Porous Membrane Culture Substrate	1300 - 1315	Nicolin Tirtaatmadja (Abstract 167) - Mixed Micelles to Deliver Drugs for Enhancing Skeletal Muscle Regeeration
1300 - 1315	Adisorn Hanworawang (Abstract 15) - The Efficacy of Dermal Extraced-Bone Powder Scaffold on the Healing of Rat's Calvarial Bone Defects	1300 - 1315	Joanne McBane (Abstract 72) - Influence of Co-Culturing Monocytes with Smooth Muscle Cells: Effects on Cell Attachment, Phenotype, Metabolism and Migration into a Degradable Polyurethane Scaffold for Vascular Tissue Regeneration	1315 - 1320	Luis Rodriguez-Lorenzo (Abstract 248) - Preparation of Cryopolymers for Bone Engineering	1315 - 1330	Kerry Daly (Abstract 142) - The Effect of the áGal Epitope in the Response to ECM in a Nonhuman Primate Model
1315 - 1330	Keivan Davami (Abstract 2) - A Review of Nanostructure in Bone Tissue Engineering	1315 - 1330	Cara Young (Abstract 204) - Poly(Vinyl Alcohol) Hydrogel Microsphreres for Cell Microencapsulation Produced by Submerged Electrospraying	1320 - 1325	Kama Bistrai Muhammad (Abstract 131) - In Vitro Comparative Study of MSC Seeding on White and Dark Poly(caprolactone) Trifumarate Scaffold		
				1325 - 1330	Taeyun Kang (Abstract 186) - Investigation of Oxygen Diffusion Through Porous Scaffold for Simulating Mass Transport Phenomenon		
1330 - 1430	***LUNCH & POSTERS***						
1400 - 1430	**TERMIS-AP Membership Business Meeting (*Hyde Park Room, Level 2*)**						
1430 - 1600	**CONCURRENT SESSION 9A: CRANIOFACIAL 3** ***(Grand Ballroom II, Level 2)***		**CONCURRENT SESSION 9B: BIOLOGICAL SCAFFOLDS 3** ***(Hyde Park Room, Level 2)***		**CONCURRENT SESSION 9C: STEM CELLS 4** ***(Grand Ballroom I, Level 2)***		**CONCURRENT SESSION 9D: EMBRYONIC STEM CELLS IN TISSUE ENGINEERING** ***(Phillip Room, Level 2)***
	Chair: Patrick Warnke & Yin Xiao		*Chair: Michael Raghunath & Ferry Mechels*		*Chair: Andrea O'Connor & James Goh*		*Chair: Guangdong Zhou & Gary Brooke*
1430 - 1500	KEYNOTE: Toshiyuki Yoshida (Abstract 162) - Periodontal Regeneration with Periodontal Ligament-Derived Cell Sheets: A Translational Research for the Clinical Trial	1430 - 1500	KEYNOTE: Tony Weiss (Abstract 275) - Elastic Biomaterials That Utilize Human Tropoelastin	1430 - 1445	Peng-Yuan Wang (Abstract 149) - Mesenchymal Stem Cell Differentiation on Laterally Graded Porous Silicon with Submicron Pore Sizes	1430 - 1445	Yao Chen (Abstract 67) - Multipoint Mesenchymal-Like Stem Cells Derived from Human Parthenogenetic Embryonic Stem Cells
1500 - 1515	Maria Woodruff (Abstract 312) - Resorbable Composite Scaffolds for Craniofacial Bone Tissue Engineering	1500 - 1515	Russell Lyons (Abstract 181) - Current Progrss Towards Understanding and Re-Engineering Resilin-Inspired Proteins and Biomaterials	1445 - 1500	Tzu-Hao Wang (Abstract 257) - Combined Analyses of MicroRNAs and mRNA Transcriptomes in Mesenchymal Stem Cells Derived from Umbilical Cords, Milk Teeth and Adult Teeth	1445 - 1500	Jennifer Turner (Abstract 121) - Cell Substrate Influences the Motility and Mode of Colony Formation of Human Embryonic Stem Cells
1515 - 1530	Ryo Takagi (Abstract 60) - GMP-Grade Cultures Human Oral Mucosal Epithelial Cell Grafts for Treatment of Esophageal Ulceration by Endoscopic Submucosal Dissection	1515 - 1530	Jerome Werkmeister (Abstract 41) - Design of Novel Collagen-Like Protein Scaffolds from Streptococcus Pyogenes	1500 - 1515	Geraldine Mitchell (Abstract 102) - Studies on Liver Progenitor Cell Spheroids In Vitro and in a Murine In Vivo Vascularized Chamber Model	1500 - 1515	Lynn Yap (Abstract 174) - Defining a Threshold Surface Density of Vitronectin for the Stable Expansion of Human Embryonic Stem Cells
1530 - 1545	Han Tsung Liao (Abstract 43) - Pig Mandibular Reconstruction by Adipose-Derived Stem Cells and Functionalized Laser-Sintered Porour PCL Scaffold with Platelet Rich Plasma: In-Vitro and In-Vivo Study	1530 - 1545	Nuno Neves (Abstract 307) - In Vivo Tissue Response and In Vitro Degradation of Chitosan-PBS Based Scaffolds	1515 - 1530	Shapour Shahgasempour (Abstract 341) - Bone Marrow-Derived Mononuclear Stem Cell Implantation in Patients with Buerger's Disease	1515 - 1530	Zheng Yang (Abstract 76) - Substrate Stiffness Affects the Chondrogenic Differentiation in Embryonic Stem Cells
1545 - 1600	Shinji Kamakura (Abstract 21) - Bone Regeneration by Octacalcium Phosphate (OCP) Collagen Composites in Canine Alveolar Cleft Model	1545 - 1600	Jelena Rnjak (Abstract 297) - Dermal Repair Electrospun Elastin-Based Dermal Substitutes for the Treatment of Severe Burns	1530 - 1545	Dehua Chang (Abstract 20) - Cell Sheet Therapy for Myocardial Infarction in a Pig Model	1530 - 1545	Kaiming Ye (Abstract 258) - Generation of Mature Beta Cells from Embryonic Stem Cells

		1545 - 1550	Naoko Maruyama (Abstract 119) - Differentiation of Side Population Cells Isolated from Human Amniotic Mesenchymal Cell into Vascular Endothelial Cells Under Hypoxic Condition	1545 - 1600	Yoh-Ichi Tagawa (Abstract 232) - High Hepatic Potentials in Liver Tissue Micro-Sized Flow-Culture System Using Murine or Human ES/iPS Cell
		1550 - 1555	Po-Yen Shen (Abstract 196) - Can Visible Light Enhance Pluripotent Gene Expression and Improve the Differentiation Ability of Amniotic Fluid Stem Cells?		
		1555 - 1600	Lei Yang (Abstract 85) - Culture and Identification of Bone Marrow Mesenchymal Stem Cells on Poly (N-Isopropylacrylamide) Copolymer Film		
1600 - 1700	***AFTERNOON TEA & PANEL DISCUSSION - CHAIR: PROFESSOR DIETMAR HUTMACHER (Grand Ballroom I, Level 2)***				

POSTERS

The below posters are on display from **Wednesday, 15 September 2010 at 0900 hours to Thursday, 16 September 2010 at 1300 hours.**

Poster No.	Presentation Title
1.	Abstract 14: Alternative Therapeutic Strategy for Aortic Aneurysm Using Doxycycline Controlled Releasing Biodegradable Fiber
2.	Abstract 16: Effect of Leucine Coating for Bone Formation in Porous Hydroxy Apatite Scaffold
3.	Abstract 20: Cell Sheet Therapy for Myocardial Infarction in a Pig Model
4.	Abstract 22: Effect of Transforming Growth Factor Beta3 on Rat Vocal Fold Fibroblasts
5.	Abstract 23: Malfunction of Mesenchymal Stem Cells in Hutchinson-Gilford Progeria Syndrome
6.	Abstract 28: Lithium Chloride (Licl) Improves Adipose-Derived Stem Cells (Asc) Proliferation and Affects its Adipogenic/Osteogenic Differentiation
7.	Abstract 29: The Biological Function of Adipose Derived Stem Cells Following Labeling with 18f-Fdg for In Vivo Tracking with Micropet Imaging
8.	Abstract 35: Isolation of Myogenic Progenitors From Human Amniotic Fluid
9.	Abstract 37: A Porous Poly(L-Lactic Acid) Membrane Designed for Culturing Human Periosteal Sheets as an Osteogenic Grafting Material
10.	Abstract 38: The Effects of α and β Poly (Vinylidene Fluoride) Phase Conformations on Cell Attachment and Proliferation
11.	Abstract 42: In Vitro Cytotoxicity and Genotoxicity of Silver-Nanoparticles Containing Hydrogel in Hela Cells
12.	Abstract 48: Porous Bioactive Glass Ceramics for Bone Tissue Regeneration
13.	Abstract 50: In Vitro and In Vivo Proliferation and Differentiation of Human Adipose-Derived Stem Cells in a Novel Injectable Temperature-Sensitive Hydrogel
14.	Abstract 51: Remarkable Osteogenic Differentiation of Human Menstrual Blood Derived Stem Cells in Presence of Human Platelet Releasate
15.	Abstract 52: Preparation of Polymer-Based Multimodal Imaging Agent to Visualize the Process of Bone Regeneration
16.	Abstract 53: Molecular Design of Ultrasound-Responsive Delivery System for Thrombolytic Therapy
17.	Abstract 56: Comb-Type Grafted Poly(N-Isopropylacrylamide) Gel Modified Surfaces for Rapid Detachment of Cell Sheet
18.	Abstract 65: Evaluation of Polymeric Dermal Wound Dressing Contained Allicin
19.	Abstract 74: The Combination of Stromal Cell-Derived Factor-1 and Bone Morphogenetic Protein-2 for Bone Tissue Engineering
20.	Abstract 75: Localisation of Human Bone Marrow-Derived Osteoblasts in Both Ectopic and Orthotopic Osteogenic Process
21.	Abstract 77: The Effects of the Electrospun Silk Fibroin Nerve Guidance Conduit on Nerve Regeneration of Rat's Defected Sciatic Nerve
22.	Abstract 79: Analyses of the Cell Viability and Metabolism of Multi-Layered Cell Sheets and a Possible Solution for Overcoming the Diffusion Limit
23.	Abstract 84: Differentiation into Neural Cells from Human Amniotic Mesenchymal Cell in Serum Free Medium
24.	Abstract 87: Boron Doped Nanocrystalline Diamond Films as Potential Substrate for Bone Tissue Regeneration
25.	Abstract 90: Development and Evaluation of a Novel Automatic Cell Culture System of Perfusion Flow Using Bone Marrow Stem Cells
26.	Abstract 93: Preparation, Fabrication and Biocompatibility of a Novel Injectable Temperature-Sensitive Chitosan-Based Hydrogel
27.	Abstract 94: Nanofibrous Plga Scaffolds Loaded with Nanodiamond as Substrates for Adhesion and Growth of Human Bone-Derived Cells
28.	Abstract 95: Vascular Endothelial and Smooth Muscle Cells in Static and Dynamic Cultures and Cocultures on Electrospun Nanofibrous Scaffolds
29.	Abstract 104: Development of Fast-Hardening Calcium Phosphate Bone Cement with Sintered Animal Bones
30.	Abstract 108: Influence of Deacetylation in Chitosan-Based, Laser-Activated Surgical Adhesive on Cell Growth and Attachment
31.	Abstract 112: Nanoscale Presentation of Cell Adhesion Motifs Via Block-Copolymer Self Assembly: Effect of Ligand Spacing on Mesenchymal Stem Cell Behaviour
32.	Abstract 113: Effect of Lithium Chloride on ß-Catenin Expression, Proliferation and Osteogeneic Differentiation of Human Menstrual Blood Derived Stem Cells

Poster No.	Presentation Title
33.	Abstract 115: Temporal Response of Zonal Human Osteoarthritic Chondrocytes to Compressive Stimulation
34.	Abstract 119: Differentiation of Side Population Cells Isolated from Human Amniotic Mesenchymal Cell into Vascular Endothelial Cells Under Hypoxic Condition
35.	Abstract 120: Thermo-Sensitive Hydrogel for Drug and Cell Delivery
36.	Abstract 121: Cell Substrate Influences the Motility and Mode of Colony Formation of Human Embryonic Stem Cells
37.	Abstract 123: Multilayer Cell Sheet Therapy for Myocardical Infarction Model
38.	Abstract 133: Porous Poly(L-Lactide) Scaffolds with Bmp-2 Releasing Surface-Immobilized Nano-Hydroxyapatite
39.	Abstract 140: Fabrication of Chitosan-Alginate-Hyaluronate-Heparan Scaffolds for Neural Stem/Progenitor Cell Culture
40.	Abstract 146: In Vitro Three Dimensional Chondrogenesis of Murine Bmscs Purified by Hypoxia Culturing and Macs with Lineage
41.	Abstract 147: Current Topics on the Japanese Regulation for the Marketing Approval of Human Cell/Tissue-Based Medical Products: Pmda's Perspective and Action on Their Evaluation and Development
42.	Abstract 158: Rheological and Stress Relaxation Properties of Phema and Phema-Tio2 Composite Hydrogels
43.	Abstract 161: Polyhydroxybutyrate and Ethyl Cellulose Composites as Biomaterials
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46.	Abstract 172: Spinner Flask Culture Induces Redifferentiation of Dedifferentiated Chondrocytes
47.	Abstract 175: Fabrication of Polycaprolactone/Elastin Composite Scaffolds for Tissue Engineering
48.	Abstract 177: Preparation Self-Emulsifying Drug Delivery System for Improvement Stability & Solubility of Docetaxel
49.	Abstract 179: Preparation and Characterization of Polymeric Micelles Consisting of Poly(Propylene Glycol) and Polyesters
50.	Abstract 180: Local Delivery of Antibiotics from a Laser Activated Surgical Adhesive
51.	Abstract 188: Proteomic Analysis of Rat Podocytes Under High Concentrations of Glucose
52.	Abstract 193: Influence of Bme on the Neurogenesis of Bmscs with Plga/Bme Film
53.	Abstract 194: Autologous and Functional Bladder Tissue Engineered in a Bioreactor Imitating the Physiological Pressure Environment
54.	Abstract 198: Effect of Silk Content on the Proliferation and Phenotype of Nucleus Pulposus Cells on Silk/Plga Scaffolds
55.	Abstract 199: Production of an Autologous Porcine Fibroblasts Sheet in Tissue Enginineering

POSTERS

The below posters are on display from **Thursday, 16 September 2010 at 1400 hours to Friday, 17 September 2010 at 1700 hours**.

Poster No.	Presentation Title
1.	Abstract 205: The Effect of Proportion of Acellular Dermal Matrix and Demineralized Bone Matrix on the Bone Regeneration in the Rat Skull Defects
2.	Abstract 210: Novel Vitrification of Human Induced Pluripotent Stem Cells
3.	Abstract 213: Variations in the Ratios of Co-Cultured Bone Marrow Stem Cells and Chondrocytes in Purified Alginate Microcapsules for Tissue Engineering
4.	Abstract 214: Effects of Sis/Plga Film on Proliferation and Phenotype of Retinal Pigment Epithelial Cells
5.	Abstract 215: Fabricating Microfibers Based on the Biomimetic Hydrogels with 3-Dimensional Bioprinting System
6.	Abstract 216: Regeneration of Internertebral Disc Using Nucleus Pulposus Cell on Purified Alginate Microcapslues
7.	Abstract 217: Compressive Strength of Poly(L-Lactide-Co-Glycolide) Scaffolds Seeded Nucleus Pulposus Cells Depending on Pore Size: In Vivo Experiment
8.	Abstract 223: Determination of Effective Action Distance In Vitro Between Umbilical Cord Blood-Derived Hematopoietic Stem/Progenitor Cells and Human Adipose Derived Stem Cells
9.	Abstract 225: Suitability of Storage Containers for Cell-Therapy Product
10.	Abstract 228: Application of Human Tooth Derived Materials for Tooth and Bone Regeneration
11.	Abstract 229: In Vitro Characterisation of Human Adipose Stem Cells in Fetal Bovine Serum and Allogeneic Human Serum
12.	Abstract 231: Comparing the Protein Expression Levels of Tumorigenic and Non-Tumorigenic Hepatocellularcarcinoma Cells
13.	Abstract 233: Exogenous Rhtrx Reduces Lipid Accumulation Under Inflammatory Conditions
14.	Abstract 234: Annexin A2 is a New Regulator of H2o2-Induced Premature Senescence in Human Mesenchymal Stem Cells
15.	Abstract 239: Ex Vivo Induction of Vascular Network Within the Engineered 3D Myocardial Tissues
16.	Abstract 245: Design and Fabrication of a Computer-Controlled Bioreactor and Biomechanical Testing Device for Bladder Tissue Engineering
17.	Abstract 248: Preparation of Cryopolymers for Bone Engineering
18.	Abstract 250: The Value of 3D Multislice CT in Fine Evaluation of Maxillary Bone Grafting
19.	Abstract 253: Laryngeal Regeneration Using Tissue Engineering Techniques in a Canine Model
20.	Abstract 255: A Comparative Study of Different Mechanical Conditioning Regimes for the Development of Tissue Engineered Anterior Cruciate Ligament
21.	Abstract 256: The Regeneration of Chronic Tympanic Membrane Perforation Using Egf Blended Chitosan Patch Scaffolds
22.	Abstract 265: Branched Oligomerization of Cell-Permeable Peptides Markedly Enhances Transduction Efficiency of Adenovirus into Mesenchymal Stem Cells
23.	Abstract 276: Hepatocyte Surface Modification for Liver Regenerative Medicine
24.	Abstract 278: The Creation of Bronchial Chondrocyte Sheets for Airway Reconstruction
25.	Abstract 279: Effects of Fluoride Application on Phosphoric Acid Etching in Primary Teeth Enamel
26.	Abstract 280: Effects of Neuroprotection by Acupuncture in a Global Ischemic Hyperglycemia Rats
27.	Abstract 282: The Effect of Formalin Fixation on Equilibrium Partitioning of an Ionic Contrast with Microcomputed Tomography (Epic-µct) Imaging of Osteochondral Samples
28.	Abstract 285: Cardiac Differentiation of Mouse ES Cells by Patterning Culture
29.	Abstract 289: Electrospraying, a Reproducible and Non-Toxic Method for Production of Microspheres Loaded with Growth Factors
30.	Abstract 290: An Experimental Comparison of Ovine Mandibular and Tibial Osteoblasts
31.	Abstract 291: Novel Strategy for Directing the Differentiation of Human Mesenchymal Stem Cells into the Myogenic Lineage on Dendrimer-Immobilized Surface with D-Glucose Display
32.	Abstract 292: Culture Surface Design for Regulating Cell Morphologies and Functions Based on Cell Anchoring Mechanisms
33.	Abstract 295: Transplantation of Cardiogenic Cells Differentiated from Mesenchymal Stem Cells Restore Electromechanical Function in the Infarcted Rat Heart
34.	Abstract 304: Construction of Vascularized Myocardial Tissue Based on a Cell Sheet Engineering

Poster No.	Presentation Title
35.	Abstract 305: Calcium Sulfate Coated Polymer-Ceramic Composite Plugs for Alveolar Crest Preservation After Tooth Extraction: Biocompatibility Assessment
36.	Abstract 306: Increasing the Porosity of Electrospun Scaffolds by Collecting Onto Electro-Conductive Structured Substrates
37.	Abstract 319: Cell Alignment and Elongation in Unidirectional Porous Collagen Scaffolds
38.	Abstract 320: Inhibition of Aortic Valvular Interstitial Cell Degeneration by Ecm Modification - Implications for Heart Valve Engineering
39.	Abstract 326: Controlled Temporal Release of a Model Protein from Plga / Plga-Peg-Plga Microparticles
40.	Abstract 329: Enhanced Osteogenesis Using Cell Permeable Transcriptional Coactivator Protein
41.	Abstract 335: Signaling for Neovascularization of an Arterio-Venous Loop-Containing Tissue Engineering Chamber: Role of Nadph Oxidase Type 4
42.	Abstract 336: The Effect of Human Adipose Tissue-Derived Mesenchymal Stem Cells on Avascular Necrosis of the Femoral Head in Nude Rats
43.	Abstract 337: The Role of Human Umbilical Cord-Derived Endothelial Progenitor Cells and Mesenchymal Stem Cells Infusion in the Functional Improvement of the Chronic Renal Failure in the Nod/Scid Mice
44.	Abstract 340: Cell Permeable Magnetic Nanoparticles for Efficient Labeling of Mesenchymal Stem Cells
45.	Abstract 348: Improving the Bioactivity and Mechanical Properties of Injectable Chitosan
46.	Abstract 350: A Novel Fabrication Process Of Wound Dressing Contained Aloe
47.	Abstract 351: Structure and Mechanical Properties of Pga/Gelatin Electrospun Nanofibers Scaffolds for Vascular Tissue Engineering
48.	Abstract 355: The Study of Photocrosslinked Peg Based Polyester Hydrogel as Scaffold for Bone Tissue Engineering
49.	Abstract 357: Tissue Engineering An Arterial Wall Model
50.	Abstract 360: Tissue-Engineered Bioabsorbable Scaffold Using Induced Pluripotent Stem Cells For Treatment of Peripheral Nerve Defect
51.	Abstract 363: Cryopreserved Gfp-Labeled Human Umbilical Cord Blood Derived Mesenchymal Stem Cells Could be Used as Seed Cells for Bone Tissue Engineering
52.	Abstract 372: Development of an Ultra-Thin Fibroin Membrane for Rpe Cell Transplantation
53.	Abstract 373: Chitosan-Collagen Composite Scaffolds for Tissue Engineered Skin
54.	Abstract 376: Fibroin-Based Materials Support Co-Cultivation of Limbal Epithelial and Stromal Cells
55.	Abstract 413: Simultaneous Expansion and Harvest of Hematopoietic Stem Cells and Mesenchymal Stem Cells Derived from Human Umbilical Cord Blood

PLENARY ABSTRACTS

(in presentation order)

PLENARY ADDRESS
Wednesday, 15 September 2010: 0900 – 0940 hours

IMPORTANCE OF VASCULARIZATION OF TISSUE ENGINEERED CONSTRUCT FROM A CLINICAL POINT OF VIEW

Wayne Morrison
O'Brien Institute, Fitzroy, VIC, Australia

No abstract provided.

PLENARY ADDRESS
Wednesday, 15 September 2010: 0940 – 1020 hours

IN VITRO AND IN VIVO IMAGING OF VASCULARIZATION OF A TISSUE ENGINEERED CONSTRUCT

Robert E Guldberg
Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanda, GA, USA

Advanced imaging tools play an increasingly important role in the rigorous quantitative evaluation of tissue regenerative strategies. Microcomputed tomography (micro-CT) imaging offers excellent resolution for quantifying 3D tissue morphology and composition but has typically been restricted to analysis of x-ray attenuating tissues such as bone. Even with this limitation, micro-CT analysis has been a valuable evaluation technique for tissue engineering studies to monitor mineralized matrix synthesis in vitro and quantify functional bone regeneration in vivo. Unfortunately, soft tissues such as blood vessels and cartilage alone are not sufficiently radiodense relative to surrounding tissues to allow micro-CT analysis. However, vascular ingrowth into scaffolds or regions of tissue injury may be imaged nondestructively following perfusion of a radiodense contrast agent. Subsequent morphological analysis can provide quantification of 3D vascular volume, vessel thickness and density, and vascular network connectivity. Recently developed in vivo micro-CT systems and circulating contrast agents offer the potential for longitudinal assessment of vascularization changes over time. Micro-CT imaging in combination with an appropriate contrast agent thus overcomes some of the shortcomings of other vascular imaging techniques because it provides high resolution, efficient, volumetric, and quantitative analyses. Challenges associated with micro-CT imaging of vascularization include resolution of very small vessels, contrast agent settling or leakage, and segmentation between vasculature and bone. Furthermore, while micro-CT analysis provides excellent 3D morphologic detail, it does not directly measure blood flow function. Despite these limitations, contrast-enhanced micro-CT imaging offers unique advantages to assess vascularization in preclinical models of tissue repair and evaluate therapeutic angiogenesis strategies.

PLENARY ADDRESS
Wednesday, 15 September 2010: 1020 – 1100 hours

ENGINEERING OF VASCULARIZATION IN A TISSUE ENGINEERED CONSTRUCT

Teruo Okano
Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo, Japan

Our research has been focused on constructing a novel form of co-culture consisting layered tissue structure. For our goal, we first developed unique tissue culture dishes which equipped their inner-bottom surface coated with the temperature-responsive polymer poly(*N*-isopropyl acrylamide) (PIPAAm). The "intelligent surface" of these dishes possessed the hydrophobicity similar to regular tissue culture polystyrene dishes at 37°C. However, the surface reversibly became hydrophilic at a lower temperature and spontaneously released the cultured cells as a single layer without the need for trypsin or EDTA, thus leaving the cell layer with extracellular matrix (ECM) on the bottom side intact. All the cultured confluent cells were harvested as a single contiguous cell sheet from the temperature-responsive culture dishes and readily applied to other biological and non-biological surfaces, thanks to the intact ECM. We here propose this novel system of cells and cell-layers arrangement called "cell sheet engineering."

We initiated human clinical studies of cell sheet engineering therapy using oral mucosal cell sheet for treatment of cornea epithelium deficient disease and recovery from endoscopic submucosal dissension surgery for esophageal epithelial cancer, and we also succeeded in treating cardiomyopathy using myoblast cell sheet. These two dimensional manipulations of the cultured cell sheets became successful applications and prompted us to the development of three dimensional applications, but without vascular networks, there remained a limitation for tissue thickness, approximately 80-100 μm, by diffusion parameters, due to necrosis within the engineered cell sheet structures. Adequate vascular networks were vital for the survival and development of constructed biological structures in order to deliver essential substances such as nutrients and oxygen. Overcoming the limitation of tissue thickness is the key to develop new procedures to treat or replace dysfunctional organs and tissues in human patients, the next stage of cell sheet engineering; therefore, our search was initiated for a method to induce angiogenesis within the engineered constructs.

First of all, one of our findings indicated that with the presence of sufficient amount of endothelial cells, the cadiomyocyte cell sheets were able to form the endothelial cell networks within the cultured cell layer in vitro, allowing immediate angiogenesis in the host environment after transplantation. Secondly, the tissue thickness limit could be solved by performing multistep transplantations at intervals which allowed sufficient neovascularization within the layered cell sheets. For example, the first three layers of the cardiomyocyte cell sheet were stacked and transplanted into the subcutaneous space, and then the second set of three-layer cardiomyocyte cell sheet was placed directly over the first set after allowing vascularization to occur in the first set. With this approach, we succeeded in constructing approximately 1 mm-thick cardiac tissue, far exceeding the original limit of 80-100 μm tissue thickness. Another finding demonstrated that we were able to introduce controlled formation of endothelial-cell networks, which transformed to tubular capillary-like structures, over prolonged in vitro culture by developing the micropatterned and microtextured culture surface with the thermo-sensitive PIPAAm. This procedure resulted in creation of five-layer tissue constructs micropatterned endothelial cells sandwiched between fibroblast cell sheets from the process called "cell sheet stamping."

Lastly, in order to overcome the challenges of developing crucial and functional prevascularied networks in an organ which required an even much denser capillary system, such as the liver, first the copolymers with different phase transition temperatures were coated on the surface of culture dishes to produce a patterned dual phase thermo-responsive cell culture surface using electron beam polymerization method and porous metal masks. In one of the experiments, site-selective adhesion and growth of hepatocytes and endothelial cells on the dual-phase surface of the dishes yielded a patterned co-culture layer based on the hydrophobic-hydrophilic surface chemistry regulated by simple temperature change. In addition, like the cell sheets from the single-phase surface, the recovered co-cultured cell sheets could be further modified and multilayered, and other types of cells could even be inserted between the cell sheets. Overall, the experimental results demonstrated that the methods applying cell sheet engineering could provide a new approach for the development of organ-like structures with the function of angiogenesis found in normal human organs. Therefore, cell sheet engineering with the intelligent surface is a highly promising tool for tissue engineering and regenerative medicine.

PLENARY ADDRESS
Thursday, 16 September 2010: 0900 – 0940 hours

POTENTIAL SOURCES AND APPLICATION MODES FOR MSC IN TRAUMA

Heinz Redl
Ludwig Boltzmann Institute For Exp. & Clin. Traumatology, Vienna, Austria

No abstract provided.

PLENARY ADDRESS
Thursday, 16 September 2010: 0940 – 1020 hours

REGENERATIVE MEDICINE: BEYOND CELLS, SCAFFOLDS AND GROWTH FACTORS

James CH Goh
Division of Bioengineering, Faculty of Engineering, Department of Orthopaedic Surgery, YLL School of Medicine, Tissue Engineering Programme, Life Sciences Institute, National University of Singapore, Singapore

Recent successes in regenerative medicine have led many to agree that what was then a concept, is now a promising reality; which might shape the future of clinical practices. With the increasing expectation on regenerative medicine to deliver prompt repair and solution to diseased organs, injured tissues and congenital anomalies, the challenge will then be to translate current research approaches to practical clinical methods.

Amongst the contributing factors, cell source remains limiting towards the realization of "off the shelf" therapeutic products. Although autologous transplantation strategies may be ideal in avoiding immune rejection risks, it is often associated with individualized application involving high cost and long lead time. Stem cell approach, on the other hand, has demonstrated success in allogeneic transplantations, making it a more direct route towards clinical translation. Adult stem cells, in particular, are lineage restrictive and non-tumorigenic, unlike embroyonic stem cells, making them safer for use as bioengineered products.

Of the various adult stem cell classes, our group has been working with the mesenchymal stem cells (MSCs) for tendon/ligament, cartilage and bone tissue engineering. It has been shown that MSCs and its differentiated derivatives can escape allogeneic immune response and acquire immunomodulatory activity to block such responses. It is thus envisioned that allogeneic MSCs can be preemptively expanded, cultured in a three-dimensional scaffold and conditioned dynamically *ex vivo* to form a functional tissue construct. Consequently, surgery to remove diseased or damaged tissue and replacement of a functional tissue engineered construct can be performed *in situ* without the need for prior autologous tissue extraction and culture. Minimally invasive techniques and bioimaging methods can be employed thereafter to assess further tissue regeneration, maturation and function. It is clearly a multidisciplinary challenge to breakthrough, translate and upscale valuable tissue engineering knowledge to practical clinical applications of regenerative medicine.

PLENARY ADDRESS
Thursday, 16 September 2010: 1020 – 1100 hours

POTENTIAL OF ADULT STEM CELLS IN THE REGENERATION OF THE CARDIOVASCULAR SYSTEM

Silviu Tescu
Mesoblast Limited, Melbourne, VIC, Australia

No abstract provided.

CONCURRENT SESSION ORAL ABSTRACTS

(in session then presentation order)

KEYNOTE ORAL PRESENTATION – ABSTRACT 369
Session 1A: Cartilage TE 1
Wednesday, 15 September 2010: 1130 – 1200 hours

TOWARDS THE ENGINEERING OF CARTILAGE GRAFTS WITH ZONAL ORGANIZATION

Jos Malda[1], Wouter Schuurman[1,2], P. René van Weeren[2] & Wouter J.A. Dhert[1]
[1]*Dept of Orthopaedics, University Medical Center Utrecht, The Netherlands*
[2]*Dept. Equine Sciences, Utrecht University, The Netherlands*

It is well established that articular cartilage has limited regenerative capacity and successful treatment of cartilage defects is still a challenge worldwide. None of the current treatment modalities has demonstrated formation of a histologically optimal repair tissue that mimics the characteristic complex zonal architecture of hyaline articular cartilage and hence can be supposed to produce the desired optimal clinical long-term results.

To further mimic the zonal organization, an increasing number of investigations is currently directed towards the development of zonal tissue-engineered cartilage implants. To achieve this, we are employing organ printing technology or "bioprinting", which combines the deposition of specific cell populations with the simultaneous deposition of biomaterials. This allows the development of zonal cartilaginous grafts and by using hydrogels a more physiological environment can be created. Biomechanical strength can be imparted and modified by mixing cell suspensions into *in situ* cross-linkable hydrogels (*e.g.*, gelatin, agarose, alginate or PEG) in a cartridge, after which the suspension is printed following a programmed 3D pattern. Adding biologically active components, such as proteins, peptides, DNA, hormones and natural or synthetic polymers to these water-based "bio-inks" will further enhance and direct the behavior of the cells. We characterized the use of bioprinting technologies to design and build heterogeneous cell-laden 3D structures and evaluated these *in vitro* and *in vivo*.

ORAL PRESENTATION – ABSTRACT 145
Session 1A: Cartilage TE 1
Wednesday, 15 September 2010: 1200 – 1215 hours

THE IMPACT OF PLGA SCAFFOLD ORIENTATION ON STRUCTURE AND MECHANICAL PROPERTY OF ENGINEERED CARTILAGE

Yingying Zhang[1,2] , Guangdong Zhou[1,2] ,Wei Liu[1,2] ,Wenjie Zhang[1,2] ,Yilin Cao[1,2]
1 Department of Plastic and Reconstructive Surgery, Shanghai 9th People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Key Laboratory of Tissue Engineering, Shanghai, P.R. China
2 National Tissue Engineering Center of China, Shanghai, P.R. China

Aim:
Cell orientation has an important influence on both the structure and function of engineered tissues. Some studies have shown that oriented scaffolds can regulate cell orientation, however, whether the oriented scaffolds can improve the structure and function of engineered cartilage remains unclear. This study explored the impact of PLGA scaffold orientation on structure and mechanical property of engineered cartilage.

Methods:
Microtubule-oriented PLGA scaffolds and unoriented scaffolds were fabricated into a cylinder shape with the same size. Porcine chondrocytes were seeded onto both kinds of scaffolds and cultured in vitro for 12 weeks followed by another 12 weeks of subcutaneous implantation in nude mice. Structures and functions related to cartilage formation were compared between two groups to evaluate the impact of PLGA scaffold orientation.

Results:
During in vitro culture, all the specimens in oriented group roughly maintained their original size and shape with relatively homogeneous cartilage formation. Contrarily, the specimens in unoriented group showed obvious shrinkage with inferior cartilage formation.

Quantitative analysis manifested that larger size, higher contents of GAG and total collagen, and stronger mechanical property were observed in oriented group than in unoriented group. After 12 weeks of implantation, both groups showed remarkable improved cartilage formation but the difference still remained. Scanning electron microscopy demonstrated that the oriented scaffolds prominently enhanced cell diffusion, orientation, and matrix production.

Conclusions:
These results indicate that microtubule oriented scaffolds can improve both the structure and function of engineered cartilage and thus provides a potential scaffolds for cartilage engineering.

ORAL PRESENTATION – ABSTRACT 33
Session 1A: Cartilage TE 1
Wednesday, 15 September 2010: 1215 – 1230 hours

CHONDROGENIC TRANSDIFFERENTIATION OF HUMAN DERMAL FIBROBLASTS STIMULATED WITH CARTILAGE DERIVED MORPHOGENETIC PROTEIN1

Shuo Yin[1,2], Lian Cen[2], Chen Wang[1,2], Guiqing Zhao[1,2], Jian Sun[2], Wei Liu[1,2], Yilin Cao[1,2] & Lei Cui[1,2]
[1] *Department of Plastic and Reconstructive Surgery, Shanghai 9th People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China*
[2] *National Tissue Engineering Center of China, Shanghai, China*

Aim:
This study aimed to investigate chondrogenic transdifferentiation potential of human dermal fibroblasts (DFs) by stimulation with cartilage derived morphogenetic protein 1 (CDMP1).

Method:
Use CDMP1 (100ng/ml) we induced human DFs at passage 5 in both monolayer and micromass culture. Chondrogenic specific markers were detected via immunochemistry, RT-PCR and Western-blot analysis in the collected specimens. The expression profile of adhesion molecules including integrin α5, β1 and N-cadherin of DFs in accompanying with chondrogenesis was further investigated.

Results:
After 7days of induction in monolayer culture, DFs acquired the polygonal chondrocyte-like shape with positive expression of chondrogenic specific markers. Such a phenotypic transition of DFs was lost at 14days. However, in micromass culture the chondrogenic transdifferentiation of DFs can be maintained even assayed at 14 days. No chondrogenesis was detected in DFs without CDMP1 treatment under both culture conditions. By neutralization assay with blocking antibodies, it was further revealed that integrin α5 expression was in a direct proportion to the degree of chondrogenic differentiation.

Conclusions:
Based on our findings, it can be ascertained that DFs are capable of transdifferentiating into a chondrogenic lineage by stimulation with CDMP1 in vitro. The integrin α5 mediating cell-cell and cell-matrix interactions is required for maintaining chondrogenic phenotype of DFs.

ORAL PRESENTATION – ABSTRACT 221
Session 1A: Cartilage TE 1
Wednesday, 15 September 2010: 1230 – 1245 hours

ENGINEERING PHYSEAL TISSUE USING HUMAN BONE MARROW DERIVED MESENCHYMAL STEM CELLS

Jacqueline Schmit, Kwee Hua See, James Hui & Eng Hin Lee
Department of Orthopedic Surgery, NUS Tissue Engineering Programme, National University of Singapore, Singapore

Aim:
In the growth plate (physis), chondrocytes differentiate and mature in defined linear zones consisting of resting chondrocytes, proliferating chondrocytes, and hypertrophic chondrocytes which progress to apoptosis, matrix mineralization and bone formation. The aim of this study was to determine if human bone marrow derived mesenchymal stem cells (hBMSC) can differentiate into physis-like cartilage using an agarose scaffold.

Method:
hBMSCs were embedded in agarose with only the surface of the construct in contact with the culture medium. The constructs were chondrodifferentiated in medium with TGFβ for 14 days then transferred to medium containing β-glycerophoshate and either TGFβ, BMP2 or both growth factors for up to 28 days. Differentiated tissues were analyzed by histology and real-time RT-PCR.

Results:
Collagen type II and glycosaminoglycan expression confirmed the presence of chondrocytes. The hypertrophy marker, collagen type X, was detected with highest levels seen in the BMP alone samples. Markers of early bone formation were highest in the samples treated with BMP alone and only these samples showed apoptosis and matrix mineralization. A physis-like linear zonal arrangement of the differentiated cells was suggested by the localization of collagen type X expression, matrix mineralization and apoptosis to the surface of the BMP constructs, with less mature chondrocytes showing higher proliferation localized to the base of the constructs.

Conclusion:
The described protocol for the chondrodifferentiation of hBMSCs provides a method to study the factors and conditions that influence the differentiation, maturation and zonal alignment of physeal chondrocytes.

ORAL PRESENTATION – ABSTRACT 298
Session 1A: Cartilage TE 1
Wednesday, 15 September 2010: 1245 – 1300 hours

EXPRESSION OF CELL SURFACE MARKERS IN CULTURED HUMAN ZONAL CHONDROCYTES

Karsten Schrobback[1], Kunnika Kuaha[1], June E Jeon[1], Ross W Crawford[1,2], Dietmar W Hutmacher[1] & Travis J Klein[1]
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[2] *Orthopaedics, The Prince Charles Hospital, Brisbane, QLD, Australia*

Aim:
Cell surface markers could be used to identify and isolate chondroprogenitors from *ex vivo* expanded chondrocytes, and lead to more consistent cartilage formation in regenerative medicine-based approaches. Moreover, the different zones of articular cartilage contain phenotypically distinct subpopulations of chondrocytes. However, little is known about cell surface marker expression in chondrocytes from the various cartilage layers. Therefore, we evaluated the expression of surface markers in *ex vivo* cultured zonal human chondrocytes.

Method:
Chondrocytes were isolated from superficial and middle/deep zones of macroscopically normal osteoarthritic cartilage (9 donors, 62±9y). Following monolayer expansion (two passages), immunophenotypes were determined by flow cytometry. Propagated cells were also embedded in 2% alginate and cultured under chondrogenic conditions over four weeks. DNA and GAG contents were quantified biochemically.

Results:
Flow cytometry revealed high levels of the putative stem cell markers CD44 and CD105 as well as the fibroblast cell marker CD90 in propagated chondrocytes. CD14 was not detectable. Although no zonal differences were found in expression levels of CD44 and CD90, superficial chondrocytes expressed higher levels of CD105 compared to middle/deep cells. Conversely, GAG contents in conditioned media were higher in middle/deep cells than in superficial chondrocytes after two weeks of redifferentiation.

Conclusions:
These data confirm that zonal chondrocytes retain zonal differences after *in vitro* expansion. Therefore, levels of putative stem cell markers may reflect inherent differences in phenotypes of zonal chondrocytes rather than the chondrogenic potential of *in vitro* expanded cells. This should be considered in the use of chondroprogenitor markers for cartilage repair.

ORAL PRESENTATION – ABSTRACT 342
Session 1B: Regenerative Medicine on Pancreas
Wednesday, 15 September 2010: 1130 – 1145 hours

STUDIES ON POLY(VINYL ALCOHOL)-MACRO-ENCAPSULATED ISLETS FOR DIABETES MELLITUS

Shoichiro Sumi[1], Kaichiang Yang[1], Zhi Qi[2], Yanna Shen[3], Goichi Yanai[1], Yasumasa Shirouzu[1], Akihito Hiura[1]
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[2]*Department of Histology and Embryology, Nankai University School of Medicine, Japan*
[3]*Department of Microbiology, Kyoto University Graduate School of Medicine, Japan*

Aim:
In pancreas or islet transplantation for diabetes mellitus (DM), donor shortage and adverse effects of immunosuppression are inevitable. Alternatively, micro-encapsulated porcine islets are intraperitoneally transplanted in clinical trials. However, they are not retrievable after transplantation. Poly(vinyl alcohol) (PVA) is a water soluble polymer that becomes gel by freezing and thawing (F/T). In combination of this nature and islet cryopreservation technique, we developed PVA-macro-encapsulated islets (PVA-MEIs) that are retrievable and exchangeable and verified their possibility.

Method:
Poly(ethylene terephthalate) meshes-reinforced PVA-MEIs were made by F/T of rat islets suspended in cryopreservation fluid containing 3% PVA in a sheet-shaped mold. In *in vitro* studies, glucose challenge was done and islet-protecting effect against flesh human plasma (FHP) was tested. In *in vivo* studies, rat PVA-MEIs were intraperitoneally transplanted to streptozotocine-induced DM mice or rats. PVA-MEIs cryopreserved either for 1, 7 or 30 days were compared *in vitro* and *in vivo*.

Results:
PVA-MEIs maintained glucose-responsive insulin secretion even after 14-day culture. They did but free islets did not survive 2-day culture in medium containing FHP. Transplantation of PVA-MEIs corrected hyperglycemia and relieved hyperglycemic renal dysfunction in DM mice. Both syngeneic (between Lewis rats) and allogeneic (Lewis islets to Wistar rat) transplantation of PVA-MEIs similarly decreased blood glucose levels for 24 weeks. These decreases were less significant than syngeneic free islet transplantation, whereas allogeneic free islets were readily rejected. Between 1- and 7-day cryopreservation groups, there was no significant difference in *in vitro* insulin secretion and glycemic control in DM mice. Similar but slightly weaker functions were shown in the 30-day cryopreservation group.

Conclusions:
PVA-MEIs enable allo- and xeno-transplantation without immunosuppression. They can be cyopreserved, which is a unique advantage that allows storage, shipping and quality control. PVA-MEIs seem promising and may replace islet transplantation.

ESTABLISHMENT OF DIABETIC ANIMAL MODEL FOR STUDY

Tzong-Fu Kuo
Department and Graduate Institute of Veterinary Medicine, School of Veterinary Medicine, National Taiwan University, China

Aim:
It is very difficult to have an idiopathic diabetic animal for diabetic research so that to develop a reliable diabetic animal for the study is an urgent and necessary topic. Over the past few years, several spontaneous diabetic animals (such as cat or dog) have been donated to the animal hospital by the owner. However, the number is not enough to reach the statistical standard. In addition, the follow up is sometimes difficult once animal somehow improvement and recover from the diabetic that stop owner send animal back to hospital/lab for further examinations. In this short presentation, I am going to introduce several animal models for diabetic mellitus study.

Method and Results:
Wild type C57B1/6 mice carry the Hp 1 allele with homology to human Hp 1 allele, DM was induced at 2-3 month of age by IP injection of 50 mg/Kg of streptozotocin (STZ) on 5 consecutive days. Blood glucose levels were monitored once a week with DM being defined as a consistent blood glucose level higher than 200 mg/dl. Male BALC/c mice, 6-8 weeks of age, was used as islet donors or recipients in the study of islet transplantation. Mice were rendered diabetic by IP injection of streptozotocin (275mg/Kg BW) 3 days before transplantation.

Rattus norvegicus Albinus, Wistar strain, weighing 200-250g, after 16 hours of fasting, was used as experimental animal by injecting streptozotocin to induce a DM animal. Female Wistar rats weighing 200g were induced to be diabetics with a single IV injection of streptozotocin (60mg/Kg). The diabetic state was confirmed by measurement of nonfasting glucose 3-4 weeks after STZ injection. Adult male albino rats were IP injection with 40 mg/Kg streptozotocin for 5 days to create DM condition. To decrease the death rates of rats by diabetic ketoacidosis, blood glucose levels were regulated by 0.5 U mixtard insulin (70% NPH and 30% regular insulin) for 6 weeks. In another experiment, male Fischer rats were rendered diabetic with a single IP injection of 1.3% streptozotocin in citric buffer (45 mg/Kg). These rats were kept without insulin therapy for 2 weeks. Diabetic state was confirmed by repeated measures of glucosuria and by plasma glucose concentrations in excess of 300mg/dL in one group. Mail Sprague-Dawley rats weighing 200-250 g and aged 3 months were given a single dose of intravenous 45 mg/Kg streptozotocin in sodium citrate buffer through the tail vein. Syrian golden hamsters were treated with streptozotocin 50mg/Kg IP daily × 3 consecutive days.

Healthy adult Beagle dogs weighing 8.4-12.8 Kg were treated with a IV bolus dose of streptozotocin 50 mg/Kg and alloxan 50 mg/Kg. Dogs showing FSG of 10 mmol/L or greater were measured three and ten days following diabetes induction. Another study, DM was induced in 3 dogs by IV injection of alloxan tetrahydrate (100mg/Kg). The dogs were fasted 12 h before and after alloxan injection. Alloxan solution was made by dissolving in normal saline at a concentration of 100mg/ml, this prepared solution was given intravenously at a dose of 80-100 mg/kg of alloxan for the dogs immediately after preparation. If the blood glucose levels were too low after injection of alloxan, a glucose solution (5-10%) was given IV. Dogs without acute renal failure or any other side effects were selected to continue the experiment.

Male castrated juvenile domestic cross-bred pigs, 12 to 14 weeks of age with body weight of 30.7 ± 1.9 Kg were induced DM with a total intraveneous dose of 150 mg/kg streptozotocin. Domesti male pigs (*Sus scrofa domestica*) or male Sinclair miniature weighing about 50 kg were used for inducing diabetes by injected with single dose of alloxan (150 mg/kg) dissolved in 0.9% saline through tail vein. Male Yucatan mini swine were used at age 8 months using a single intravenous injection of alloxan (150 mg/kg). Pigs that achieved and maintained blood glucose levels greater than 200 mg/dl were included in the diabetes group. Another study, Male Yucatan swine (aged 8-12 mo, 35-45 Kg) was induced by the IV administration of 100 mg/kg of the pancreatic β-cell toxin alloxan monohydrate. Pigs were monitored closely for 24 h after alloxan administration to prevent the life-threatening drop in blood glucose levels triggered by the massive insulin release associated with alloxan-inducedβ-cell death. Pigs were maintained with fasting blood glucose levels between 300 and 400 mg/dl for the duration of the study using daily insulin injections as required.

Conclusions:
In general, streptozotocin is used to induce diabetes in small animals such as mouse and rat, and alloxan is used in big animals such as pig and dog. The systematic toxicity of alloxan is lower for big animals when compared with streptozotocin, and the cost of alloxan is much cheaper. The reliable diabetic animals are crucial for diabetes researches.

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Session 1B: Regenerative Medicine on Pancreas
Wednesday, 15 September 2010: 1200 – 1215 hours

OPTIMIZATION OF ISLET TRANSPLANTATION BY PRECONDITIONING STRATEGY

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Background and Aim:
Islet and pancreas transplantation is an alternative treatment for patients with type I diabetes. Ischemia and inflammatory injury make lots of islets loss of function and apoptosis during pancreas harvest, islet isolation, or after islet transplantation through portal vein. Preconditioning management to protect heart, liver, lung, kidney, small intestines, skeletal muscle, and pancreas from ischemia-reperfusion injury have a lot of research evidences and several clinical trials ongoing. Recently, more evidences about remote preconditioning also could protect organs or tissues from ischemia or inflammatory injury. Remote preconditioning has more possibility of clinical application due to its non-invasive procedure. Till now, there're only few research published about using the preconditioning strategy on islet transplantation.

Method:
We systematically reviewed the literature searching on Pubmed with the keyword "Islet and Preconditioning", and "Pancreas and Preconditioning".

Results:
Different preconditioning methods will be listed. Results of which using preconditioning strategy on islet or pancreas transplantation will be compared. The evidence of improving engraftment and function of the transplanted islets by using the preconditioning strategy on donor and recipient will be provided. We will further discuss the possible molecular mechanism of the protection effect of the preconditioning strategy on islet or pancreas transplantation.

Conclusions:
Using the preconditioning strategy on donor and recipient during islet transplantation is a promising method to optimize the outcome of islet transplantation.

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Wednesday, 15 September 2010: 1215 – 1230 hours

UNDERSTANDING THE ROLE OF NON-CODING (NC)RNAS IN REGENERATIVE BIOLOGY OF ENDOCRINE PANCREAS

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The mammalian pancreas is known to show a remarkable degree of regenerative ability. We and others have demonstrated that the mammalian pancreas can regenerate in normal as well as diabetic conditions. Recently we also demonstrated that miR-195 family microRNAs, a class of non-coding (nc) RNAs, target the pro-endocrine transcription factor neurogenin 3 (ngn3) in regenerating mouse pancreas thereby promoting the regenerative pathway rather than a stem cell pathway for generation of new islets. Present studies carried on human islet-derived progenitor cells suggest that regulating the levels of islet-specific microRNAs in human islet-derived progenitor cells may help to promote better differentiation into endocrine pancreatic lineage. Transient inhibition of miR-195 family microRNAs using locked nucleic acids (LNAs) followed by forced expression of miR-30, miR-7 and miR-375 leads to efficient differentiation of islet-derived progenitor cells. These observations suggest that human islet-derived pancreatic progenitors could be used for generation of insulin-producing cells. Such insulin-producing islet-like cell aggregates may have tremendous potentials for cell replacement therapy in diabetes.

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Session 1B: Regenerative Medicine on Pancreas
Wednesday, 15 September 2010: 1230 – 1245 hours

CALCIUM PHOSPHATE CEMENT CHAMBER AS IMMUNO-ISOLATIVE DEVICE FOR BIOARTIFICAL PANCREAS: AN IN VIVO STUDY ON DIABETIC CANINE

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Aim:
Fibrous tissue outgrowth and hypoxia are the major restrictions for the application of bioartificial pancreas (BAP). Accordingly, the intramedullary cavity is proposed as an implant site and a BAP constructed of calcium phosphate cement (CPC) chamber was implanted.

Method:
Mouse insulinoma cells were encapsulated in agarose gel and then enclosed in a CPC chamber to fabricate a BAP. BAPs were implanted in the femoral intramedullary cavity of diabetic dogs. Pre- and postprandial blood glucose levels were monitored perioperatively. Blood samples were collected for the analysis of C-peptide level, and physiological conditions were observed at pre-determined intervals. BAPs were retrieved at 12 weeks post-operatively for histological examination.

Results:
Preprandial blood glucose level of diabetic dogs decreased from 420±25 to 223±47 mg/dl at one day post-operatively and was maintained in the range of 259±36 mg/dl for 12 weeks. As serum C-peptide level increased from 5.3±2.8 to 105.7±19.4 pmol/l, the rate of decrease of postprandial blood glucose was accelerated. Histological examination revealed that recipient bone tissues were binding to the surfaces of BAPs directly; there was no development of fibrous tissue. Immunohistochemical stain was positive for insulin in the enclosed insulinoma cells.

Conclusions:
This study demonstrated that BAPs implanted into the intramedullary cavity functioned well during the experimental period. Thus, the intramedullary cavity can serve as an implant site for BAPs.

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Wednesday, 15 September 2010: 1245 – 1300 hours

DECELLULARIZED PORCINE-DERIVED BLOOD VESSEL MATRIX GRAFT FOR URETHRAL REPLACEMENT IN A RABBIT MODEL

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Aim:
To evaluate a xenographic urethral replacement model utilizing porcine derived, decellularized blood vessel matrices in rabbits.

Method:
In 17 male rabbits, a 1 cm tubular segment of porcine, acellular blood vessel matrix replaced a 1 cm urethral defect without a postoperative catheter. The animals were sacrificed at varying intervals (1, 3, and 6 months) and assessed for graft patency and integration properties.

Results:
All but one animal survived. One animal died of unknown etiology one month after surgery. In all 17 rabbits, the urethra was patent without evidence of stricture formation as confirmed by gross inspection and passage of a 10Fr catheter at the time of euthanasia/tissue harvest. At one month, histological examination revealed epithelialization, host cell infiltration, angiogenesis and migration of smooth muscle. Neovascularization and smooth muscle bundles were more organized by 6 months. No significant fibrosis or stricture was observed in the anastomotic area.

Conclusions:
We show successful reconstruction and integration of an unseeded, xenograft urethral substitution that would ultimately be an off-the-shelf product. Prior research using seeded and unseeded allograft has shown mixed results. This highly translational and easily reproducible model would obviate the need for a harvest procedure and prior cell seeding technology, decrease morbidity, operative time and would require only a single-stage urethral reconstruction. To our knowledge this is the first report of a xenograft blood vessel matrix for urethral substitution.

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Session 1C: Obstetrics & Gynaecology
Wednesday, 15 September 2010: 1130 – 1200 hours

THE USE OF XENOGENIC IMPLANTS IN PELVIC FLOOR SURGERY

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Primary repair for symptomatic pelvic organ prolapse (POP) is associated with up to 30% of recurrence. To reduce this implant materials can be used to reinforce a native tissue repair or to simply replace a fascial structure. There is now evidence that such strategy is effective, at least for anterior repairs[1]. This may lead to graft related complications (GRC), in particular erosion complicating 10% of cases. In an effort to reduce this risk, xenografts rather than synthetic non-resorbable meshes have been proposed as they would lead to a different host respone. We conducted a number of experiments demonstrating that acellular collagen matrices (ACM) indeed provoke a lesser more anti-inflammatory response type. Also non-cross linked products were biomechanically less performing, whereas cross linking does not guarantee product. Clinically the use of SIS (non-cross linked) did not to improve the long term results of neither anterior[2] or posterior repair[3]. Meschia used porcine cross-linked dermal collagen and observed significantly less recurrences when (RR 2.72)[4]. Conversely, our results with sacropexy with ACM show that long term anatomical results are less good, neither does it prevent GRC, even not in patient at higher risk[5, 6]. These findings demonstrate that (1) similar materials may not provide similar results when used in other sites within the body; (2) experimental findings and clinical studies complement each other. Clinical studies remain a necessary second step, prior to generalized use of implants.

1 Xia et al, BJOG 2008
2 Chaliha 2006
3 Paraiso 2006
4 Meschia 2007
5 Deprest 2009

ORAL PRESENTATION – ABSTRACT 330
Session 1C: Obstetrics & Gynaecology
Wednesday, 15 September 2010: 1200 – 1215 hours

FUNCTIONAL IMAGING OF THE INTACT AND DAMAGED PELVIC FLOOR

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The diagnostic assessment of pelvic floor function and anatomy is attracting increasing attention in urogynaecology and female urology. This is largely due to the realisation that delivery-related pelvic floor trauma is common, even if almost universally overlooked, and that it is a major factor in the causation of pelvic organ prolapse. Modern imaging methods such as magnetic resonance and 3D ultrasound have enabled us to diagnose such abnormalities reliably and accurately, most commonly in the form of an avulsion of the puborectalis muscle off its insertion on the os pubis.

In addition, modern imaging allows assessment of the levator hiatus and its distensibility on Valsalva, providing an indirect measure of elasticity. The hiatus is important since it consitutes the largest potential hernial portal in the human body, and since it's properties are essential in childbirth and for continence.

In this talk I will try to summarize current knowledge of pelvic floor functional anatomy, with particular emphasis on diagnosis of delivery-related damage to the levator ani muscle. I will also discuss potential therapeutic approaches.

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Session 1C: Obstetrics & Gynaecology
Wednesday, 15 September 2010: 1215 – 1230 hours

SCAFFOLDS FOR THE REPAIR OF PELVIC ORGAN PROLAPSE

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Aim:
Pelvic organ prolapse (POP) is the herniation of the pelvic organs into the vagina. Mesh materials currently used to support prolapsed organs typically lead to problems of vaginal wall erosion and poor mesh integration, partly due to inferior mechanical properties. The aim of this work was to design a novel mesh of similar mechanical properties to vaginal tissue, which can accommodate adult stem cells for regeneration of the pelvic floor.

Method:
Non-biodegradable monofilament polyurethane was knitted and thermally bonded. Gelatin nanofibres were then electrospun onto the surface of the knitted mesh.

The breaking load and strain to break of the polyurethane mesh were assessed and compared to the measured biomechanical properties of prolapsed human vaginal tissue, in both the longitudinal and transverse directions. The mechanical properties of a clinically used POP mesh was also assessed.

Results:
Average (n=3) breaking loads of 26 N and 54 N, with strains of 1.85 and 3.34, were determined for the knitted mesh in the machine and cross directions, respectively. The clinical mesh was determined to have a breaking load of 114 N in the longitudinal and 104 N in the transverse direction, with strains of 0.67 and 0.81, respectively.

The highest breaking loads determined for the prolapsed vaginal tissue were 43 N (2.91 MPa) and 21 N (1.34 MPa), with strains of 1.12 and 0.94, in the longitudinal and transverse directions, respectively.

Conclusions:
The fabricated mesh had similar breaking loads, with higher extension to break, compared to prolapsed human vaginal tissue.

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Session 1C: Obstetrics & Gynaecology
Wednesday, 15 September 2010: 1230 – 1245 hours

POTENTIAL OF HUMAN ENDOMETRIAL MESENCHYMAL STEM CELLS FOR TISSUE ENGINEERING THE PELVIC FLOOR

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Mesenchymal stem/stromal cells have been identified in the highly regenerative human endometrium, the mucosal lining of the uterus which sheds and grows 4-10 mm each month as part of the menstrual cycle. Endometrial mesenchymal stem/stromal cells (eMSC) are a small subpopulation of stromal cells with similar properties and surface marker phenotype as bone marrow MSC. Properties of eMSC include colony forming unit (CFU) activity, self renewal in vitro, high proliferative potential, multipotency, with capacity to differentiate into adipogenic, myogenic, osteoblastic and chondrogenic lineages, reconstitution of endometrial stromal tissue in vivo, and perivascular location in both the shed and germinal layers of the endometrium. EMSC can be prospectively isolated and partially purified by their co-expression of CD146 and platelet-derived growth factor receptor-β (PDGFR-β) from the endometrial stromal fraction ($CD45^-EpCAM^-$), enriching 10 fold for CFU activity over unsorted stromal cells. Further, we have now identified a single marker (W5C5) that partially purifies eMSC allowing a simpler isolation procedure with greater cell viability using magnetic beads rather than cell sorting (FACS). Since endometrial biopsy tissue is easily obtainable in an office procedure with less morbidity than bone marrow or adipose tissue, we propose to use $CD146^+PDGFR\beta^+$ or $W5C5^+$ eMSC delivered in novel scaffold materials, designed to match the biomechanical properties of vaginal tissue, as a tissue engineering construct for autologous MSC-based therapy for augmenting pelvic organ prolapse repair surgery. Thus eMSC-seeded scaffolds may provide an innovative regenerative medicine therapy for pelvic organ prolapse, a major clinical unmet need.

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Session 1C: Obstetrics & Gynaecology
Wednesday, 15 September 2010: 1245 – 1300 hours

CLINICAL USE OF HUMAN AMNIOTIC EPITHELIAL CELLS IN MATERNAL-FETAL MEDICINE

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Over recent years it has become apparent that the human placenta may be a ready source of a variety of stem cells or cells with stem cell-like capability. In addition to umbilical cord blood stem cells, pluripotent cells may be derived from the cord itself, the placenta and the fetal membranes. Our group has been interested in human amnion epithelial cells – cells from the amnion layer of the amniotic membranes from normal term pregnancies. We have characterized these cells in detail and developed GMP-compliant procedures for their isolation, cryostorage, and application. We have shown that these human amnion epithelial cells (hAECs) are able to differentiate down endodermal, mesodermal and ectodermal lineages *in vitro*, and that the cells are karyotypically stable, maintain long telomere lengths in culture, and, unlike embryonic stem cells, do not form teratomas. These properties suggest that hAECs may be ideal for regenerative medicine applications. We have been studying the regenerative potential of hAECs using an *in utero* ovine model of ventilation-induced lung injury (to mimic the bronchopulmonary dysplasia seen in very preterm infants requiring respiratory support) and various mouse models of acute lung injury. In these studies, we have shown that hAECs mitigate/prevent lung injury and restore normal lung architecture and function. We continue to explore the mechanisms whereby hAECS exert their effects but these would appear to include *in vivo* differentiation into lung cells and modulation of the host responses to injury. We are preparing to undertake a clinical trial with these cells later this year.

KEYNOTE ORAL PRESENTATION – ABSTRACT 374
Session 1D: Cardiac Tissue Engineering
Wednesday, 15 September 2010: 1145 – 1215 hours

SUPPORTIVE ENVIRONMENTS FOR CARDIAC TISSUE ENGINEERING IN VIVO

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Aim:
The human heart has little capacity to regenerate muscle tissue, whether from cardiomyocytes or from local stem cell populations. We have developed in vivo models for cardiac tissue engineering as a surgical alternative for myocardial restoration. These models are in turn limited by difficulty in donor cardiomyocyte supply, so we developed methods for adult stem cell differentiation and applied these into our tissue engineering model. Our method supports stem cell survival for cardiac tissue engineering in vivo.

Method:
Cardiomyocyte differentiation from adult mesenchymal stem cells (human adipose, rat bone marrow and mouse cardiac-tissue derived) was evaluated in vitro comparing various strategies, including epigenetic modification, modified culture media and co-culture methods. The co-culture model was adapted for application to a vascularised tissue engineering chamber in nude rats. Histological and molecular methods were used to evaluate cell survival, cell fate and tissue formation.

Results:
Cardiomyogenic differentiation of stem cells in vitro was evident with increased expression of cardiac muscle mRNA and protein markers. The most effective method was direct contact co-culture of stem cells with neonatal rat donor cardiomyocytes. Cell tracking with fluorescent dye, together with species-specific markers for human or mouse (immunohistochemistry/in situ hybridisation) were able to show survival of implanted stem cells for 6 weeks. Cell fate included differentiation to adipose, vascular and cardiac cell types. The implanted cells supported assembly and contraction of cardiac tissue.

Conclusions:
Tissue engineering environments combined with vascularisation can support prolonged stem cell survival in vivo and allow differentiation of implanted cells. Cardiac tissue engineering is a feasible approach to use of stem cells for myocardial restoration.

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Session 1D: Cardiac Tissue Engineering
Wednesday, 15 September 2010: 1215 – 1230 hours

INJECTABLE CELL DELIVERY SYSTEMS FOR MYOCARDIAL TISSUE ENGINEERING: CELL SHEET FRAGMENTS, CELL BODIES AND CELL RODS

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Aim:
Cell transplantation is a promising approach for patients with myocardial infarction. However, following injection, retention of the transplanted cells in the injected area remains a central issue, which can be deleterious to cell-transplantation therapy. We hypothesized that the use of cell-sheet fragments, with the preservation of extracellular matrix (ECM), may significantly increase cell retention and thus improve cell therapy.

Method:
Mesenchymal-stem-cell (MSC) sheet fragments with ECM were fabricated. Experimental myocardial infarction was created in male syngeneic Lewis rats. Thirty minutes after myocardial infarction, an intramyocardial injection was conducted with a needle directly into the peri-infarct areas. There were four treatment groups ($n \geqq 10$): sham; PBS; dissociated MSCs and MSC sheet fragments. Echocardiography and pressure measurements were assessed postoperatively.

Results:
At retrieval, the hearts were fixed for histological evaluation. After injection, the MSC sheet fragments maintained intact, while the complete cell sheets were torn into pieces. The results obtained in the echocardiography and pressure measurements revealed a superior heart function in the MSC-sheet-fragment group compared with the dissociated-MSC group ($P < 0.05$). The MSC sheet fragments were able to conform and align their inherent ECM along with the interstices of the muscular tissues at the injection sites, while only a few cells were identified in the dissociated-MSC group at 12 weeks postoperatively. Additionally, transplantation of the MSC sheet fragments stimulated a significant increase in vascular density ($P < 0.05$) and enhanced the graft/host cell connection.

Conclusions:
The MSC sheet fragments may serve as a cell-delivery vehicle by providing a favorable ECM environment to retain the transplanted cells and improve the efficacy of therapeutic cell transplantation.

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Wednesday, 15 September 2010: 1230 – 1235 hours

MULTILAYER CELL SHEET THERAPY FOR A MYOCARDIAL INFARCTION MODEL

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Aim:
It is critical for a multilayer cell sheet to attach on infracted myocardial tissue within a limited time to maximize the cell sheet based therapy. Our previous study showed that it takes 30 min for bone marrow stem cells (BMSC) to attach firmly onto the normal heart tissue of pig. The goal of this study is to optimize suitable timing for the attachment of multilayer BMSC sheet on the infracted myocardial tissue.

Method:
BMSC were harvested from the bone marrow of pig. Once the cells reach confluence for 7 days after being cultured in mesenchymal stem cell (MSCs) basal medium, a monolayer BMSC detached itself spontaneously from temperature-responsive culture dish when the temperature was reduced from 37 °C to 20 °C. Myocardial infarction was created by the ligation of the left anterior descending branch of the left coronary artery in pig. A multilayered cell sheet was then transplanted onto the ischemia area on left ventricle.

Results:
A multilayer of BMSC was prepared by stacking three-monolayer of cells. Histological analyses and scanning electron microscopy demonstrated that a firm adhesion was formed between MSCs sheets and the ischemia heart tissue within 30 minutes, which is the same as normal heart tissue.

Conclusions:
The timing of cell sheets adhesion provides an important information for the clinical application of BMSC sheet-based therapy for heart infraction tissue repairing.

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Wednesday, 15 September 2010: 1235 – 1240 hours

SYNERGISTIC EFFECT OF ADIPOSE-DERIVED STEM CELL THERAPY AND BONE MARROW PROGENITOR RECRUITMENT IN ISCHEMIC HEART

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Aim:
Human multipotent adipose-derived stem cells (hMADSCs) have been isolated and shown its therapeutic effect on ischemic heart. However, the mechanistic insight has poorly been understood. We investigated to explore the biological role of transplanted hMADSCs in ischemic myocardium.

Method:
Nude rats were either transplanted with hMADSCs or PBS in myocardium immediately following myocardial infarction. Cardiac function, infarct size and vascularity in peri-infarct area were assessed by echocardiography and immunostaining 28 days after surgery. We also examined mRNA expressions of cytokine in hMADSs by real-time RT-PCR. Tie2/LacZ bone marrow (BM) transplanted nude mouse model was used for detection of BM-derived endothelial progenitor cells (EPCs) in ischemic myocardium.

Results:
The cardiac function was significantly greater with increased capillary density and reduced fibrosis area in hMADSC group than that in control group. However, remarkable differentiation of hMADSCs into cardiac cell lineages was not detected. Although hypoxia decreased the mRNA expressions, upregulations of VEGF and bFGF were detected in hMADSCs. Moreover, stem/progenitor chemokine SDF-1 mRNA expression was also high and stable even under hypoxic conditions. -gal+ EPCs were frequently observed in ischemic myocardium in hMADSC group compared to control group, and the promotional effect of hMADSCs on EPC recruitment was cancelled by SDF-1 gene silencing with siRNA technique.

Conclusions:
hMADSCs exhibited a therapeutic effect on ischemic heart via its paracrine effect rather than direct contribution to tissue regeneration. hMADSC SDF-1 may play a key role in synergistic effect of transplanted hMADSs and recruited EPCs in ischemic myocardium.

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Session 1D: Cardiac Tissue Engineering
Wednesday, 15 September 2010: 1240 – 1245 hours

NOVEL STRATEGY FOR DIRECTING THE DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS INTO THE MYOGENIC LINEAGE ON DENDRIMER-IMMOBILIZED SURFACE WITH D-GLUCOSE DISPLAY

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Aim:
The current study reports the cellular responses of human mesenchymal stem cells (hMSCs) cultured on the dendrimer-immobilized surface with D-glucose display. Based on examining morphological behaviors, moreover, fundamental mechanisms of cell and surface interaction are discussed in aspects of cytoskeletal formation and differentiation of MSCs.

Method:
Culture surfaces were designed by immobilizing dendrimer with D-glucose display, that is, 1st-generation (G1), 3rd-generation (G3) and 5th-generation (G5) dendrimer surfaces[1, 2]. For all experiments, hMSCs were seed on the surfaces and cultured for specified days in DMEM supplemented with 10% FBS and antibiotics. To evaluate cytoskeletal formation and differentiation, immunostaining and real time-PCR were performed using the standard protocol[2].

Results and Discussion:
To compare the dynamics of cell behaviors on different culture surfaces, the time-lapse observation of hMSCs on the PS, G1, G3 and G5 surfaces was conducted. The cells on the G5 surface showed more dynamic behaviors of temporal stretching and contracting associated with stimulated migration (such as “self-mechanotransduction”), as compared with the cells on the G1 and plain surfaces. On the G5 surface, moreover, a frequency of round-shaped cells increased, and spreading of the cells was appreciably suppressed. From the cytoskeletal staining of F-actin, it was found that the immature stress fibers were of significance in the cells on the G5 surface. In addition, the cells on the G5 surface expressed RhoA inactivation and Rac1 activation during the culture, indicating that the G5 surface permits the regulation of RhoA and Rac1 expression associated with altering in cellular morphology and migratory behaviors. It was also found that cardiac troponin T expression was, in particular, promoted within cell aggregates on the G5 surface, thus supporting the consideration that a balance of Rho family GTPases activation induces cardiomyogenesis in hMSCs.

Conclusions:
The current results suggest that the dendrimer surface can be a potential tool for the guided differentiation of hMSCs directing to cardiomyocyte-like cells in the absence of an aqueous cardiomyogenesis-inducing factor. These findings suggest that the dendrimer surface with D-glucose display offers a model of designing a substrate, based on the self-mechanotransduction in microenvironment, as a tool for guiding proper lineage specification in an *ex vivo* stem cell culture system.

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ORAL PRESENTATION – ABSTRACT 285
Session 1D: Cardiac Tissue Engineering
Wednesday, 15 September 2010: 1245 – 1250 hours

CARDIAC DIFFERENTIATION OF MOUSE ES CELLS BY PATTERNING CULTURE

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Aim:
Formation of ES cell aggregates promotes their differentiation into cardiomyocytes. ES cell aggregates are generally prepared via suspended spherical aggregates called embryoid bodies (EBs). Because the differentiation efficiency depends on the size of EBs, it should be controlled for the efficient differentiation. Although a hanging drop method is widely used to prepare size-controlled EBs, this method is unfavorable for the mass preparation because of its labor-intensive procedure. In this study, we developed a simple and scalable method to prepare size-controlled ES cell aggregates directly onto a substrate, by using cell-patterning technology.

Method:
Cell-patterning substrates were fabricated by a photolithography-based method. On the surface of the substrates, cell-adhesive circular domains (100-400 □m in diameter) were arrayed in the rest of non-cell-adhesive surface. Undifferentiated mouse ES cells were seeded onto the substrate and cultured for differentiation in □-MEM supplemented with FBS. The efficiency of cardiac differentiation was analyzed by flow cytometry.

Results:
ES cells seeded on the substrate proliferated within the circular domains and formed three-dimensional cell aggregates. Around day 7, some of the aggregates began to beat. Flow cytometry revealed that the optimal diameter of circular domains was 200 □m for efficient cardiac differentiation. We investigated culture conditions such as FBS concentrations for efficient cardiac differentiation. The percentage of cardiomyocytes reached about 7% at present.

Conclusions:
The cell-patterning method is useful for the simple mass preparation of cardiomyocytes from ES cells, and will strongly promotes the use of these cells in tissue engineering and transplantation experiments.

ORAL PRESENTATION – ABSTRACT 320
Session 1D: Cardiac Tissue Engineering
Wednesday, 15 September 2010: 1250 – 1255 hours

INHIBITION OF AORTIC VALVULAR INTERSTITIAL CELL DEGENERATION BY ECM MODIFICATION – IMPLICATIONS FOR HEART VALVE ENGINEERING

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Aim:
Recent data from our group showed a rapid *in vivo* re-endothelialisation of decellularized heart valves after modificatoion of the basal lamina by coating with bioactive substances. The enhancement of valvular interstitial cell (VIC) calcification by TGF-beta1 (TGF-ß1) and the endothelial inducing effect of vascular endothelial growth factor (VEGF) have been demonstrated. Based on these rationales we sought to investigate the modulating properties of extracellular matrix coating on VIC calcification in the presence of TGF-ß1 and VEGF.

Method:
Ovine aortic VIC cultured on collagen, fibronectin, laminin or uncoated surfaces were exposed to TGF-ß1, VEGF or left untreated for 7 days. Western blot analysis, live/dead assay, annexin V staining, alizarin red S staining and Real time PCR were performed after treatment.

Results:
Calcific nodule formation was dependent upon the ECM coating protein and growth factor exposition. VEGF significantly inhibited the formation of calcific nodules independent of ECM protein coating ($P<0.05$). TGF-ß1 exposition resulted in the formation of calcific nodules on collagen, laminin and uncoated control surfaces. In contrast, fibronectin coating resulted in significantly reduced nodule formation despite TGF-ß1 administration. Cell viability and apoptosis analysis showed a marked a increase of apoptotic and dead cells inside calcific nodules. Furthermore, these events correlated with the expression and not the gene amplification of the alpha smooth muscle actin gene as a marker of VIC activation and calcific nodule formation.

Conclusions:
An additive protective effect on VIC can be achieved by providing specific growth factors or a specific ECM environment. While certain soluble factors, e.g. VEGF are known for inhibiting calcification and apoptosis, the modification of culture substrate chemistry with TGF-ß1-binding ECM components like fibronectin offers the potential to alter availability and presentation of TGF-ß1 delivered to the cell surface receptors. ECM coating strategies, e.g. with fibronectin represent a promising and feasible tool for modification of heart valve scaffolds for tissue engineering purposes and preclinical trials.

ORAL PRESENTATION – ABSTRACT 249
Session 2A: Tendon/Ligament/Meniscus
Wednesday, 15 September 2010: 1400 – 1415 hours

ENGINEERING OF BONE, CARTILAGE AND TENDON USING CELL FREE SCAFFOLDS

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Aim:
Present work describes the feasibility of an cross-linkable injectable hyaluronan hydrogel for bone, cartilage and tendon repair. The hydrogel used is a two-component system based on aldehyde-modified hyaluronan and hydrazide-modified polyvinyl alcohol, which are rapidly cross-linked *in situ* upon mixing.

Method:
A system was designed to provide for a minimally invasive technique for delivery of drug and material into the desired area, reducing the risks involved in open surgery. We have investigated BMP-2-induced chondrogenic differentiation of chondrocytes and mesenchymal cells cultured in the hyaluronan hydrogel *in vitro* and the effects of the hydrogel, with and without growth factors, on healing of a full-thickness osteochondral defect in the rabbit knee, bone formation as augmentation of mandible, to regenerate cranial defects, long bone defects in the rat model, in the large animal and the translation to the clinic.

Results:
An injectable scaffold for minimal invasive delivery has been demonstrated suitable for bone formation by induction using BMP2 and by conduction in the absence of growth factors. These findings have been verified in large animals and translated to human trials. I*n vivo* experiments in the rabbit knee showed that treatment with hyaluronan gel, with and without BMP-2, led to cartilage regeneration of a higher quality with increased type II collagen content as compared to sham controls. Tendon and enthesis was formed upon mechanical stimulation.

Conclusions:
Bone, cartilage and tendon regeneration can be achieved using endogenously recruited cells.

ORAL PRESENTATION – ABSTRACT 386
Session 2A: Tendon/Ligament/Meniscus
Wednesday, 15 September 2010: 1415 – 1430 hours

HUMAN KNEE MENISCAL CELLS: CHARACTERIZATION OF THEIR PRIMARY CULTURE AND THEIR RESPONSE TO BIOMIMETIC SURFACES IN 2D AND 3D GROWTH ENVIRONMENTS

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With the aim of developing a tailored 'smart' scaffold for meniscus repair and partial replacement, we have firstly characterised the phenotype/gene expression of human knee meniscal cells and their changes during traditional monolayer expansion. Thereafter, we have investigated the effects of a number of biomimetic surfaces on the growth, morphology, matrix production and gene expression of the dedifferentiated meniscal cells in 2D and 3D environments. The expression of ECM, transcription factors and transforming growth factors (TGFs) in non-arthritic human menisci (n=6) and monolayer primary cultures were evaluated using quantitative real-time reverse transcriptase polymerase chain reaction (RT-qPCR). The dedifferentiated cells were then seeded on PLGA coated coverslips or PLGA porous scaffolds with self-assembled biomimetic surfaces. Cellular responses were examined using RT-qPCR, histology, DNA quantification and a dimethylmethylene blue (DMMB) assay. We observed that cells recovered from the inner zone of human menisci expressed a prominent level of mRNA for collagen I (COLI), and to a lesser extent collagen II (COLII) and aggrecan. Only low levels of Sox-9, Runx-2 and TGFs were detected in the initial harvest. All the genes, except COLI and Runx-2, were downregulated during monolayer expansion on TCP and the cells gradually lost their characteristic morphology. All of the biomimetic surfaces investigated supported cell growth. The cells formed colonies and exhibited the highest sulfated glycosaminoglycan (sGAG)/DNA ratio, COLII gene and TGF upregulation on surfaces that presented mixtures of proteins and a GAG known to exist in meniscal tissue, whether presented in 2D or 3D. Overall, our results indicate that primary human meniscal cells undergo dedifferentiation during traditional monolayer expansion and that this process could be reversed by culturing these (dedifferentiated) cells on our biomimetic surface.

ORAL PRESENTATION – ABSTRACT 267
Session 2A: Tendon/Ligament/Meniscus
Wednesday, 15 September 2010: 1430 – 1445 hours

STEM CELL BASED THERAPY RETARDS THE PROGRESSION OF OSTEOARTHRITIS AND PROMOTES REPAIR MENISCUS INJURY OF SHEEP MODEL KNEE JOINT

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Articular cartilage has a limited ability to heal after trauma or maintain its function with increasing age due to the avascular nature of the tissue. Defects can lead to the development of osteoarthritis (OA). OA may also issue after ligament rupture, and will progress more rapidly after meniscectomy. OA is characterized by damage of articular cartilage and changes in the subchondral bone. This study was designed to determine if autologous bone marrow mesenchymal stem cells (BMSCs) cultured in chondrogenic medium could repair surgically induced osteoarthritis. To achieve this goal, sheep BMSCs were cultured first in Dulbecco's Modified Eagle Medium (DMEM) high glucose containing 1% antibiotic-antimycotic and 10% fetal bovine serum (FBS). After 10 days BMSCs were trypsinized as they reached confluence. The viability and total number of cells were recorded, and plated in T-75 Flask at 2,000,000 cells/ flask with medium containing 5ng/ml Transforming growth factor beta 3 (TGFβ3) + 50 ng / ml insulin like growth factor (IGF-I), 1 ml insulin transferrin selenium (ITS), 5mg/ml Ascorbic Acid-2 phosphate, 40 µg/ml L-proline and 10^{-7} M Dexamethasone for 3 weeks. OA was induced surgically in unilateral sheep model by meniscectomy and complete resection of anterior crutiate ligament (ACL). Three weeks post OA induction sheep subjected to exercise for three weeks. After 6 weeks a single dose of autologous BMSCs suspended in 5 ml DMEM at density of 2 million cells/ml was injected intra-articularly into the osteoarthritic knee joint. After 6 weeks, the distal head of the femur and the proximal tibial plateau were removed, fixed 24 hours in 10 % formaldehyde, photographed, and morphologically graded by an assessor based on international cartilage repair society (ICRS) grading system, decalcified in 0.5 EDTA containing 8% Hydrochloric Acid for 3 weeks and then specimens sectioned into 5 µm and stained with H & E and Safranin O. The result demonstrated that Gross and histological observation of osteoarthritic knee joint treated with autologous BMSCs cultured in chondrogenic medium showed clear evidence of articular cartilage regeneration when compared with other groups.

ORAL PRESENTATION – ABSTRACT 138
Session 2A: Tendon/Ligament/Meniscus
Wednesday, 15 September 2010: 1445 – 1500 hours

ELONGATED MORPHOLOGY AND UNIAXIAL MECHANICAL LOADING REGULATE TENOCYTE PHENOTYPE

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Aim:
To investigate the role of cell shape and mechanical loading in regulating tenocyte phenotype and function.

Methods:
Primary porcine tenocytes of newborn pigs were cultured respectively on culture dish (Group A), smooth (Group B) and microgroove silicone membrane (Group C, forcing cells in an elongated morphology) and were mechanically loaded either parallel with or perpendicular to microgroove direction followed by RT-PCR and quantitative PCR.

Results:
With passage, the gene expression of tenomodulin (marker molecule for mature differentiated tenocytes) was lost and collagen I (functional molecule) expression was significantly reduced ($p<0.05$) in spread cells of Groups A and B, but was well maintained in elongated cells of Group C. Interestingly, the lost expression of tenomodulin in Group B cells was restored after being switched to microgroove culture condition. Significantly increased RhoA-GTP level ($p<0.05$) and reduced ROCK activity ($p<0.05$) were found in Group C cells, whereas artificially activating RhoA with LPA or inhibiting ROCK activity with Y-27632 could lead to significantly increased tenomodulin expression in spread cells ($p<0.05$), and no or minor increase in Group C cells. In addition, the tenomodulin and collagen I gene expression level was the highest in parallel loading and the lowest in perpendicular loading and middle in non-grooved membrane.

Conclusion:
These data confirm that elongated cell morphology is essential for tenocytes to maintain their phenotype and function, can redifferentiate the dedifferentiated tenocytes, and RhoA/ROCK pathway participates in the regulation. Mechanical loading along with the axis of elongated cells further enhances the phenotype and function.

ORAL PRESENTATION – ABSTRACT 129
Session 2A: Tendon/Ligament/Meniscus
Wednesday, 15 September 2010: 1500 – 1515 hours

THE EFFECT OF MECHANICAL LOADING ON TISSUE ENGINEERIED TENDON FORMATION: STRUCTUAL AND PROTEOMIC ANALYSES

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[2] *National Tissue Engineering Center of China, Shanghai, China*

Aim:
Previous studies have demonstrated that mechanical loading was beneficial for engineered tendon formation. Nevertheless, the underlying mechanism remains unexplored. This study employed proteomic analysis tool to investigate how the mechanical loading changes the protein expression profile of engineered tendons comparing with non-loaded tendons.

Method:
Human tenocytes and fibroblasts were isolated, expanded and respectively seeded on polyglycolic acid fibers to form cell-scaffold constructs, which were in vitro cultured for total 14 weeks with either static or dynamic loading followed by histology, ECM and proteomic analysis (2D-LC-MS/MS approach).

Results:
The engineered tendons with dynamic mechanical stimulation achieved better quality than those with static loading including histology, collagen fibril diameter and mechanical property. In proteomic assay, a total of 1333 proteins were identified, and over 200 proteins were up-regulated(R>5) or down-regulated (R<0.2). The majority of the up-regulated proteins and the down-regulated proteins are related to binding and catalytic activity. Importantly, tendon related matrix molecules such as collagen I(α1, 2), collagen VI(α1, 3) were dramatically up-regulated after dynamic mechanical loading both in fibroblast and tenocyte engineered tendons, but no significant change in collagens III, IV, XII and XIV. In addition, decorin, biglycan, tenascin and lumican were significantly up-regulated in fibroblast engineered tendons, indicating tenogenic transdifferentiation.

Conclusions:
These findings indicate that dynamic mechanical stimulation could enhance engineered tendon formation by dermal fibroblasts with more mature tissue structure and modified extracellular protein profile approaching to the profile of tendon tissue, which is likely to be caused by transdifferentiation of fibroblasts to tenocytes under dynamic loading.

ORAL PRESENTATION – ABSTRACT 255
Session 2A: Tendon/Ligament/Meniscus
Wednesday, 15 September 2010: 1515 – 1520 hours

A COMPARATIVE STUDY OF DIFFERENT MECHANICAL CONDITIONING REGIMES FOR THE DEVELOPMENT OF TISSUE ENGINEERED ANTERIOR CRUCIATE LIGAMENT

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Aim:
A comparative study is performed between three conditioning regimes of continuous levels (high and low intensity) and rehabilitative regime, with static culture as control.

Method:
Scaffolds of knitted silk fibroin with aligned electrospun silk fibroin (SF-AL) were seeded with rabbit bone marrow stromal cells (rBMSCs, P2, $1.5x10^6$/scaffold) and cultured statically for 3 days before loading into a customized bioreactor. Continuous low and high intensity, with different cyclic frequency (0.1Hz (4320cycles/day) and 0.5Hz (21600 cycles/day) respectively), were applied for the two groups ("Low" and "High") through the 28 days study. The group undergoing rehabilitative regime ("Rehab") would be exposed to "Low" (D3-D7) and "High" (D7-D28). All other parameters were constant.

Results:
Cell proliferation: "High" showed significantly lower proliferation rates than other groups from D3 to D7. "Rehab" showed a deviation from its proliferative phase from D14.

Collagen deposition: Significantly more collagen deposition in "Rehab" between D14 and D28 as compared to other groups, indicating the group undergoing a differentiative phase.

Mechanical properties: "Rehab" had significantly higher strength and stiffness than other groups by D21 with breaking load and elastic stiffness at D28 measured as 238 ± 19 N and 44.4 ± 2.8 N/mm respectively.

Histology: Sections in "Rehab" and "Low" showed aligned collagen deposition with "Rehab" having larger bands of collagen fibrils with crimp pattern at D28.

Conclusions:
Through this comparative study, a preliminary outlook of a suitable mechanical stimulation regime was presented. "Rehab" was shown to be a better stimulation approach of MSCs towards the regeneration of ligament/tendon tissue when compared to other continuous stimulation regimes.

ORAL PRESENTATION – ABSTRACT 264
Session 2A: Tendon/Ligament/Meniscus
Wednesday, 15 September 2010: 1520 – 1525 hours

MODULATION OF GAP JUNCTION EXPRESSION IN INJURED EQUINE SUPERFICIAL DIGITAL FLEXOR TENDON (SDFT)

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Aim:
Determine connexin (Cx) expression during repair of spontaneous equine SDFT injuries; establish a tissue explant model of SDFT injury and determine the effects of knockdown of specific connexins in this model on collagen synthesis using antisense oligodeoxynucleotide (asODN).

Method:
Connexin gene expression in injured and normal SDFT was evaluated using RT PCR. Connexin and collagen gene expression was measured in injured SDFT and age-matched controls using qRT-PCR. Connexin 43 and 32 and procollagen aminopropeptides I and III were labelled followed by qCLSM. Collagen content and solubility were determined by dye-binding assay. SDFT explants with [10μM] and [30μM] asODNCx43 in pluronic gel were compared with controls and pluronic gel-only; analyses included histopathology, qRT-PCR (Cx43, 32; collagens I, III), immunohistochemistry (Cx43, 32; procollagen aminopropeptides I, III) and biochemistry (soluble and insoluble collagens).

Results:
RT PCR identified Cx 43, 32 and 37 in injured SDFT; only Cx43 and 32 in normal SDFT. Connexin43 and 32 protein expression, quantified by plaque numbers and total area per tenocyte, were diffusely upregulated in injured SDFT, most significantly ($p=0.003$) at the central zone of injury. Down-regulation of Cx43 occurred at 8h ($p=0.05$) and 24h ($p=0.01$) post-application of [30μM] asODNCx43 in pluronic gel to the SDFT explants. Soluble collagen was increased ($p=0.03$) 5 days post-asODNCx43 application.

Conclusions:
Gap junction activity is upregulated in injured SDFT. Equine SDFT explants provided useful information concerning tenocyte metabolism and ECM synthesis. Downregulation of Cx43 occurred in the explants following application of [30μM] asODNCx43 in pluronic gel, which influenced subsequent collagen synthesis.

KEYNOTE ORAL PRESENTATION – ABSTRACT 321
Session 2B: Biomaterials_Based RM
Wednesday, 15 September 2010: 1400 – 1430 hours

APPLICATION FOR BIOMATERIALS-BASED REGENERATIVE MEDICINE FOR ORTHOPAEDIC SURGERY

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Bone grafting are often used for large amounts of bone defects after fracture, which is one of the challenging for orthopaedic surgeons. Ligament rupture and cartilage damage are also common injuries. However, the healing potential of the injured tissue is extremely poor. Recently, tissue regenerative medicine has been becoming very attractive in orthopaedic field. Responsive cells, regulatory factors and scaffold are the important factors for tissue regeneration. Several growth factors have been reported to play an important role for cartilage, bone, ligament and tendon healing. Gelatin hydrogel, which control the release of containing growth factors, is very attractive biomaterials for tissue regeneration. We applied FGF-2, Granulocyte colony-stimulating factor (G-CSF), which is known to stimulate the development of progenitor cells to neutrophils and Platelet-Rich Plasma (PRP) for bone, meniscus and ligament injuries in animal model. It is likely that the controlled release using gelatin hydrogel enables the FGF-2 and PRP growth factors to enhance their biological activities for cartilage tissue regeneration. G-CSF has recently been proved to contribute to angiogenesis in an appropriate environment and reported to regulate the proliferation, migration, or differentiation of the adult stem cells. A local application of G-CSF with drug delivery system (DDS) using gelatin hydro-gel significantly accelerated bone-tendon interface strength via enhanced angiogenesis and osteogenesis in animal study. Growth factors with DDS could be one of attractive therapy for orthopaedic field.

ORAL PRESENTATION – ABSTRACT 380
Session 2B: Biomaterials_Based RM
Wednesday, 15 September 2010: 1430 – 1445 hours

BIOMATERIAL TECHNOLOGY TO MANIPULATE STEM CELLS FOR REGENERATIVE MEDICINE AND THERAPY

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As the third advanced medical therapy following reconstruction surgery and organ transplantation, a new therapeutic trial based on the natural-healing potential of body itself to induce tissues regeneration and repairing, has been recently expected. To realize this regeneration therapy, there are two practical approaches; cell therapy and tissue engineering. The tissue engineering is a biomaterial technology or methodology to artificially create a local environment which manipulates the proliferation and differentiation of cells for cell-induced tissue regeneration. If a key growth factor or gene acts on cells at the right site, the right time period, and concentration, it is no doubt that the inherent potentials of cells themselves can be physiologically promoted, resulting in the natural induction of cell-based tissue regeneration and repairing. For example, one of the biomaterial technologies to enhance the in vivo activity of growth factor or gene is drug delivery system (DDS). Biodegradable hydrogels for growth factor and gene release have been explored to demonstrate the feasibility in the regeneration and repairing of various tissues. This DDS technology can be combined with stem cells to significantly enhance the efficacy of cells transplanted in regeneration therapy. In addition, it is also effective as a research tool of stem cell biology. The technology enabled plasmid DNA and small interference RNA (siRNA) to enhance or manipulate stem cells for activation of the biological functions. In this paper, our recent results of biomaterial technology to manipulate the biological functions of stem cells are presented.

ORAL PRESENTATION – ABSTRACT 183
Session 2B: Biomaterials_Based RM
Wednesday, 15 September 2010: 1445 – 1500 hours

ELECTROSPUN "GREEN" SOY PROTEIN-BASED SCAFFOLDS FOR SKIN TISSUE ENGINEERING AND WOUND HEALING

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Aims:
1)To develop a bioactive plant protein-based electrospun scaffold for wound healing applications.
2)To characterize its mechanical properties and biocompatibility *in vitro*.
3)To evaluate its effect on wound healing rate *in vivo*.

Methods:
Soy protein isolate (SPI) was dissolved in 1,1,1,3,3,3-Hexafluoro-2-propanol with <0.1% poly(ethylene oxide) (PEO), and electrospun into submicron-fibrous scaffolds. Tensile properties were tested and ability to support cell culture for 8 days was demonstrated using primary human dermal fibroblasts. Fibroblasts were also cultured on SPI or collagen I for 2h and 24h to compare focused gene-expression profiles by PCR. Ability of SPI/PEO scaffolds to promote wound healing was tested in full-thickness excisions in hairless rats as compared to Tegaderm® (control).

Results:
SPI/PEO scaffolds possess mechanical properties similar to human skin when hydrated and promote the morphotypic culture of human dermal fibroblasts. No substrate-dependent differences in adhesion- and ECM-related gene-expression profiles were seen between fibroblasts cultured on SPI and collagen I. On both substrates, some genes including MMP-10, MMP-1, integrin alpha-2, collagen VII, and laminin beta-3 were significantly upregulated at 24h. SPI/PEO scaffold-treated wounds exhibited slightly faster initial healing but no statistically significant difference in healing rate compared to control.

Conclusions:
1)Electrospun SPI/PEO scaffolds support adhesion, spreading and proliferation of primary human dermal fibroblasts.
2)SPI/PEO scaffolds possess mechanical properties comparable to human skin and induce "normal" cell-biomaterials interactions *in vitro*.
3)SPI/PEO scaffolds promote slightly faster initial wound healing response but otherwise similar healing rates as the standard of care. An impaired healing animal model is needed to clearly identify differences in healing rates.

ORAL PRESENTATION – ABSTRACT 269
Session 2B: Biomaterials_Based RM
Wednesday, 15 September 2010: 1500 – 1515 hours

CELL SHEET ENGINEERING TOWARD BIOENGINEERING OF FUNCTIONAL NEO-ISLETS

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Aim:
The present study discusses a novel tissue engineering approach for DM by fabricating a monolayered tissue sheet composed of dissociated single pancreatic islet cells for the creation of functional and transplantable neo-islet constructs.

Experimental Methods:
Temperature-responsive culture dishes specific to islet cell culturing were prepared by covalent immobilization of the temperature-responsive polymer poly(*N*-isopropylacrylamide) (PIPAAm) to the plastic dishes followed by coating with laminin-5. Dissociated pancreatic islet cells were obtained from Lewis rats and were then plated onto the laminin-5-PIPAAm dishes. After the cells reached confluency, cultured islet cells were harvested as a uniformly connected tissue sheet by lowering the culture temperature. The functionality of the harvested islet cell sheet was examined by histological examination, cell culture conditions, and functional activity following transplantation into diabetic individuals.

Results:
Histological examination showed that the harvested cell sheet had a monolayered 2-D structure. Immunohistological staining revealed that the islet cell sheet predominantly comprised of insulin- (76%) and glucagon- (22%) staining positive cells, respectively. Upon re-plating of the tissue sheet onto new culture dishes, we detected a positive response of the tissue sheet to a glucose-challenge test. Therapeutic effectiveness of the islet cell sheets was confirmed in the transplantation study to the Streptozotocin-induced diabetic immunodeficient mice.

Conclusions:
The study in this report describes a new proof-of-concept approach to generate monolayered functional neo-islets *in vitro*. In all, this repot serves as the introduction for the creation of islet cell-based therapy for DM to provide patients an alternative method for *de novo* production of insulin.

ORAL PRESENTATION – ABSTRACT 106
Session 2B: Biomaterials_Based RM
Wednesday, 15 September 2010: 1515 – 1530 hours

LASER-ACTIVATED CHITOSAN-BASED BIOADHESIVE FOR OCULAR TISSUE REGENERATION IN WOUND HEALING APPLICATIONS

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Penetrating corneal wound repair remains reliant upon sutures although healing frequently leads to the formation of opaque scar tissue and decreases in visual acuity. We have developed a laser-activated chitosan-based biomaterial that effectively seals corneal wounds, resisting greater leakage pressures than commercial fibrin glue or sutures while reducing operational times. Furthermore, this easily applied bioadhesive film is enzymatically biodegradable by lysozyme, a natural ingredient of tears. Its potential in corneal wound healing and ocular tissue regeneration is under investigation.

Cultivation of human stromal fibroblasts on the chitosan-based bioadhesive appeared to promote cell growth and attachment when compared to chitosan films. Electron microscopy showed normal cell morphology and proliferation across the surface of the lasered biomaterial. Similarly, cell cycle analysis revealed no significant deviations from asynchronous growth in the DNA content of stromal fibroblasts in the presence of biomaterial. Comparative expression proteome profiling further confirmed the presence of adhesion proteins such as Fibronectin and many other actin binding proteins responsible for cellular adhesion such as Caldesmon, Filamin-A, Filamin-C, Galectin-1, Plectin-1, Profilin-1, Calreticulin and Calumenin. The results suggest that the biomaterial has significant potential in the field of ophthalmic surgery and can support ocular tissue regeneration while overcoming the disadvantages associated with sutures.

KEYNOTE ORAL PRESENTATION – ABSTRACT 230
Session 2C: Bone 1
Wednesday, 15 September 2010: 1400 – 1430 hours

LOWERING THE EFFICACIOUS DOSE OF BMP-2 USING HEPARAN GLYCOSAMINOGLYCANS

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Aim:
Autologous bone graft remains the most reliable method for treatment for orthopaedic trauma, however donor-site morbidity and insuffient graft material limit its use. Graft substitutes containing BMP-2 are efficacious, however supraphysiological doses are required due to its inherent instability. As such, BMP-2 treatment is expensive and associated with unwanted osteopathologies. The ability to lower the efficacious dose of BMP-2 represents an important therapeutic aim. Heparin has been shown to bind and enhance growth factor activity. Due to >90% sulfation, heparin readily interacts with various proteins to both agonize and antagonize them. Heparan sulfate (HS), a structural analog of heparin, may provide greater selectivity in protein interactions due to its lower, yet greater specificity of sulfation that can be exploited to improve the utility of BMP-2.

Method:
In this study we compared heparin and HS on their ability to enhance BMP-2 activity. Osteoprogenitor cells were treated with varying doses of BMP-2 in the presence or absence of heparin/HS and the mechanism of action determined. Effects were assessed by FACS, histochemistry, Western blot, ELISA and qPCR assays.

Results:
HS dose-dependently enhanced BMP-2-induced osteogenic differentiation in a manner comparable to heparin. Importantly, BMP-2 stability and activity was greatly enhanced in the presence of heparin/HS through a mechanism that involved the inhibition of noggin's antagonism of BMP-2 activity resulting in sustained ALP activity in the presence of increasing concentrations of noggin. Co-immunoprecipitation revealed that heparin/HS reduces noggin/BMP-2 interactions. Heparin/HS increased the bioavailability of BMP-2 and sustained pSMAD 1/5/8 signalling over a 72h period resulting in accelerated osteogenic differentiation.

Conclusions:
These results suggest that heparin/HS interacts with BMP-2 and enhances its activity. This provides a means to reduce the efficacious dose of BMP-2 that is currently being explored in pre-clinical animal models in our laboratory.

KEYNOTE ORAL PRESENTATION – ABSTRACT 406
Session 2C: Bone 1
Wednesday, 15 September 2010: 1430 – 1500 hours

NEW BIOMATERIALS STRATEGIES BONE ENGINEERING

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This talk will provide an overview of our recent developments in bio-inspired materials and tissue regeneration of bone. Engineering of large-volumes of bone in vivo will be presented using relatively simple yet effective biomaterials approaches based on hydrogels or strontium containing bioceramics. Strontium ranelate has found great success as an oral anti-osteoporosis drug with effects on both osteoblasts and osteoclasts. We have developed strontium containing bioceramics that in an ovine sheep model result in significantly improved quality of bone regeneration compared to a commercially available bioceramic. Additionally a thorough materials analysis of tissue engineered bone will be presented. Indeed many different cells are used in bone regeneration applications but it is not always clear if they produce a material that mimics the structural and compositional complexity of native bone. By applying multivariate analysis techniques to micro-Raman spectra of mineralized nodules formed in vitro, we have revealed cell-source-dependent differences in interactions between multiple bone-like mineral environments. These recent findings will be discussed here. Understanding the biological mechanisms of bone formation in vitro that contribute to cell-source-specific materials differences may facilitate the development of clinically successful engineered bone.

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ORAL PRESENTATION – ABSTRACT 189
Session 2C: Bone 1
Wednesday, 15 September 2010: 1500 – 1515 hours

DEVELOPMENT OF HYDROXYAPATITE SCAFFOLDS FOR BONE TISSUE REGENERATION

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Aim:
To regenerate tissue or organs, many groups have researched scaffolds, which constitute one of the main components of tissue engineering. A scaffold must meet several criteria to enable tissue regeneration. The scaffold should have a suitable pore size, interconnected pores, sufficient mechanical strength, and biocompatibility. Using hydroxyapatite (HA) ceramic biomaterial, which is the main component of real bone tissue, we fabricated HA scaffolds with the desired pore size, interconnected pores, and sufficient mechanical strength.

Method:
A projection-based microstereolithography (pMSTL) system was used to fabricate the HA scaffolds. The pMSTL system uses a digital micromirror device (DMD) that generates a two-dimensional (2D) pattern. The desired three-dimensional (3D) structure is fabricated by stacking 2D patterns. The material used in the pMSTL system should be photocurable. We used an HA slurry (10% v/v) consisting of HA powder and photopolymer resin to fabricate the HA scaffolds. After fabricating the 3D HA/polymer composite structure, it was sintered to remove the photopolymer in the 3D structure and attach the HA particles to each other. In the sintering process, various conditions (maximum temperature, dwelling time) were used to increase the mechanical strength of the HA scaffolds.

Results and Conclusions:
In this study, HA scaffolds were fabricated successfully using a pMSTL system and HA slurry. Scanning electron microscopy showed that the fabricated scaffolds had the desired pore size and fully interconnected pores. In addition, scaffolds were made using various sintering conditions and their mechanical strengths were determined by the bonding force of the HA particles.

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Session 2C: Bone 1
Wednesday, 15 September 2010: 1515 – 1520 hours

RH-OP-1 ASSOCIATED WITH MESENCHYMAL STEM CELLS PROMOTE BONE ALLOGRAFT INTEGRATION

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Aim:
The main goal of this study was to test whether Mesenchymal Stem Cells (MSC) and Bone Morphogenetic Protein 7 (BMP-7, also known as OP-1) added to a massive bone allograft can promote complete bone-allograft integration.

Method:
A 3 cm full size intercalary bone defect was created in the mid-diaphysis of the metatarsal bone of the sheep and it was replaced with an allograft alone (Control Group), or either allograft associated with MSC (MSC Group), OP-1 (OP-1 Group), or MSC and OP-1 (MSC+OP-1 Group). Plain X-Ray, mechanical screw extraction torque test, histology and histomorphometry were performed in order to evaluate: the bone-allograft integration, the mechanical strength of the allograft, and the amount of newly formed bone in the allograft together with the length of new vessels penetration inside the allograft.

Results:
Radiographic results showed a faster and complete integration of the allograft in the MSC+OP-1 Group. Histology demonstrated that the amount of new bone was significantly greater inside the graft together with a longer vessel penetration in the MSC+OP-1 Group in comparison to the others. Mechanical strength of the allograft was not compromised by the high rate of bone remodeling showed in groups treated with OP-1.

Conclusions:
These results demonstrated that the association of MSC and OP-1 improve bone allograft integration promoting an almost complete bone restoring.

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Session 2C: Bone 1
Wednesday, 15 September 2010: 1520 – 1525 hours

REMARKABLE OSTEOGENIC DIFFERENTIATION OF HUMAN MENSTRUAL BLOOD DERIVED STEM CELLS IN PRESENCE OF HUMAN PLATELET RELEASATE

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Aim:
Menstrual blood has been identified as an easily accessible, non-controversial and renewable stem cell source. So, the challenge remains to develop robust protocols to generate different lineages such as osteoblasts from menstrual blood derived stem cells (MenSCs). The aim of this study was to find out substitution effect of fetal bovine serum (FBS) with human platelet releasate (HPR) as a major growth factor source during osteogenic differentiation of MenSCs.

Methods:
Human MenSCs were isolated of menstrual blood by discontinuous density gradient centrifugation and plastic adherence. After characterization of isolated cells, osteogenic differentiation of MenSCs was performed in a medium including ß-glycerophosphate, ascorbate and dexamethasone. Parallel histochemical and molecular experiments were carried out using either culture media containing FBS or HPR- supplemented media.

Results:
Flow cytometric analysis illustrated that MenSCs were positive for stem cells markers such as CD9 (95%), CD29 (99%), CD44 (99%) and CD73 (98%) while negative for CD34(0.6%), CD45 (0.22%) and CD38(0.06%) and mildly positive for stro1 (2%) and CD133(7%). Unlike MenSCs differentiated in medium containing FBS, nodule-like structures were observed in induced cells in presence of HPR. Mineralization as judged by Alizarin red staining was strongly more positive in cells differentiated in presence of HPR than that fortified with FBS (60±10% in HPR group against 5±4% in FBS group). The supporting role of HPR was further confirmed by the mRNA expression of osteobalst specific markers including alkaline phosphatase and osteocalcin.

Conclusion:
HPR is an efficient and potent substitute for FBS during osteogenic differentiation of MenSCs.

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Session 2C: Bone 1
Wednesday, 15 September 2010: 1525 – 1530 hours

THE OSTEOCONDUCTIVITY OF BIOMATERIALS IS REGULATED BY BMP2 AUTOCRINE LOOP INVOLVING A2B1 INTEGRIN AND MAPK/ERK SIGNALING PATHWAYS

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Aim:
The aim of this study is to investigate the mechanism(s) by which β-tricalcium phosphate (β-TCP), a clinically used bone graft substitute, exerts its osteoconductivity on primary human osteoblasts (HOBs).

Methods:
First, after HOBs were seeded on β-TCP scaffolds and cultured for 1 and 7 days, the gene expression including osteogenesis-related genes, BMP2 and its receptors (BMPR-1a, BMPR-1b and BMPR-2) and phosphorylated Smad1/5 protein levels were compared to those at day 0. Second, osteogenesis-related gene expression and phosphorylated Smad1/5 protein levels were compared between the groups with or without adding BMP2 singaling inhibitor (Noggin). Third, BMP2 and its receptors gene expression and phosphorylated Smad1/5 protein levels were compared between the groups with or without the blocking of α2β1 integrin (by adding blocking antibody) or the blocking of mitogen-activated protein kinase (MAPK)/extracellular related kinase (ERK) signaling pathway (by adding specific inhibitor: PD98059).

Results:
HOBs cultured on β-TCP scaffold for 1 and 7 days induced the gene expression of BMP2 and its receptors and activated its downstream Smad1/5 signaling pathway, which were accompanied with induced osteoblastic differentiation. Blocking BMP2 activity by Noggin led to the abrogation of osteoblastic differentiation and partially-inhibited Smad1/5 signaling pathway. The blocking of α2β1 integrin, or the inhibition of MARK/ERK signaling pathway, attenuated the induction of gene expressions of BMP2 and its receptors, and the activation of Smad1 signaling pathway.

Conclusion:
β-TCP scaffold promotes osteoblastic differentiation by a BMP2 autocrine loop, a process involving α2β1 integrin and MAPK/ERK signaling pathway. The findings of this study might shed the light for fabricating or designing an ideal scaffold for bone tissue engineering.

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Session 2D: Enabling Technology for TE
Wednesday, 15 September 2010: 1400 – 1430 hours

BIOMATERIAL INDUCED STEM CELL RECRUITMENT FOR *IN SITU* TISSUE REGENERATION

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Classic tissue engineering approaches have been employed to overcome the present challenges by implementing biomaterial scaffolds that are seeded with desired cell types. These constructs are used to generate functional tissues that progressively mature when introduced *in vivo*. Although the principle of this technology has been demonstrated in various preclinical and clinical studies in different tissue systems, this approach typically requires a donor tissue biopsy, followed by isolation and expansion of cells from the biopsied tissue. If these processes were simplified by eliminating the need for tissue biopsy and *in vitro* cell manipulation, a more efficient means of developing biological substitutes for functional tissue restoration *in vivo* could be developed. This type of tissue restoration may be possible if the body's innate regenerative systems, which have all the biological resources necessary for tissue regeneration, could be tapped. It is widely accepted that almost every tissue in the body contains some type of stem or progenitor cells, including brain, liver, circulating blood, heart, skin, fat, and muscle. It would seem that these cells are part of underlying regenerative machinery that is responsible for daily maintenance activities, including repair of normal tissue wear and tear, as well as small, non-life-threatening types of injuries. However, when extensive tissue damage occurs and large tissue defects are present, the regenerative response is probably overwhelmed, and an immune-based reparative response takes over to maintain some level of function.

The presence of an underlying regenerative mechanism in the form of tissue-specific stem/progenitor cells suggests that there may be a potential opportunity to bias the host response toward repair and replacement of large tissue defects. We investigated whether host biologic resources and environmental conditions could be used for *in situ* tissue regeneration, which may eliminate the need for donor cell procurement and subsequent *in vitro* cell manipulation. To address this aim, we implanted a common biomaterial into mice and characterized the infiltrating cells to determine their regenerative potential. We showed that host cell infiltrates are not entirely comprised of inflammatory and fibroblast-like cells and the normal inflammatory process can be altered by incorporating anti-inflammatory agents that influence the formation of scar tissue. In addition, the infiltrating cells are capable of differentiating into multiple cell lineages, including osteogenic, myogenic, adipogenic, and endothelial lineages, if appropriate conditions are provided. These results suggest that it is possible to recruit a predominance of cells with multilineage potential into a biomaterial scaffold. Therefore, it may be possible to enrich the infiltrate with such cell types and control their fate, provided the proper substrate-mediated signaling can be imparted into the scaffold for *in situ* tissue regeneration.

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Session 2D: Enabling Technology for TE
Wednesday, 15 September 2010: 1430 – 1445 hours

OXYGEN GENERATING MATRIX FOR REGENERATIVE MEDICINE

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Oxygen plays an essential role for respiration and bioenergetic metabolism in animals. Under normal physiological conditions, oxygen is delivered to the body tissues via blood circulation that comes under the control of the respiratory system. The concentration level of oxygen on the tissues is tightly regulated to maintain a balance, which can be altered during many pathophysiological states. However, if the tissues face some damages, then the normal functionality of the biological system will fail, followed by deterioration of tissues and necrosis. In such cases, the recovery of the tissues through natural mean is often difficult especially if the area affected is large. Therefore, the issue of supplying sufficient oxygen for the growth of the cells and regeneration of tissue and organs still remains a great challenge. In this paper, a novel microencapsulation of hydrogen peroxide into PLGA via a modified solvent evaporation is presented including optimisation of ingredients ratios, methodologies, and experimental conditions. The addition of hydrogen peroxide and its amount in secondary stirring solution during the synthesis were found crucial for the microencapsulation process. The presence of hydrogen peroxide and release kinetics of oxygen through microspheres were monitored indirectly via measuring the concentration of dissolved oxygen in the incubation solution in vitro and the effect was investigated in vivo. The results shows the microspheres have low or no toxicity effect in vitro and in vivo at a controlled condition, and the developed smart biomaterials slowly producing oxygen have various applications as oxygen providing system for regenerative medicine.

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Session 2D: Enabling Technology for TE
Wednesday, 15 September 2010: 1445 – 1500 hours

AN AUTOMATED PERFUSION BIOREACTOR FOR ONLINE, REAL-TIME, NON-INVASIVE MONITORING OF pH AND pO_2 WITHIN MULTIPLE INDEPENDENT CHAMBERS

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Aim:
Based on a previously validated device for cartilage and cardiac tissue engineering, in this study, we present a fully automated perfusion bioreactor enabling online, real-time, non-invasive monitoring of critical culture parameters (pH and pO_2) within multiple, independent chambers.

Methods:
A programmable, automated system was developed to drive one pH and one pO_2 optical sensor (Fluorometrix) to serially interrogate up to 18 parallel culture chambers in an incubator-compatible bioreactor. Validation of pH sensing was performed using buffers. Primary human articular chondrocytes were dynamically seeded and cultured for 7 days on collagen (Ultrafoam) scaffolds (N=6, Φ8mmx3mm, $4*10^6$cells/chamber). Perfusion seeded and statically cultured constructs were used as controls (N=2).

Results:
The system allowed to accurately monitor (up to every 5s) induced pH variations within the range 7.0-7.6, showing excellent correlation with a standard pHmeter (±0.05). Progressive pH drop during cell dynamic culture was observed (pH=-0.22±0.076), and externally induced environmental changes promptly detected, e.g. initial pH balancing, partial or complete medium change, incubator door opening. pO_2 monitoring allowed the detection of an induced bacterial contamination (10^7E.Coli/chamber), showing a 14% pO_2 drop in 190min. In line with Alamar Blue and MTT data, the set-up confirmed the beneficial effects of interstitial perfusion, applied by our bioreactor, over conventional static protocols, when engineering cartilage tissue.

Conclusions:
The study presents a novel perfusion bioreactor with automated, online monitoring of pH and pO_2 within independent chambers, opening new perspectives for more rational experimental designs and representing a step forward towards traceable, and thus clinically sustainable, tissue engineering applications.

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Session 2D: Enabling Technology for TE
Wednesday, 15 September 2010: 1500 – 1515 hours

DIAGNOSTIC MICROBIOREACTOR ARRAYS FOR MULTIPLEXED MICROENVIRONMENTAL SCREENING OF PLURIPOTENT STEM CELL EXPANSION, MAINTENANCE, AND DIFFERENTIATION

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Self-renewal and differentiation of stem cells are processes which depend on a delicate balance of key signalling pathways. Accurate control of these fates is paramount for cellular therapies based on stem cells, however, this is limited by several factors, including: use of undefined media and substrata, autocrine/paracrine action of cultured cells, temporal variation in microenvironmental composition, and downstream cross-talk between signalling pathways. To address these issues while progressing towards highly-controlled, multiplexed screening platforms for stem cells, we have fabricated firstly, microbioreactor arrays generating linear and logarithmic flowrate conditions for optimising physical culture parameters, and secondly, a scalable, full-factorial microbioreactor array generating every combination of 3 concentrations each of 3 soluble factors (27 discrete culture conditions in total). This platform has been combined with *in situ* immunofluorescence to create a screening platform under completely defined surface and soluble conditions, with accurate spatiotemporal control of the cellular microenvironment. MEL-2 human embryonic stem cells (hESC) were screened for maintenance of pluripotency markers TG30 and Oct-4 against b-FGF and TGF-β1 in a chemically-defined medium background, with retinoic acid included as an internal pro-differentiation control. Factorial analysis of the results revealed the main and interaction effects of tested factors in maintaining hESC pluripotency. This same platform is being applied to optimising directed neuronal differentiation of pluripotent stem cell-derived neural progenitors. We envision that this microbioreactor array will constitute a new discovery platform to probe cells in fundamentally distinct, highly-controlled conditions, which can be applied to elucidate complex processes such as self-renewal, differentiation, and development.

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Session 2D: Enabling Technology for TE
Wednesday, 15 September 2010: 1515 – 1530 hours

EFFICACY AND SAFETY OF ENGINEERED SKIN SUBSTITUTE ON SKIN WOUND HEALING

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Aim:
The aim of this study is to construct a bio-active, hybrid scaffold that is biodegradable, biocompatible and porous in structure to support skin cell growth and wound healing.

Method:
3D collagen/polycaprolactone(PCL) skin substitutes were prepared by lyophilisation and solvent evaporation techniques. Surface morphology was analyzed by SEM and cells variability on skin substitutes were assessed using human dermal fibroblasts. Efficacy and safety of the engineered skin substitute on skin wound healing was examined in vivo in a mouse model.

Results:
SEM analysis of scaffolds revealed a characteristic morphology of porous collagen/PCL surface morphology. Collagen/PCL scaffolds exhibited a burst phase of around 50% during first 4hrs in PBS followed by plateau release up to day 14, whereas cross-linked collagen/PCL scaffold displayed gradual and sustained release of the protein phase over 21 days. Human dermal fibroblasts colonized efficiently on the external surface of collagen/PCL with/without cross-linking. Surface modification of collagen/PCL scaffold enhanced the cell growth on the scaffold while cell numbers were doubled on DMEM modified collagen/CPL scaffolds compared with control collagen/PCL showing its potential for skin regeneration. The scaffolds with/without skin cells are currently assessed in an animal model to examine its role for skin regeneration and wound healing. This study will provide significant information on the feasibility, efficacy and safety of collagen/PCL scaffold, skin substitutes and dressing materials.

Conclusions:
Our present study can potentially lead to the development of an improved non-toxic permanent autologous skin substitute that would impact the clinical treatment of deep burns.

KEYNOTE ORAL PRESENTATION – ABSTRACT 141
Session 3A: Cartilage TE 2
Wednesday, 15 September 2010: 1600 – 1630 hours

IN VITRO ENGINEERING OF HUMAN EAR-SHAPED CARTILAGE ASSISTED WITH CAD/CAM TECHNOLOGY

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Due to the lack of appropriate scaffolds, the *in vitro* engineering of cartilage tissue with a sophisticated structure, such as a human ear, remains a great challenge. Although polyglycolic acid (PGA) has become one of the most successful scaffolds for cartilage regeneration, how to overcome its limitations in achieving desirable mechanical strength and accurate control over shape remains an unsolved problem. In this study, the mechanical strength of PGA scaffold was enhanced by coating with polylactic acid (PLA). The content of PLA was optimized by balancing the scaffold's biocompatibility and mechanical strength. The PLA/PGA scaffold was then fabricated into a human ear shape mirror-symmetrical to a normal ear by pressing the scaffold in the ear negative molds, which were fabricated by the computer aided design and manufacturing (CAD/CAM) technique according to the CT scan data from the normal ear. The ear-shaped scaffold reached a similarity level of over 97% compared to the positive ear mold by the shape analysis using a 3D laser scan system. Most importantly, after chondrocyte seeding, the constructs largely retained the original shape during culture with a similarity level of over 84%. Furthermore, the constructs formed ear-shaped cartilage-like tissues at 12 weeks, which revealed a tissue structure with abundant cartilage extracellular matrices and mature lacuna. Additionally, the ear-shaped cartilage at 12 weeks also exhibited fine elasticity and good mechanical strength. These results may provide a useful strategy for reconstructing cartilage tissue with complicated shapes such as a human ear by an *in vitro* engineering approach.

ORAL PRESENTATION – ABSTRACT 308
Session 3A: Cartilage TE 2
Wednesday, 15 September 2010: 1630 – 1645 hours

POLYCAPROLACTONE NANOFIBER MESHES AS SCAFFOLDS FOR CARTILAGE TISSUE ENGINEERING USING A DYNAMIC CULTURE SYSTEM

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Aim:
The aim of the present work was to determine if, using low seeding densities of human bone marrow-derived MSCs in poly(ε-caprolactone) (PCL) nanofiber meshes cultured dynamically in an in-house developed bioreactor (PT patent nº 104155), we could obtain suitable cartilage constructs for future implantation.

Method:
Adherent, colony-forming fibroblastic-cells were isolated from human bone marrow aspirates, from patients undergoing knee arthroplasties, and the MSCs phenotype characterized by flow cytometry. Cells were seeded onto electrospun PCL nanofiber meshes and cultured in a perfusion bioreactor to determine their ability to promote the production of a cartilagineous extracellular matrix. Constructs were cultured in a dynamic environment during 4 weeks.

Results:
Results indicate that the flow perfusion bioreactor increased the chondrogenic differentiation of hBM-MSCs, as confirmed either by morphological analysis and by Real Time-PCR analysis. Cartilage-related gene expression analysis showed that aggrecan, collagen type II and Sox9 were highly expressed, which is consistent with the positive staining for proteoglycans and the immunolocalisation of collagen type II. Furthermore, static cultures indicate a tendency to lead to fibrocartilage and dynamic culture to lead to hyaline-like cartilage tissue.

Conclusions:
Flow perfusion bioreactor enhanced the chondrogenic differentiation of hBM-MSCs by shortening the culturing time and promoting a stronger staining by toluidine blue and safranin-o indicating enhancing ECM production as compared with static conditions.

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Session 3A: Cartilage TE 2
Wednesday, 15 September 2010: 1645 – 1700 hours

DIFFERENT CHONDROGENIC PATTERN OF HUMAN AND PORCINE CHONDROCYTES CULTURED IN A POROUS CARTILAGE-DERIVED MATRIX

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Aim:
Chondrocyte is an important cell source for cartilage tissue engineering. However, whether cell behavior in large animal models can be readily applied to human studies is largely unknown. We developed a porous scaffold derived from native pig articular cartilage and hypothesized that human and porcine articular chondrocytes may exhibit different growth and differentiation pattern in this cartilage-derived matrix (CDM).

Method:
We examined physical properties and feasibility of cell penetration of CDM with different concentration. The biochemical contents, histology, immunohistochemistry, and mechanical properties of the chondrocyte-seeded scaffolds were examined at different time points.

Results:
The scaffold material supported proliferation of both types of chondrocyte, though the human chondrocyte group had higher DNA content in earlier time points. Total collagen and glycosaminoglycan contents of the cell-seeded constructs did not differ over the culture period of 28 days. Day 28 histology and immunohistochemistry of both groups showed abundant cartilage-specific macromolecules deposition, such as collagen type II and proteoglycan. However, the distribution pattern was very different: cartilaginous tissue spread throughout the whole human chondrocyte-seeded construct, while porcine chondrocyte-seeded scaffold developed a dense, cartilage-like tissue in the periphery. Human chondrocyte-seeded CDM also had a significantly lower aggregate modulus and hydraulic permeability at day 28.

Conclusions:
The CDM scaffold helped maintain the chondrogenic phenotype of both human and porcine chondrocytes without the use of exogenous growth factors. These data further supported the interspecies differences when applying chondrocytes in cartilage tissue
engineering. Hence, experimental results with large animal models should be cautiously interpreted to humans.

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Session 3A: Cartilage TE 2
Wednesday, 15 September 2010: 1700 – 1715 hours

MASS-SCALE FABRICATION OF CELL PELLETS FOR HIGH THROUGHPUT SCREENING AND 3D ASSEMBLY OF TISSUE ENGINEERED CARTILAGE

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Aim:
The use of pellet culture systems as a 3D culture model for understanding fundamental processes in chondrogenesis is well established. A simple, high-throughput process for fabricating large numbers of macro-sized pellets would provide significant time and cost advantage to current methods, as well as open the possibility for 3D assembly of pellets consisting of various cell types (e.g. chondrocytes, MSCs, zonal compositions of cells).

Method:
Expanded (P2) human nasal chondrocytes (HNCs) were centrifuged at 300g for 5 minutes at $5x10^5$ cells/pellet and micro-mass pellet cultivated for 7, 14, 21 and 28 days in bovine serum containing DMEM supplemented with TGFβ-1 (10ng/ml), insulin (0.01mg/ml) and ascorbic acid (0.1mM) (chondrogenic media). The 4 different formats for pellet fabrication included standard conical 15ml polypropylene tubes containing either 1ml (1T) or 300µl (300T) chondrogenic media (positive controls), and v- (300V) and round-bottom (300R) polypropylene 96-well plates (Greiner) containing 300µl chondrogenic media. At each timepoint, pellet size and shape, as well as tissue quality was assessed via histology, biochemistry (glycosaminoglycan (GAG), DNA content) and collagen type I and II mRNA expression.

Results:
Pellets cultured in 15ml tubes at 1ml media (1T) were significantly larger (Ø1.15–1.45mm) compared to all other formats at all timepoints. GAG/DNA was greater in 1T pellets at early timepoints, but was only significant at d21. Although delayed slightly, tissue formation (as assessed via histology and GAG/DNA) in 300V and 300R 96-well plates was equivalent to tubes, but allowed large numbers of pellets to be fabricated with greater ease and significantly lower volumes of media/growth factors and at reduced cost.

Conclusions:
We describe a high-throughput 96-well plate culture method as a lab-scale substitute to current cartilage pellet culture techniques. V- and round-bottom plates allow regular sized pellets to be generated in a mass scale, and can be applied to a range of cell types.

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Session 3A: Cartilage TE 2
Wednesday, 15 September 2010: 1715 – 1730 hours

PRELIMINARY CHARACTERISATION OF A NOVEL-DESIGNED MECHANICAL CONDITIONING BIOREACTOR TO DELIVER BIAXIAL COMPRESSION ON CHONDROCYTES SEEDED IN 3D AGAROSE SCAFFOLD

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Aim:
The development of a mechanical conditioning bioreactor will bring great benefits to research in tissue engineering as it will enable research studies to be undertaken to fully characterise the response of chondrocytes, permitting a more advanced understanding of the complex interactions involved in cartilage growth and remodelling. The primary objective of this study is to show the reproducibility of our novel bioreactor in delivering a variety of multi-dimensional loading regimes to chondrocytes seeded within agarose hydrogel constructs. Our hypothesis is that cells will modulate ECM synthesis patterns upon bi-axial dynamic loading.

Method:
Chondrocytes were seeded in 4% agarose. 3-D agarose constructs were attached to porous glass endplates at both ends. A novel bioreactor was used to deliver 10% direct and 2% shear compression onto the cell-seeded constructs. The load was delivered for 12 hours, at a frequency of 1Hz; and the constructs were left unstrained for the following 12 hours. Biochemical analysis to measure GAG concentration of the cells was done after 48 hours of stimulation.

Results:
GAG levels in constructs subjected to dynamic bi-axial loading have shown 80-90% increment compared to the constructs that were not loaded. T-test analyses of the results were statistically significant at $p=0.05$ ($n=12$).

Conclusions:
A preliminary study of the effect of dynamic biaxial loading on chondrocytes has shown its ability to up-regulate cell metabolism to produce a higher concentration of proteoglycans which in turn leads to ECM formation. Consistent and significant results have been reproducibility produced by the novel mechanical-conditioning bioreactor.

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Wednesday, 15 September 2010: 1730 – 1745 hours

IN VITRO ENGINEERING OF HUMAN EAR-SHAPED CARTILAGE ASSISTED WITH CAD/CAM TECHNOLOGY

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Aim:
In vitro engineering is becoming an important trend to promote clinical application and industrialization of tissue engineered cartilage. However, due to the difficulties in accurate shape control and inferior mechanical strength of the most widely used scaffolds in cartilage engineering (such as polyglycolic acid (PGA)), in vitro engineering a cartilage with an accurate desired shape is still a great challenge. Therefore, the aim of this study is to in vitro engineer a cartilage with sophisticated ear-shape by enhancing the scaffold's mechanical strength and accurately manipulating the scaffold shape.

Method:
The mechanical strength of the PGA scaffold was enhanced by coating PGA fibers with polylactic acid (PLA). The amount of PLA in the scaffold was optimized by balancing the scaffold's biocompatibility and mechanical strength. The PLA/PGA scaffold was then fabricated into an ear shape that is mirror-symmetrical to a normal ear by pressing the scaffold in a set of ear negative molds, which were fabricated by the computer aided design and manufacturing (CAD/CAM) technique according to the CT scan data from the normal ear.

Results:
The ear-shaped scaffold reached a similarity level of over 97% compared to the positive ear mold by the shape analysis using a 3D laser scan system. Most importantly, after chondrocyte seeding, the cell-scaffold constructs largely retained the original shape during in vitro culture with a similarity level of over 84% compared to the positive mold. Furthermore, the constructs formed ear-shaped cartilage-like tissues after 12 weeks of culture, which showed not only abundant cartilage extracellular matrices (ECM) and mature lacuna but also fine elasticity and a certain mechanical strength.

Conclusions:
This study may provide a useful clue for future strategies of reconstructing the external ear or other tissues with complicated shapes by an in vitro engineering method.

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Wednesday, 15 September 2010: 1745 – 1800 hours

CHONDROGENIC DIFFERENTIATION OF MENSTRUAL BLOOD DERIVED STEM CELLS ON A NANOFIBROUS SCAFFOLD

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Aim:
Recently, menstrual blood derived stem cells (MenSCs) have been identified as unique population of stem cells created enormous promise for the cell based therapies. However, much more studies are needed to introduce these cells suitable for tissue engineering and regenerative medicine. In this study, chondrogenic differentiation of MenSCs has been evaluated on a nanofibrous scaffold with high infiltration capacity.

Methods:
MenSCs were isolated of menstrual blood by discontinuous density gradient centrifugation and plastic adherence. After characterization of isolated cells, cells differentiation into chondrocytes was investigated on a nanofibrous scaffold with specific growth and differentiation factors. The scaffold was prepared from polycaprolactone and surface modified by plasma treatment and collagen grafting.

Results:
The isolated cells were positive for stem cells markers such as CD9 (95%), CD29 (99%), CD44 (99%) and CD73 (98%) as judged by flow cytometric analysis. Based on scanning electron microscope images, the scaffold had a highly porous scaffold that cells adhered, penetrated and proliferated properly. The scaffold contained an extensive cartilage-like extracellular matrix with more than 50% greater glycoseaminoglycan content than control MenSCs differentiated in standard cell pellet culture ($P<0.05$). Proteoglycan production by cells differentiated on the scaffold was more demonstrated by alcian blue staining. Unlike non differentiated MenSCs, imunoreactivity of cells with monoclonal antibodies against for collagen type II was strongly positive in cells differentiated on the scaffold.

Conclusion:
The evidences presented in this study introduce MenSCs as an suitable stem cell population candidate for cartilage tissue engineering.

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Session 3B: Silk
Wednesday, 15 September 2010: 1600 – 1630 hours

SILK FIBROIN SOURCE, MOLECULAR STRUCTURES AND PROCESSING: HOW DO THEY AFFECT ITS BIOMEDICAL BEHAVIOUR?

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Scaffolds for tissue engineering should be designed in terms of chemistry, structure and mechanical properties to act as a template to enable cells adhesion, activation and natural extra-cellular environment production leading to the regeneration of healthy tissues. Taking as a model the natural ECMs, biopolymers matrices, and in particular proteins, are attracting the interest of many researchers. Proteins constitute the basic building blocks of the biological systems, and possess a hierarchical structure with multi-functional properties able to interact with the chemical-physical environment. Among proteins, silk-based materials are increasingly investigated for the fabrication of scaffolds suitable for the regeneration of various mammalian tissues such as bone, cartilage, tendon and skin. Silk fibroin can be processed to obtain powders, films, gels, nano- and micro-nets, sponges, therefore suitable for scaffolds whose properties can be tailored and designed to specific applications. However, silk materials can differ widely in composition, structure and properties depending on their specific source as well as on the applied conversion/fabrication process. Protein conformation is critical to biological functions, triggering and controlling the interaction with body proteins and/or cell material cross talk.

For instance, as recently shown, tailoring of the crystallinity degree and morphologies can be exploited to induce different blood responses in terms of platelet adhesion and activation. Different cell behaviours in terms of adhesion, proliferation and ECM production were observed, as well as different integrins involvement in the cell-material interaction.

Experimental examples will be given and discussed.

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Wednesday, 15 September 2010: 1630 – 1700 hours

SILK FIBROIN-PVA HYDROGELS FOR BIOMEDICAL APPLICATIONS

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Aim:
Hydrogels are 3D polymer networks widely used in biomedical applications. The aim of this work is to characterize the properties of a poly (vinyl alcohol) (PVA) and silk fibroin (SF) hydrogel, as well as illustrate its potential as a drug releasing agent.

Method:
PVA was functionalized with photopolymerisable groups (~4 crosslinkers/chain), blended with SF and crosslinked into hydrogels. The total macromer content was 10wt% with ratios of PVA to SF ranging from 100 to 50/50. The hydrogels were investigated to determine their morphology, crystallinity, stability, swelling and mass loss. The FITC–Dextrans (FD-4, FD-20 and FD-40; 0.125 wt %) were chosen as model drugs, and their release was measured via spectrofluorometry.

Results:
Hydrogels containing different SF percentage show difference in pore size and distribution. XRD analysis showed amorphous SF in PVA is crystallized to β-sheet secondary structure upon gelation. The sol fraction, water uptake and fibroin mass loss increased with higher fibroin content within the gels (Table1). As expected, FDs released at a faster rate from gels with higher fibroin content and thus higher swelling. The percent cumulative release over 7 days of FDs increased with decreasing FD molecular weight (see Figure1 for the 60/40 PVA/SF hydrogel).

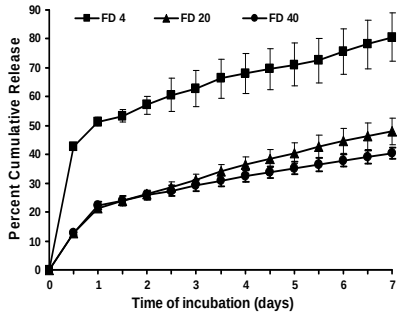

Figure 1: Cumulative FITC-Dextran (FD4, FD 20 and FD 40) release from PVA–SF (60/40) hydrogels.

Hydrogel (PVA/SF)	%Sol fraction	Swelling ratio	% fibroin loss
100/00	18.89 ± 1.34	07.89 ± 0.34	–
90/10	23.56 ± 2.98	12.56 ± 1.28	15.78 ± 1.56
80/20	26.69 ± 2.75	13.19 ± 1.75	16.75 ± 1.10
70/30	35.12 ± 3.90	15.12 ± 0.90	17.47 ± 0.89
60/40	41.56 ± 3.20	17.56 ± 1.20	21.86 ± 1.80
50/50	44.56 ± 4.15	20.56 ± 1.15	24.86 ± 1.85

Table 1: % Sol fraction, swelling ratio and % fibroin loss from the co-polymer hydrogels after 7 days of incubation

Conclusions:
PVA and SF are copolymerized to fabricate hydrogels which can release encapsulated model compounds in controlled manner, thus demonstrating its potential use in drug delivery [Indo-Australia Biotechnology Fund, Department of Biotechnology and Australia India Strategic Research Fund (AISRF) funded this work].

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Wednesday, 15 September 2010: 1700 – 1715 hours

VASCULARIZATION OF SILK FIBROIN BASED MATERIALS: FROM CELL-MATERIAL INTERACTIONS TO PREVASCULARIZED TISSUE CONSTRUCTS

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Aim:
Silk fibroin as a scaffold material can be tailored for specific biomedical applications by physical modification, such as fibers in the nanometric or micrometric scales. The aim of our studies is to optimize endothelial cell growth and to improve the vascularization in silk fibroin constructs by so called prevascularization strategies. In these approaches inclusion of endothelial cells or prevascular structures is hypothesized to favor a fast anastomomis of the construct with blood vessels of the host peri-implant tissue. In this context outgrowth endothelial cells are a promising autologous cell source due to their origin from an easily accessible source and their good expansion capacity. Nevertheless, the angiogenic potential might be triggered at different levels including material variation and crosstalk with other cells, which was the focus of our studies summarized in this abstract.

Methods:
Preparation of micro- or nanometric silk fibroin scaffolds, cell culture and seeding procedures were performed according to previous published protocols (*1-3*)

Results:
Nanometric and micrometric silk fibroin constructs supported endothelial cell growth and showed angiogenic potential in response to proangiogenic stimulation. On the nanometric net each fiber served as a potential adhesion point, reflected by a higher expression of ß-1 integrin and the formation of focal adhesions at the contact zones. In co-cultures of OEC and primary osteoblasts on silk fibroin the angiogenic activation of OEC was achieved without addition of external angiogenic growth factors or matrix components, while maintaining also the osteogenic potential of primary osteoblasts in the constructs. Angiogenic structures in the constructs increased over culture time. Furthermore the co-culture approach also supports the formation of perfused vessels *in vivo* as recently shown for starch poly(caprolactone) fiber meshes and matrigel plugs (*4*). These findings can also be transferred to silk-fibroin constructs as shown by recent studies from our group.

Conclusions:
Silk fibroin fiber meshes fulfill a series of prerequisites for successful vascularization. Their high interconnectivity facilitates the formation of angiogenic structures using co-cultures, either as advanced *in vitro* models or as a therapeutical tool to trigger angio-genesis.

References:
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Wednesday, 15 September 2010: 1715 – 1730 hours

PROTEIN FIBRE PARTICLES FOR BIOMEDICAL APPLLICATIONS

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Aim:
Ultrafine protein particles have been fabricated from natural protein fibres, such as silk and wool[1]. Our current study investigates assessments of biocompatibility, biodegradability, reversible sorption behaviour and applications in composite scaffolds for tissue engineering.

Method:
Particles of different sizes were prepared from protein fibres using cutter milling, attritor milling and air jet milling. Particle size distribution was measured using laser light scattering and scanning electron microscopy. Surface area was determined using nitrogen adsorption. AFM imaging was used to study particle surface contours. Surface charges were measured using a Zetasizer. *In-vitro* degradation kinetics was studied using protease XIV and inflammatory responses were measured by estimating proinflammatory cytokines by *RT-PCR.*

Results:
A combination of wet attritor milling, spray drying and air jet milling produced particles with a volume average particle size less than 1 µm and a surface area up to 20 m^2/g. Fabrication methods influenced particle shape, surface roughness, and binding behaviour. Solubility and degradation behaviour changed during milling as a result of changes in microstructure. Particles were used to toughen macro-porous protein composite scaffolds for tissue engineering. Reinforcement increased specific compressive modulus and strength of the composites by up to 40 times without a significant reduction in the porosity[2].

Conclusions:
The potential of using milled protein fibre particles for biomedical applications, such as tissue engineering was demonstrated. The option to use protein particle reinforcement of biomaterials provides various paths forward related to control of mechanical properties, degradation lifetimes and drug delivery.

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Wednesday, 15 September 2010: 1730 – 1745 hours

HUMAN BONE MARROW STEM CELL RESPONSE TO SILK FIBROIN/CARBON NANOTUBE COMPOSITES

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Aim:
Natural silk fibroin, which is derived from the cocoon of the silk worm Bombyx mori, has excellent tensile strength and elasticity, good thermal stability, hygroscopicity, microbial resistance and biocompatibility. However, the mechanical properties of regenerated silk fibroin for various applications are reduced by a conformation transition and a decrease in molecular weight. Multiwalled carbon nanotubes (MWCNTs) are ideal reinforcements in composites on account of their remarkable structural, mechanical, electrical and thermal properties.

Method:
Fegenerated silk fibroin/MWCNT nanocomposite films were prepared using a solvent system with pre-dispersed MWCNTs. Their biocompatibility was examined in vitro using human bone marrow stem cells (hBMSCs), and their physical properties were assessed under a range of conditions.

Results:
The incorporation of MWCNTs in a silk film induced the crystallization of that. The MWCNT-incorporated silk film increased the stability of the films in water. Furthermore, the strong interactions between the carboxylic acid group of MWCNTs and the amide group of silk fibroin lead to an improvement in the mechanical properties. The biocompatibility of the MWCNTs-incorporated silk fibroin films were examined in vitro using hBMSCs. The hBMSCs have favorably adhered and proliferated to them. There were no significant differences between the pure silk fibroin films and the MWCNTs-incorporated silk fibroin films.

Conclusions:
In this study, water insoluble silk films were prepared by incorporating MWCNTs in an all aqueous process. The biocompatibility of the silk fibroin/MWCNT films were examined in vitro using hBMSCs. The hBMSCs adhered favorably and proliferated to a similar level on both the silk only and MWCNT-incorporated silk films. This approach should allow MWCNTs to be used in a wide range of biomedical applications.

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Wednesday, 15 September 2010: 1745 – 1800 hours

BONE-LIGAMENT-BONE TISSUE ENGINEERING IN A RABBIT MODEL USING BMSC-SEEDED TRIPHASIC SILK SCAFFOLDS

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Aim:
The fibrocartilage interface (enthesis) between ligament and bone is responsible for high insertional strength and avulsion resistance. To overcome the poor long-term outcome that affects donor and tissue engineered ligament grafts that cannot generate this native interface, a tissue engineered bone-ligament-bone (BLB) graft, incorporating the ligament-bone interface, was developed in this study

Method & Results:
A novel triphasic 3D silk scaffold, with three different zones suitable for bone and ligament regeneration, was developed by coating a knitted silk scaffold with lyophilized silk fibroin sponge and further coating with hydroxyapatite (for the two bone ends). Bilineage co-cultures were established between BMSCs and osteoblasts/ ligament cells wherein cells could effectively communicate via functional gap junctions that were demonstrated using dye transfer techniques; gene expression analysis indicated chondrogenic differentiation of co-cultured BMSCs. A trilineage co-culture of BMSCs, osteoblasts and ligament cells was established on the triphasic scaffold to generate a BLB construct. Cells remained viable and proliferated on the 3D scaffold, and gene expression analysis demonstrated that, compared with BMSCs monocultures on the scaffold, BMSCs in the trilineage co-culture on the scaffold showed fibrocartilaginous differentiation. A tissue engineered BLB graft was next used to repair a full length longitudinal hemi-defect in the rabbit medial collateral ligaments, wherein it was shown to successfully regenerate both the collagenous ligament mid-substance and the ligament-bone interface in the graft.

Conclusions:
Such BLB grafts with regenerated interfacial tissue are expected to have better functional outcome than ligament-alone constructs for long-term repair of severely injured ligaments.

KEYNOTE ORAL PRESENTATION – ABSTRACT 388
Session 3C: Mammalian Regeneration
Wednesday, 15 September 2010: 1600 – 1630 hours

CHARACTERIZING THE IMMUNOLOGICAL RESPONSE TO WOUNDING AND REGENERATION IN THE SALAMANDER

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Aim:
Salamanders have the highest regenerative capacity of any vertebrate and can regenerate a range of important tissues even as an adult. Limb regeneration in the salamander is the most extensively characterized context of adult regeneration and this has been shown to be dependant on the formation of a temporary structure known as the wound epithelium. After similar injuries in mammals this structure is not formed and typical wound healing can be characterized by a slower epithelial migration rate and the formation of fibrotic scar tissue. The immune system in mammals has been implicated in directing these fibrotic processes and could potentially inhibit a latent regenerative program. This work aims to characterize the immune response during salamander limb regeneration and identify key differences to that observed in mammalian wound healing.

Method:
Salamander cytokine genes have been cloned and real time PCR assays performed under different wounding scenarios. Macrophage populations have been labeled (uptake by phagocytosis) with fluorescent beads and assessed for their presence in regenerative tissues.

Results:
Crush injuries have the capacity to induce the rapid upregulation of major inflammatory cytokine pathways showing that axolotls are capable of mounting effective inflammatory responses. Amputated limbs show low levels of inflammation but are characterized by a rapid up-regulation of the collagenase MMP-9. Fluorescently labeled macrophages appear in the regenerative wound environment early and may play an important role in supporting the regenerative response.

Conclusions:
The different cytokine responsiveness and timing in the appearance of immune cell subclasses to the site of injury may be important factor for the successful regenerative response in salamanders. These immunological signals may provide a favorable environment for formation of the Wound Epidermis and the prevention of fibrosis. Transgenic axolotls are in construction to genetically dissect these pathways

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Session 3C: Mammalian Regeneration
Wednesday, 15 September 2010: 1630 – 1645 hours

DIRECTED DIFFERENTIATION OF HESC INTO SKELETAL MUSCLE: A POTENTIAL SOURCE OF MUSCLE PRECURSOR CELLS FOR THERAPEUTIC APPLICATIONS

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Aim:
Muscular dystrophies (MD) are inherited diseases characterized by progressive weakness and degeneration of the skeletal muscles that control movement. Despite the substantial advances made in identifying the genetic defects causing these diseases, there is currently no effective treatment available.

Due to their early embryonic origin, human embryonic stem cells (hESC) can be induced to recapitulate in vitro the earliest stages of human development and can be differentiated into a variety of tissues and specialized cells. In our laboratory, we are using this model to generate skeletal muscle cells with the aim of using them in the future for the treatment of MD and other severe myopathies.

Method:
Selective culture conditions are set up to direct hESC to recapitulate in vitro the earliest stages of embryonic development, including somitogenesis. A FACS sorting strategy is then used to isolate putative myogenic precursors, which are expanded in culture for molecular and cellular analyses aimed at confirming their cell identity.

Results:
We were the first to report the derivation of mesenchymal precursors from hESCs able to further differentiate into skeletal myoblasts in vitro. Here, we present the latest strategy developed in the lab to isolate skeletal muscle precursors from hESC. This strategy is based on the use of FACS sorting to purify cells expressing a muscle specific reporter gene for tracking the emergence of the earliest myogenic cells.

Conclusions:
Using the selective method we developed, we provide support for the future use of hESC as an important source of cells for muscle repair.

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Session 3C: Mammalian Regeneration
Wednesday, 15 September 2010: 1645 – 1700 hours

ENHANCED WOUND REPAIR THROUGH THE COALESCENCE OF URINARY BLADDER MATRIX AND LASER ACTIVATED CHITOSAN BIOADHESIVE

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Tissue repair and reconstruction requires an ideal scaffold that is biocompatible with the host tissue for effective repair and regeneration. The extracellular matrix derived urinary bladder matrix (UBM) has been employed in therapeutic applications for tissue repair and shown to facilitate recruitment of marrow-derived stem cells resulting in remodeling of damaged tissue. Current techniques for tissue reconstruction also involve the use of sutures, with documented disadvantages. SurgiLux® is a laser-activated chitosan adhesive that enhances the growth of several cell lineages. This study adopted a novel approach by incorporating SurgiLux® and UBM to fabricate an innovative therapeutic scaffold that holds considerable potential in the field of tissue repair.

Physiochemical examination of the combined adhesive properties through X-ray diffraction, confocal laser scanning microscopy and *in vitro* laser tissue repair revealed that UBM-SurgiLux® displayed increased surface rugosity, lower crystallinity and a greater wound repair strength compared to SurgiLux® films. Histological examination showed that the UBM scaffolds seal the wound properly without causing any thermal damage to tissue. Cellular response at the material interface revealed maintenance of regular morphology and enhanced growth of 3T3 fibroblasts compared to SurgiLux® films alone. Expression protein profiling of UBM composition identified two proteins, Galectin 7 and Fibulin 5 that exhibit augmentation of cell growth if implanted as nerve conduits due to their enhanced capability to repair wound.

Our results suggest that the coalescence of UBM with SurgiLux® leads to the prospect of developing successful laser activated UBM-SurgiLux® biomaterials for enhanced wound repair and tissue reconstruction.

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Session 3C: Mammalian Regeneration
Wednesday, 15 September 2010: 1700 – 1715 hours

DAMAGE ASSOCIATED MOLECULAR PATTERNS (DAMPS) AND MACROPHAGES IN THE HOST IMMUNE AND REMODELING RESPONSE TO XENOGENEIC BIOLOGICAL SCAFFOLDS

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Xenogeneic extracellular matrix (ECM) scaffolds are widely used clinically and are readily remodeled by host tissues, with few reports of adverse reactions. However, host remodeling responses may be altered by the manufacturing process and further modifications such as chemical cross-linking of ECM. Such addition modification of ECM scaffolds may induce in a more deleterious immune response and encapsulation of the implant. The specific mechanisms responsible for this difference in immune response and ultimate outcome is unclear. Immunomodulatory molecules in ECM and their interaction with host macrophages have been suggested to influence the remodeling process. Damage associated molecular pattern molecules (DAMPs) are released during non-apoptotic cell death, and may act as inflammatory mediators. Consequently, the presence of DAMPs within biological scaffolds may direct host immune and remodeling responses. In the present study, several well established DAMPs (HMGB1, S100A9, S100A8, HSP60, HSP70, heparin sulfate proteoglycan and hyaluronan) were isolated from multiple commercially available ECM scaffolds. Using an *in vitro* system and the human myelomonocytic cell line, THP1, the response to several ECM scaffolds (both chemically cross-linked and not) was examined. ECM scaffolds were found to have an immunomodulatory effect on THP1 cells, driving their differentiation towards macrophages and inducing the secretion of chemokines CCL2 and CCL4. In addition, the DAMPs HMGB1 and hyaluronan were released from these scaffolds during culture. These results suggest that immunomodulatory molecules present within biologic scaffolds may influence the host response and facilitate constructive remodeling.

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Session 3C: Mammalian Regeneration
Wednesday, 15 September 2010: 1715 – 1730 hours

EVALUATION OF GROWTH FACTORS, CYTOKINES, CHEMOKINES SECRETION AND CELLULAR MIGRATION INDUCED BY ACELLULAR COLLAGEN SCAFFOLDS AND AUTOLOGOUS ARTIFICIAL CONNECTIVE TISSUE

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Aim:
We are focused on the development of a tissue substitute, based on collagen I scaffolds and oral autologous fibroblasts. Previously, we developed autologous artificial tissue (AACT) that favors oral mucosa regeneration and established that proangiogenic, tissue-remodeling, and immune-modulating effectors are secreted by the AACT at the moment of implantation. To get insight into the reasons behind this phenomenon this work evaluated the AACT expression of 43 proangiogenic, tissue-remodeling, and immune-modulating compounds at the moment of implantation, as well as AACT and collagen I scaffold (CS) chemotactic properties.

Method:
Prior to evaluate AACT and CS chemotactic properties, AACT secretion of wound healing modulating factors was assessed as follows: medium aliquots from AACT cultures were taken at 7 d., the time at which the AACT are used to graft partial thickness wounds, and analyzed using a RayBio® Human Angionesis antibody array following manufacturer's directions. Thereafter, AACT and CS chemotactic properties were determined using a CHEMICON® QCMTM Cell Migration Assay according with fabricant instructions. Assays using human blood clot (BC), prostaglandin E2 (PGE-2), BC embedded AACT, PGE-2 embedded AACT, BC embedded CS and PGE-2 embedded CS were also included.

Results:
1) At the moment of implantation fibroblasts in the AACT secrete compounds that modulate wound healing.
2) AACT and blood clot chemotactic activity are similar and significantly higher than the one exhibited by CS.

Conclusions:
AACT therapeutic effects might be due to mediation of wound healing modulating factors that recruit cells involved in tissue formation into the organized structure provided by the collagen scaffold used to AACT manufacturing.

ORAL PRESENTATION – ABSTRACT 58
Session 3C: Mammalian Regeneration
Wednesday, 15 September 2010: 1730 – 1745 hours

PROMOTION OF MUSCLE REPAIR BY MYOBLAST TRANSPLANTATION USING THE CONTROLLED DELIVERY SYSTEM OF BFGF

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Aim:
Although myoblast transplantation is an attractive method for muscle regeneration, the survival rate of transplanted myoblasts is extremely low. The authors investigated the efficacy of myoblast transplantation using the controlled and sustained delivery system of bFGF.

Methods:
The defects of thigh muscle in SD rats were created, and myoblasts prepared from GFP-positive SD rats were transplanted. The rats were divided into three groups; in groups 1, myoblasts were transplanted with bFGF incorporated in gelatin hydrogel microspheres. In group 2, they were introduced with gelatin hydrogel microspheres only. In group3, only myoblasts were transplanted.

Results:
One week after transplantation, GFP-positive myoblasts survived in the recipients in all groups. Four weeks after transplantation, GFP-positive myoblasts were found to be integrated into the recipient muscle and to contribute to muscle fiber regeneration in group 1, while GFP-positive cells were not detected in groups 2 and 3. Furthermore, marked native myofiber regeneration characterized by centralization of the nucleus was also found to be accelerated in group 1.

Conclusion:
Our findings suggested that the controlled and sustained release of bFGF promoted muscle regeneration not only by integration of transplanted myoblasts, but also by activation of native satellite cells. This system may become a promising tool to improve the effectiveness of muscle regeneration following myoblast transplantation.

ORAL PRESENTATION – ABSTRACT 170
Session 3C: Mammalian Regeneration
Wednesday, 15 September 2010: 1745 – 1800 hours

DELIVERY OF GROWTH FACTORS CONTAINED IN PLATELET-RICH PLASMA USING HEPARIN-CONJUGATED FIBRIN FOR WOUND HEALING

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Aim:
Platelet rich plasma (PRP) is increasingly used in the treatment of wound healing. We have developed heparin-conjugated fibrin (HCF) which can exert controlled release of various growth factors contained in PRP. PRP provides physiologic mixture of growth factors required for wound healing process. In this study, we hypothesize that HCF can deliver growth factors contained in PRP for a long period and enhance the wound healing effect of PRP.

Method:
PRP was prepared by centrifuging whole blood at 2,400 rpm for 10 min and subsequently 3,500 rpm for 15 min. Full-thickness (2.0 x 2.0 cm) wounds were created on the dorsum of athymic mice. HCF mixed with PRP and thrombin was applied at the wound sites. No treatment, application of PRP with thrombin, PRP with fibrinogen and thrombin served as controls. Skin regeneration was evaluated by histological and Immunohistochemical analyses.

Results:
Initial burst release and release period dramatically differ depending on the types of the delivery system *in vitro*. At 12 days after injury, PRP with HCF group showed complete epithelialization of the wound compared to the other groups. The macroscopic wound sizes of PRP with HCF group were statistically smaller than the other groups at 12 days. The HCF with PRP groups showed excellent epithelial maturation.

Conclusions:
HCF could be useful for delivery of various heparin-binding growth factors contained in PRP to promote wound healing.

KEYNOTE ORAL PRESENTATION – ABSTRACT 409
Session 3D: Cell Therapy from Bench to Bedsite
Wednesday, 15 September 2010: 1600 – 1630 hours

CELL THERAPY IN STROKE

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Stem cell transfer has been used for treatment of ischemic disorders such as myocard infarction or stroke. However, the use of embryonic or fetal grafts in humans is restricted by ethical considerations, severe logistical problems, and the fact that intracerebral administration of homologous embryonic stem cells can lead to teratocarcinomas as demonstrated in rodents (Erdo *et al*., 2003). Therefore, alternative stem cell sources for treatment of neurological disorders have been evaluated in animal models, among them human bone marrow (Chopp and Li, 2002) and human umbilical cord blood (HUCB, Newman *et al*., 2003; Boltze and Emmrich, 2005).

In our study we investigated the potential of stem cells from different sources to promote functional recovery following experimental stroke by middle cerebral artery occlusion (MCAO). The experiments were performed in spontaneously hypertensive (SH) rats, known for a risk profile comparable to stroke patients. For cell therapy cryopreserved cells were administered systemically within the first days after MCAO. Behavioural tests (Beamwalk, RotaRod, Stairway) were performed together with a neurological severity score (mNSS) to assess neurofunctional disabilities. In addition, the experiments were carefully documented by magnetic resonance imaging (MRI).

We could demonstrate a nearly complete recovery from functional deficits with treatments performed within the first three days after artery occlusion. Additionally, in vitro experiments with a neuronal cell line demonstrated a direct protective effect conferred by our cell preparations. Less glial reactivity and smaller lesions were observed in vivo, while in the groups with little behavioural recovery (irrelevant control cells) reactive astrocytes were more numerous, and the lesions were larger (Hau et al., 2008, Kranz et al., 2010).

To prepare for clinical studies we have recently established a large animal stroke model in sheep by surgical MCAO of various sizes. Autologous bone marrow cells have been used for systemic i.v. cell therapy 24 hrs after the stroke incident (Boltze et al., 2008) Significant improvement could be observed in the following 6 weeks by functional behavioural tests as well as by sequential MRI imaging und subsequent positron emission tomography (PET).

Abbreviations:
SH = spontaneously hypertensive; MCAO = middle cerebral occlusion; CSFE = Carboxy-fluorescein diacetate, succinimidyl ester; HUCB = human cord blood, GFAP = glial fibrillary acidic protein

KEYNOTE ORAL PRESENTATION – ABSTRACT 88
Session 3D: Cell Therapy from Bench to Bedsite
Wednesday, 15 September 2010: 1630 – 1700 hours

MICROENGINEERED HYDROGELS FOR STEM CELL BIOENGINEERING AND TISSUE REGENERATION

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Micro- and nanoscale technologies are emerging as powerful tools for controlling the interaction between cells and their surroundings for biological studies, tissue engineering, and cell-based screening. In addition, hydrogel biomaterials have been increasingly used in various tissue engineering applications since they provide cells with a hydrated 3D microenvironment that mimics the native extracellular matrix. In our lab we have developed various approaches to merge microscale techniques with hydrogel biomaterials for directing stem cell differentiation and generating complex 3D tissues. In this talk, I will outline our work in controlling the cell-microenvironment interactions by using patterned hydrogels to direct the differentiation of stem cells. In addition, I will describe the fabrication and the use of microscale hydrogels for tissue engineering by using a 'bottom-up' and a 'top-down' approach. Top-down approaches for fabricating complex engineered tissues involve the use of miniaturization techniques to control cell-cell interactions or to recreate biomimetic microvascular networks within mesoscale hydrogels. Our group has also pioneered bottom-up approaches to generate tissues by the assembly of shape-controlled cell-laden microgels (i.e. tissue building blocks), that resemble functional tissue units. In this approach, microgels were fabricated and seeded with different cell types and induced to self assemble to generate 3D tissue structures with controlled microarchitecture and cell-cell interactions.

KEYNOTE ORAL PRESENTATION – ABSTRACT 410
Session 3D: Cell Therapy from Bench to Bedsite
Wednesday, 15 September 2010: 1700 – 1730 hours

SO YOU THINK YOU ARE READY FOR THE CLINIC?

Brian Newsom, Mark Bonyhadi, Eric Roos, Kelli Tanzelli & Paul Pickering
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The path to get from the research bench to the clinic can be both complex and challenging. There is a great need to find ways to make this process efficient and cost effective. While there are many regulatory guidelines and case studies to refer to, it is difficult, if not impossible, to find a "tried-and-true" process an investigator can follow to move a promising cell based modality into the clinic. The intent of this talk is to provide researchers, physicians, and industry professionals with technical, procedural and tactical information so that they can develop effective strategies for getting their concepts into the clinic. Intelligent planning will save time and money over the long run, and will help identify hurdles early enough to allow ample time to generate data critical for a successful submission for regulatory approval. Attention needs to be paid to critical tool/reagent selection, the qualification/validation of those tools, and how to mitigate risk during those processes. In addition, testing strategies for cell and stem cell therapy products, including characterization and safety assays, validation and qualification strategies, and GLP/GMP compliance considerations should be contemplated early on even if they are developed at later stages of clinical development. Preparing your strategy early in the pre-clinical stage and developing strategies for mitigating risk can help to reduce long-term costs, get into the clinic in a timely fashion, and save time and money in the long run and to better position you for an easier transition from bench to bedside.

KEYNOTE ORAL PRESENTATION – ABSTRACT 407
Session 3D: Cell Therapy from Bench to Bedsite
Wednesday, 15 September 2010: 1730 – 1800 hours

MULTI-BIOFUNCTIONAL POLYMER GELS TO AID REGENERATIVE THERAPIES

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Cell-instructive characteristics of extracellular matrices (ECM) result from a subtle balance of biomolecular and biophysical signals and must be recapitulated in engineered biomaterials to enable regenerative therapies. However, hardly any of the currently applied materials allow for the *independent* modulation of the involved molecular *and* physical cues. Addressing this challenge, we developed a rational design strategy for ECM-inspired biohybrid hydrogels based on multi-armed, end-functionalized poly(ethylene glycol), heparin, and multifunctional peptide linkers. A mean field approach was adapted to identify conditions at which the multi-component gels allow for the decoupling of biochemical and mechanical gel properties.

To support specific applications heparin-based biofunctionalization schemes were utilized to generate various gel types with fine-tuned cell adhesiveness and morphogen presentation. Bifunctional peptide crosslinker units were developed to combine matrix metalloproteinase- (MMP-) sensitivity and cell adhesive characteristics, allowing for the localized three-dimensional reorganization of the gels by invading cells. The novel materials were used in systematic studies on the exogenous stimulation of the pro-angiogenic state of endothelial cells *in vitro*. Multiple combinations of growth factor and adhesive ligand concentrations and matrix elasticity, respectively, were found to support capillary formation of human endothelial cells grown in contact with the gels. Additional applications of the modular biohybrid gel materials include culture experiments to expand and differentiate various types of human stem and progenitor cells.

In sum, our study demonstrates the power of joint theoretical and experimental efforts in creating bioactive materials with specifically and independently controllable characteristics to provoke a desired cellular response in different emerging applications.

KEYNOTE ORAL PRESENTATION – ABSTRACT 389
Session 4A: Stem Cells
Thursday, 16 September 2010: 1145 – 1215 hours

REGENERATION AND REPAIR OF CONNECTIVE TISSUES BY MESENCHYMAL PROGENITOR CELLS - NEW MODALITIES OFTREATMENT FOR DISORDERS OF THE MUSCULO-SKELETAL SYSTEM?

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Plastic adherent bone marrow derived mesenchymal mononuclear cells contain a mixed population of cells in various stages of differentiation. These different cell clones can be identified by their expression of surface antigens to which Mabs have been prepared. Secondary antibodies to these Mabs coupled to magnetic beads have facilitated the isolation of specific mesenchymal cell subsets including mesenchymal progenitor cells (MPC) that express the antigens STRO-1 and STRO-3. These MPC have extensive proliferative capacity and exhibit more potent immunosuppressive/anti-inflammatory activities than the plastic adherent mixed mesenchymal stem cell populations. We have subsequently used the STRO-1/STRO-3 immune-selected MPC in pre-clinical studies to ascertain their capacity to accelerate the repair and reconstitution of injured bone, cartilage and the intervertebral disc. In addition, we have identified factors that reduce apoptosis, promote proliferation and chondrogenic differentiation of MPC *in-vitro* and *in-vivo*. Successful cervical and lumbar spinal fusion studies in sheep models using MPC seeded into a calcium hydroxyapatite/calcium triphosphate carriers have now progressed to Phase IIa human clinical trials, while the safety and efficacy of intra-articular (IA) administered MPC into ovine stifle joints with traumatically induced osteoarthritis (OA) has supported a human Phase IIa study to evaluate the ability of IA injected MPC to preserve cartilage in young adults who have undergone surgical repair of anterior cruciate ligament (ACL) rupture. ACL deficiency is frequently associated with cartilage degeneration and the early onset of OA. More recently, we have demonstrated that six months following injection of 0.5 x 106 MPC into the nucleus pulposus (NP) of degenerate ovine lumbar discs, their pathology and disc height, as determined histologically, radiologically and by MRI, were improved. Moreover, the proteoglycans (PGs) deposited in the NP extracellular matrix of these MPC injected discs were restored to the levels close to those present in adjacent nondegenerate control discs. In contrast the NPs of non-MPC injected degenerate discs failed to show the same extent of matrix recovery.

Collectively, these clinical and preclinical studies afford evidence that MPC are capable of achieving repair and restoration of injured musculoskeletal tissues and could provide alternative therapeutic strategies for the management of these very common orthopaedic problems in the future.

ORAL PRESENTATION – ABSTRACT 57
Session 4A: Stem Cells
Thursday, 16 September 2010: 1215 – 1230 hours

ENHANCED IMMUNOSUPPRESSIVE ACTIVITY AND CHONDROGENIC POTENTIAL OF HUMAN MESENCHYMAL PROGENITOR CELLS CULTURED IN A NOVEL XENO-FREE MEDIUM

Ravenska Wagey[1] Brenton Short[1,2], Betty Hoac[1], Andy Kokaji[1], Terry Thomas[1], Allen Eaves[1,3], Maureen Fairhurst[1] & Bert Wognum[1]
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Aim:
To study the characteristics of Mesenchymal Progenitor cells (MPCs) cultured in a novel serum- and nonhuman component-free medium (MesenCult®-XF).

Methods:
Clonogenic growth was analyzed by low density plating of BM mononuclear cells and proliferation was examined by serial passage in vitro. For immunosuppressive studies, T-cells labelled using carboxyfluorescein succinimidyl ester and activated with antibodies to CD3, CD28 and CD2 were co-cultured with MPCs and their divisional history analyzed by flow cytometry. Chondrogesis was examined by micromass culture in MesenCult®-XF chondrogenic differentiation medium.

Results:
MPCs cultured in MesenCult®-XF or serum-containing medium revealed similar CFU-F frequencies but colonies generated in MesenCult®-XF were twice as large. MPCs cultured for 9 passages in MesenCult®-XF showed an average fold expansion of 8.5 ± 1.4; $\bar{x}$ ± SD (n=3) at each subculture which was ~3-fold higher than expansion of MPCs cultured in serum. MPCs cultured in MesenCult®-XF showed strong inhibition of T-cell proliferation with 100% and 80% inhibition after 3 and 7 days, respectively, whereas MPCs cultured in serum-containing medium showed weak inhibition (50% and 20% inhibition after 3 and 7 days, respectively). Chondrogenic differentiation was greatly enhanced in cells cultured in MesenCult®-XF with enhanced production of type II Collagen.

Conclusions:
MPCs expanded more extensively in serum-free MesenCult®-XF medium and exhibited enhanced immunosuppressive activity and chondrogenic potential in vitro. The ability to expand functional human MPCs in a serum and nonhuman component-free medium will enable further research into the efficacy of mesenchymal cells in clinical applications such as immuno-suppression and tissue repair.

ORAL PRESENTATION – ABSTRACT 64
Session 4A: Stem Cells
Thursday, 16 September 2010: 1230 – 1235 hours

EFFECTS OF MICROENVIRONMENTAL CHANGES ON HUMAN MESENCHYMAL STEM CELL (hMSC) BEHAVIOUR

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Human mesenchymal stem cell (hMSC) behaviour is intimately regulated by the compositional and temporal variations in the surrounding microenvironment, which includes extracellular matrix molecules, soluble factors and cell-cell interactions. This study examined the effects of (1) culture medium, (2) tailored biomimetic surfaces, (3) oxygen tension (pO_2) and (4) plating density on hMSC behaviour in monolayer culture. After 14 days, cell morphology, proliferation and sulfated glycosaminoglycan (sGAG) production were assayed using toluidine blue staining, DNA quantification and a dimethylmethylene blue (DMMB) assay. The results show that both the culture medium and the presented surfaces exerted profound effects on the morphology of hMSCs. In particular, hMSCs in Dulbecco's modified Eagle's medium with 10% serum (DMEM) retained their normal spindle-shaped morphology, whereas those cultured in chondrogenic medium (CM) or CM without TGF-β3 (CM-) appeared round or polygonal in shape. Under the latter conditions, the cells formed colonies on surfaces that presented, on a background of collagen I/II, covalently-bound chondroitin-6-sulfate (C6S) or C6S and TGF-β3. At plating densities of 2500 and 1×10^4 cells/cm^2, cultures maintained in 2% pO_2 generally exhibited greater proliferation (up to 7-fold) compared to those in 20% pO_2. This growth *advantage* was not found at higher plating density, of 5×10^4 cells/cm^2, possibly due to contact inhibition. The sGAG/DNA ratio, a marker for meniscal fibrochondrogenic differentiation, was highest (21.0 ± 6.6 μg/μg) in hMSCs seeded at 1×10^4 cells/cm^2 on the C6S surface and maintained in CM- in 20% pO_2. These optimized culture conditions enhance the suitability of hMSCs for meniscal tissue engineering.

ORAL PRESENTATION – ABSTRACT 113
Session 4A: Stem Cells
Thursday, 16 September 2010: 1235 – 1240 hours

EFFECT OF LITHIUM CHLORIDE ON ß-CATENIN EXPRESSION, PROLIFERATION AND OSTEOGENIC DIFFERENTIATION OF HUMAN MENSTRUAL BLOOD DERIVED STEM CELLS

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Aim:
Menstrual blood derived stem cells (MenSCs) appears unique stem cells that have been identified recently. The special traits of MenSCs can be related to cell signaling pathways. Lithium chloride (LiCL) has been shown to implicate the effects of Wnt signaling on cell proliferation and differentiation. In this study, we investigated the influence of lithium chloride on ß-catenin expression, proliferation and osteogenic differentiation of MenSCs.

Methods:
MenSCs were isolated from healthy women using combining gradient density centrifugation with plastic adherence. After characterization of isolated cells, cell proliferation of MenSCs in presence of 10-15 mM LiCl was evaluated by MTT assay. ß-catenin expression of treated cells was examined using immufluoroscent technique. Thereafter, osteogenic differentiation ability of MenSCs osteocytes in media containing 10-15mM liCl was traced by Alizarin red staining.

Results:
Flow cytometric analysis revealed that both mesenchymal stem cell markers including CD9, CD29, CD44, CD105, CD73 and embryonic stem cells markers such as Oct-4 are expressed on menstrual blood stem cells. MTT value was decrease depending on LiCl concentration. The proliferation of MenSCs cultivated in culture media containing 15mM LiCl was approximately twofold less than that grown without LiCl ($P<0.01$) as to nuclear accumulation of ß-catenin protein in cells treated by LiCl was greater than cells without LiCl. However, calcium assembly in differentiated cells into osteoblast judged by Alizarin red staining were not gross divergent between groups.

Conlusion:
LiCl probably suppress MenSCs proliferation via mediating nuclear accumulation of ß-catenin in a dose dependent manner but no affect on osteogenic differentiation.

ORAL PRESENTATION – ABSTRACT 309
Session 4A: Stem Cells
Thursday, 16 September 2010: 1240 – 1245 hours

CHONDROGENIC DIFFERENTIATION OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS SEEDED IN 3D POROUS SCAFFOLDS IN A FLOW PERFUSION BIOREACTOR

Marta Alves da Silva[1,2], Albino Martins[1,2], Ana Costa-Pinto[1,2], Vitor Correlo[1,2] , Paula Sol[1,2], Mrinal Bhattacharya[3], Susana Faria[4], Rui Reis[1,2] & Nuno Neves[1,2]
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Aim:
Determine the outcome of a in-house flow perfusion bioreactor in the chondrogenic differentiation of human bone marrow mesenchymal stem cells (BM-MSCs). We intended to find out if the shear stress caused by the medium perfusion trough the constructs was capable of augmenting the differentiation process, using 3D porous scaffolds.

Method:
Human BMSCs were isolated from bone marrow aspirates and were characterized by flow cytometry. After expansion, hBM-MSCs were seeded statically onto fiber meshes scaffolds, consisting of a blend of 50/50 chitosan and Poly(Butylene Terephtalate Adipate)– CPBTA. The chitosan-poly(butylene terephthalate adipate) (CPBTA) (50/50 wt%) fiber meshs were produced using a micro-extruder followed by fiber bonding. Constructs were cultured in the flow perfusion bioreactor for 28 days, using complete medium for chondrogenesis supplemented with TGF-β3.

Results:
We observed an enhanced ECM deposition and collagen type II production in the constructs cultured in the flow perfusion bioreactor when compared with the controls in static culture. Moreover, it was observed a longer period required for the differentiation of the cells in static culture. ECM accumulation in these constructs was lower than in the bioreactor and the expression of collagen type I was considerably enhanced in a statistically significant level when compared with dynamic culture conditions.

Conclusions:
In the present work, we observe statistically significant evidence that confirm our hypothesis that shear stress has a beneficial effect on the chondrogenic differentiation of hBM-MSCs when cultured in 3D porous scaffolds.

ORAL PRESENTATION – ABSTRACT 112
Session 4A: Stem Cells
Thursday, 16 September 2010: 1245 – 1250 hours

NANOSCALE PRESENTATION OF CELL ADHESION MOTIFS VIA BLOCK-COPOLYMER SELF ASSEMBLY: EFFECT OF LIGAND SPACING ON MESENCHYMAL STEM CELL BEHAVIOUR

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Aim:
Classically soluble factors have been investigated for their effects on mesenchymal stem cell (MSC) growth and differentiation, however, more recent work has shown that the mechanical environment, including substrate modulus and ligand type and availability can also influence MSC properties. Here we aim to determine how the spatial arrangement of cell adhesion ligands affects MSC behaviour.

Method:
We use self-assembled polystyrene-block-poly(ethylene oxide)-copolymers (BCP) to present RGD-peptide to MSCs with lateral spacings, ranging from 34 to 62 nm. These surfaces are used to investigate how lateral spacing of adhesion ligand affects MSC adhesion, morphology and differentiation.

Results:
We demonstrate that adhesion of MSCs to the BCP surfaces is through specific attachment to the presented RGD-motif and that this is mediated by 5, V, 1 and 3 integrins. As the lateral spacing of the peptides is increased, the ability of the MSCs to spread is diminished and that the morphology changes from a classical fibroblastic morphology and defined stress-fibres, to less-spread cells with numerous cell protrusions and few stress fibres. In addition, the ability of MSCs to form mature focal adhesions is significantly reduced on substrates with increased lateral spacing. Finally, we investigate differentiation and using qRT-PCR determination of gene expression levels and a quantative alkaline phosphatase assay, show that MSC osteogenesis is reduced on surfaces with increased lateral spacing, whilst adipogenic differentiation is increased.

Conclusions:
We show that lateral spacing of adhesion ligand affects MSC size, shape, cytoskeleton, ability to form focal adhesions and differentiation along the osteo and adipogenic lineages.

ORAL PRESENTATION – ABSTRACT 146
Session 4A: Stem Cells
Thursday, 16 September 2010: 1250 – 1255 hours

IN VITRO THREE DIMENSIONAL CHONDROGENESIS OF MURINE BMSCS PURIFIED BY HYPOXIA CULTURING AND MAGNETIC-ACTIVATED CELL SORTING WITH LINEAGE

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Aim:
Mouse is an ideal model to study cell biology and differentiation mechanisim of stem cells in tissue engineering. However, due to lack of appropriate methods to proliferate and purify murine bone marrow stromal cells (mBMSCs), three dimensional (3D) cartilage construction based on mBMSCs has never been accomplished. This study explored the feasibility of *in vitro* 3D chondrogenesis of mBMSCs purified by hypoxia culturing and magnetic-activated cell sorting (MACS) with lineage.

Methods & Results:
The current results demonstrated that mBMSCs cultured under hypoxia (4%) had stronger capacity of proliferation compared to normal oxygen (20%) group. Furthermore, hypoxia cultured BMSCs included a lower level of hematopoetic lineage, suggesting that hypoxia culture can purify at a certain extent mBMSCs. In vitro differentiation assays further demonstrated that hypoxia cultured BMSCs had stronger adipogenic, osteogenic, and chondrogenic potentials. In order to obtain a higher percentage of BMSCs, we further enriched mBMSCs by MACS with hematopoetic lineage markers. These enriched mBMSCs expressed higher levels of Sca-1, CD90, c-kit, and CD105 but negative for CD31, CD34, and CD45. After seeded onto PLGA scaffolds and *in vitro* chondrogenically induced for 10 weeks under hypoxia, mBMSCs formed typical cartilage-like tissue with positive expression of collagen II and chondromodulin 1. Moreover, the engineered cartilage could retain its cartilage phenotype even after 10 weeks of subcutaneous implantation.

Conclusions:
These results indicated it is feasible to engineer a 3D cartilage with purified mBMSCs by hypoxia culturing and MACS with lineage. This study provides an ideal animal model for investigating differentiation mechanism of BMSCs in the future.

ORAL PRESENTATION – ABSTRACT 165
Session 4A: Stem Cells
Thursday, 16 September 2010: 1255 – 1300 hours

COMPARISON OF DIFFERENT BIOREACTOR TO CULTURE FETAL MESENCHYMAL STEM CELLS FOR BONE TISSUE ENGINEERING

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Bioreactors provide dynamic culture conditions for efficient exchange of nutrients and the mechanical stimulus necessary for generation of effective tissue engineered bone grafts (TEBG). We have shown that biaxial rotating (BXR) bioreactor-matured human fetal mesenchymal stem cell (hfMSC) mediated-TEBG can heal rat critical sized femoral defects. However, it is not known whether optimal bioreactors exist for bone TE (BTE) applications. We systematically compared this BXR bioreactor with three most commonly used systems: Spinner Flask (SF), Perfusion and Rotating Wall Vessel (RWV) bioreactors, for their application in BTE applications.

The BXR bioreactor achieved higher levels of cellularity and confluence (1.4-2.5x, $p<0.05$) in large 785mm^3 macroporous scaffolds not achieved in the other bioreactors operating in optimal settings. BXR bioreactor treated-scaffolds experienced earlier and more robust osteogenic differentiation on von Kossa staining, ALP induction (1.2-1.6x, $p<0.01$) and calcium deposition (1.3-2.3x, $p<0.01$). We developed a MicroCT quantification method which demonstrated homogenous growth of hfMSC in BXR bioreactor-treated grafts, but not with the other three.

BXR bioreactor enabled superior cellular proliferation, spatial-distribution and osteogenic induction of hfMSC over other commonly used bioreactors. In addition, we developed and validated a non-invasive quantitative microCT-based technique for analyzing neo-tissue formation and its spatial distribution within scaffolds.

KEYNOTE ORAL PRESENTATION – ABSTRACT 411
Session 4B: Scaffold Design & Function 1
Thursday, 16 September 2010: 1130 – 1200 hours

PRINCIPLES OF SCAFFOLD DESIGN AND FUNCTION

Gilson Khang
Chonbuk National University, Korea

No abstract provided.

ORAL PRESENTATION – ABSTRACT 173
Session 4B: Scaffold Design & Function 1
Thursday, 16 September 2010: 1200 – 1215 hours

DELIVERY OF AN ANTI-INFLAMMATORY HORMONE VIA LAYER-BY-LAYER ASSEMBLY OF BIOMOLECULES

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Aim:
Many biomaterials used in tissue engineering cause inflammatory responses *in vivo,* reducing the effectiveness of tissue regeneration. Our objective was to modulate inflammation by incorporating the anti-inflammatory hormone, -melanocyte stimulating hormone (-MSH) on the biomaterial surface. Poly (lactic-*co*-glycolic) acid (PLGA) microspheres were examined as a model biomaterial for anti-inflammatory delivery.

Method:
PLGA microspheres were prepared using an oil-in-water emulsion and solvent evaporation technique. Initially, -MSH was physisorbed on the microspheres. Its bioactivity upon release was assessed using a macrophage inflammation cell assay and a subcutaneous implantation model in rats.

Multilayer assemblies of the polyelectrolytes hyaluronic acid and chitosan were used to encapsulate -MSH on the microspheres. Selected lipids were then incorporated between the -MSH and polyelectrolytes. Layer growth was assessed by quartz crystal microgravimetry and confocal microscopy. Hormone release was quantified using spectroscopy and high performance liquid chromatography.

Results:
The -MSH was bioactive and was released quickly from uncoated microspheres, showing limited effects *in vitro* and *in vivo.* To slow the release, multilayer assemblies were investigated. -MSH could not be effectively embedded within the polyelectrolytes due to its hydrophobic nature and small size. However, the addition of lipid layers improved -MSH loading and facilitated the electrostatic assembly of chitosan and hyaluronic acid. The films formed were stable with pH changes.

Conclusions:
Biomolecular multilayers can be successfully assembled on PLGA after adsorption of -MSH using a switching layer of lipids. This multilayered assembly can potentially slow the release of molecules like -MSH and be tuned to vary their release profile.

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DRUG ELUTING POLYURETHANE NANOCOMPOSITE: RELEASE AND PRELIMINARY CELL INTERACTIONS

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Aim:
Problems arising with drug eluting stents are often related to insufficient drug release or loss of the polymer coating. Nanocomposite (NC) systems have demonstrated sustained release of drugs due to presence of nanoparticles within the material. This study aims to (A) examine the influence of adding a drug on the structure and dispersion of polyurethane nanocomposites (PUNC), and (B) evaluate *in vitro* interactions of fibroblasts with drug-loaded PUNCs.

Method:
NCs were prepared using PU (Urethane Compounds, 80A), and one of two organically modified silicates (OMS): dodecylamine ($12CH_3$) and hexadecylamine ($16CH_3$). Dexamethasone acetate (DexA) was chosen as the drug. Solvent cast NCs were labeled according to %OMS and % drug. The spacing of silicates was determined by X-ray diffraction. Drug elution was determined using UV-spectrophotometry. Cell growth inhibition (CGI) was determined by a Vi-Cell viability analyser.

Results:
Improvement in dispersion by longer carbon chain modifiers was demonstrated by an increase in silicate spacing of $12CH_3$ NCs compared to $16CH_3$ NCs from 2.55 to 3.26 nm (5wt% silicate loading).

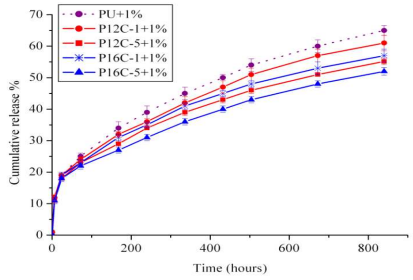

Figure 1. Cumulative release of PUNC incorporated with DexA over 5 weeks (*n*=3)

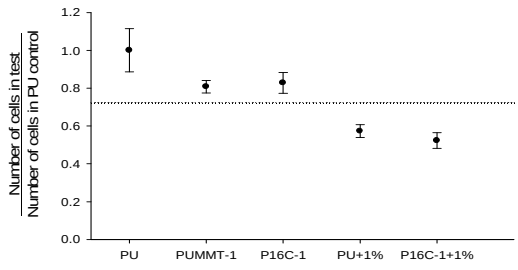

Figure 2. CGI result (*n*=3). Ratio >0.7 represents acceptable CGI

DexA release from NCs showed greater reduction as the silicate content was increased to 5wt% (Figure 1). The level of CGI suggested that the NC materials were not cytotoxic and the released DexA retained biological activity (Figure 2).

Conclusions:
Sustained release and low cell inhibition of PUNCs demonstrated attractive properties as sustained drug delivery systems.

Acknowledgement:
Research supported from the Australian Research Council (Discovery Grant DP0558561)

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APPLICATION OF NANOFIBRE MESHES FOR TREATMENT OF DIAPHRAGM HERNIA

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Aim:
Aside of basic biocompatibility, modern biomaterials are often specialized and tailored in their properties for a specific task. Nanofibres have gained interest because of their structural similarity to tissue components. However, control over their surface chemistry and thus cell adhesion has so far been limited.

Method:
We have developed a one-step preparation method for nanofibre-constructs with precise control over surface chemistry by the use of a functional prepolymer additive during electrospinning of biodegradable polyesters such as poly(D,L-lactide-*co*-glycolide) (PLGA).

Results:
The additive enriches at the surface and minimizes cell adhesion as demonstrated for human dermal fibroblasts and human mesenchymal stem cells. However, functionalization with cell-adhesion molecules such as peptides during the fibre preparation process results in specific cell adhesion due to interaction of cells with these signals [1, 2].

We present fibre preparation and characterization as well as one application of this technology for a clinical problem. Congenital diaphragm hernias are severe threats for newborns for which no adequate treatment or material approach exists. Our nanofibre technology may be developed as sustainable and resorbable air-tight mesh for such treatments.

For proof-of-principle, we have used standard hernia meshes as mechanical support. Fibres were deposited on both sides of a plasma-activated Prolene® hernia mesh (Ethicon). Constructs were implanted into 18 female Chinchilla rabbits over an artificial defect of 1 cm in diameter in the lateral left diaphragm at the interface of tendon and muscle. Animals recovered well after operation, and explantation after 3 and 6 months showed good biocompatibility and no foreign body responses.

Literature:
[1] Groll, J., Ameringer T., Spatz J.P., Möller M., Langmuir, **2005,** 21, 1991.
[2] Gasteier, P., Reska, A., Schulte, P., Offenhäuser, A., Möller, M., Groll, J., Macromol. Biosci., **2007,** 7, 1010.

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Session 4B: Scaffold Design & Function 1
Thursday, 16 September 2010: 1245 – 1300 hours

A XENOGENEIC SCAFFOLD FOR VOCAL FOLD RECONSTRUCTION: RHEOMETRIC MEASUREMENTS

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Aim:
Previous studies have shown the potential of a biodegradable, acellular xenogeneic scaffold derived from the bovine vocal fold for the repair of vocal fold injury. This study examines the impact of this scaffold on the viscoelastic properties of the vocal fold in a rabbit model.

Method:
Bovine acellular scaffolds in an injectable form were injected into the right vocal folds of six rabbits, while buffered saline was injected into the left vocal folds as sham control. At different time points (3 days, 7 days, 1 month, 2 months) following the surgery, the rabbits were humanely sacrificed and their larynges were examined. Rheometric measurements and histological examination were performed on the laryngeal sections to assess the vocal fold tissue remodeling and biomechanical changes.

Results:
Rheometric results showed that the experimental vocal folds demonstrated higher elastic shear modulus (G') and dynamic viscosity (□') than those of the sham control 7 days following the surgery. By 30 days, their G' and □' became lower than those of the sham control, whereas after 60 days the experimental and control vocal folds were similar in G' and □'.

Conclusions:
The observed viscoelastic changes of the vocal folds were likely associated with the host tissue response induced by the bovine scaffold, suggesting a positive impact on the functional biomechanical performance of the repaired vocal fold for phonation.

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Session 4C: Biomaterials for In Vitro Tissue Synthesis
Thursday, 16 September 2010: 1130 – 1200 hours

DECOUPLED CONTROL OF RIGIDITY AND PERMEABILITY OF A CELL ENCAPSULATED HYDROGEL FOR THERAPEUTIC ANGIOGENESIS

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Therapeutic angiogenesis has emerged as promising strategy to repair ischemic wounds and tissues. Various cells to endogenously express proangiogenic growth factors have been used as medicines for therapeutic angiogenesis. As the ability to sustain proangiogenic activities in the ischemic tissue is a key factor for enhancing the angiogenic therapeutic efficacy, cells were often encapsulated into a hydrogel to deliver proangiogenic factors in a local and sustained manner. However, there are several challenges that need to be resolved when using the hydrogel because of a limited viability of cells encapsulated in the hydrogels. Therefore, this talk will present a new strategy to decouple the inverse dependency between stiffness and permeability using a hydrogel of poly(ethylene glycol) dimethacrylate and methacrylic alginates [1, 2]. We will also discuss how the decoupling design improves the efficacy of encapsulated cells to stimulating neovascularization. Such biomaterial design strategy will be broadly valuable for treating other acute, chronic and malignant injuries and diseases using a wide array of bioactive molecules and cells.

References:

1. Cha, C. Kohman, R., & **Kong, H.J**. Biodegradable Polymer Cross-linker: Independent control of stiffness, toughness and degradation rate of hydrogel. *Advanced Functional Materials* 19; 3056-3062 (2009).
2. Cha, C., Kim, S., Cao, L. & **Kong, H.J.** Decoupled control of stiffness and permeability of cell-encapsulated poly(ethylene glycol) hydrogel. *Biomaterials* 31, 4864-4871 (2010).

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Session 4C: Biomaterials for In Vitro Tissue Synthesis
Thursday, 16 September 2010: 1200 – 1215 hours

SELF-ASSEMBLY OF COLLAGEN-MIMETIC PEPTIDE AMPHIPHILES INTO BIOFUNCTIONAL NANOFIBER

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Aim:

Collagen that is the most abundant protein in mammalians and the predominant component of extracellular matrices (ECMs) play an essential role in cell growth and tissue formation. Our studies focus on mimicking collagen structurally and biologically using various peptide sequences toward realizing artificial collagen-like biomaterials.

Method:

Collagen-mimetic peptides (CMPs) incorporating integrin-specific GFOGER sequence from collagen α_1 (I) have been synthesized using solid phase peptide synthesis method and been used to optimize cell adhesion, proliferation and functions. In recent study, novel collagen-mimetic peptide amphiphiles (CPAs) were prepared by conjugating single hydrophobic tail with CMPs to mimic native collagen fibrils. Circular dichroism spectroscopy and transmission electron microscopy were used to characterize physical properties of CPA whereas cell adhesion assay and immunofluorescent staining were carried out to study their biological functions.

Results & Conclusions:

Physical studies indicated that CPAs had collagen-like triple-helical conformation and were able to self-assemble into nanofiber. In addition, CPAs conjugated with integrin-specific GFOGER sequence were shown to promote collagen-like cell adhesion and development. The self-assembled peptide nanofibers had the ability to structurally and biologically mimic native collagen fibrils. Moreover, these artificial collagen fibrils were able to form hydrogel that have great potential for use as scaffolds for tissue engineering and regenerative medicine.

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CULTURE SUBSTRATES WITH IMMOBILIZED GROWTH FACTORS FOR USE IN IN VITRO EXPANSION OF HUMAN NEURAL PROGENITOR CELLS

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Aim:
Human neural progenitor cells (hNPCs) have been considered as one of the potential sources for the cell transplantation therapy of central nervous disorders and can be obtained by the standard neurosphere culture. However, there are still limitations with this method. The most critical problems may be heterogeneity of cells in a neurosphere. In addition, growth rate is limited with neurosphere culture, probably because of the presence of differentiated cells in the spheres. To overcome these limitations, we made attempts to develop culture substrates that enable to selectively expand hNPCs in adherent culture.

Method:
Epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) were fused with hexahistidine by recombinant DNA technology. These engineered growth factors were immobilized alone or in combination of two factors onto the Ni ion-bound glass surface by coordinating the terminal hexahistidine sequence. The substrates were then tested in vitro for the selective expansion of hNPCs derived from the human fetal brain.

Results:
Adhesion and proliferation of hNPCs took place most efficiently on the surface with both EGF and bFGF compared to surfaces with either factor as well as a bare glass surface. The rate of cell proliferation was more than 2-fold higher in the adherent culture on the substrate developed here than in the standard neurosphere culture. A population obtained after 5-day culture on the substrate contained nestin-expressing progenitors at a content of approximately 90%.

Conclusions:
The culture substrate with immobilized both EGF and bFGF is effective for the selective expansion of hNPCs.

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ECM SCAFFOLDS FOR THREE-DIMENSIONAL CELL CULTURE AND TISSUE ENGINEERING

Andrew CA Wan, Benjamin CU Tai, Y Shona Pek, Karthikeyan Narayanan, Chan Du, Kwong Joo Leck, Shujun Gao & Jackie Y Ying
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A major focus of our laboratory is the design and fabrication of three-dimensional (3D) scaffolds to be employed as matrices for tissue engineering. Scaffolds based on fibers that are formed by interfacial polyelectrolyte complexation (IPC) can be incorporated with extracellular matrix (ECM) and cells, while retaining the activity and viability of the encapsulated components. The influence of the relevant ECM proteins on the maintenance of primary hepatocyte function was studied in a 3D scaffold format, showing the contribution of both a 3D environment and ECM incorporation towards maintaining albumin secretion function. RGD-modified scaffolds were then used to deliver human mesenchymal stem cell (hMSC) differentiated hepatocyte-like cells to the liver in a rat partial hepatectomy model. The implanted cells were localized at the implantation site; they retained liver phenotypic markers and secreted albumin into rat sera after 2 weeks. IPC scaffolds were also shown to be effective in presenting ECM signals to differentiate stem cells. When hMSCs were cultured within scaffolds incorporated with the extracellular matrix of a preosteoblast cell line and implanted subcutaneously into nude mice, bone matrix was formed ectopically after 4 weeks. Another 3D culture system that we have developed is a thixotropic polyethylene glycol-silica gel that could accommodate the ECM secretion of cells, and which allowed the mechanical properties of the secreted ECM to be characterized.

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Thursday, 16 September 2010: 1245 – 1300 hours

SCAFFOLD FREE 3D CELL CONSTRUCTS FOR IN VITRO TISSUE SYNTHESIS

Takuya Matsumoto
Osaka University, Japan

A morphologically controlled three-dimensional (3D) cell construct composed of only cells and having no scaffold material might be a valuable biologic material for tissue engineering applications and an excellent tools for investigating tissue development. To obtain such a 3D cell construct, a 3D thermoresponsive hydrogel (pNIPAAm) was prepared as a mold material that changes its volume depending on the temperature. 3D cell constructs composed of bone marrow derived stromal cells (BMSCs) with a variety of morphologies were obtained by decreasing the surrounding temperature of the hydrogel designed with a predefined shape and formed by curing in a polymer mold manufactured via 3D printing system. The 3D cell constructs detachment resulted from a simple change in the gel volume, not by the surface chemistry of the gel, because the surface hydrophilicity of the gel was maintained over a wide temperature range. We also introduce the application of the 3D cell constructs for in vitro tissue synthesis.

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Session 4D: Neural Tissues
Thursday, 16 September 2010: 1130 – 1200 hours

REPAIRING THE BRAIN WITH BIOMATERIALS

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Repairing the brain after trauma or the effects of disease presents a number of unique challenges. In other organs, injury is followed by inflammation, a process that results in the development of a scar that is used to build a scaffold, followed by revascularisation and cell proliferation that will partially or fully restore the original tissue structure. In the case of nervous system, scaffolding is not so important, the principle cells do not regenerate and "scarring" may actually prevent vascularisation. The view is often expressed that biomaterials deployed in the brain should not engender any cellular response but this fails to acknowledge the important permissive role of astrocytes and microglia in supporting neurones and encouraging axon outgrowth. There is also very little consideration to the revascularisation of the brain, bearing in mind that in the healthy brain neurones are always coupled with blood vessels via an astrocytic intermediary.

Functionalisation of biomaterials significantly alters their effectiveness in enhancing neuronal survival and axon growth. Much more work is required to understand whether this is due to direct influence of the biomaterial on the cells or by their improved capacity to support the laying down of extracellular matrix materials.

Finally, trophic molecules facilitate the survival of neurones and directions in relation to the establishment of circuitry. Biomaterials offer the capacity to deliver these molecules to enhance or restore circuitry and connectivity.

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Session 4D: Neural Tissues
Thursday, 16 September 2010: 1200 – 1215 hours

IMPLANTATION OF POROUS PHEMA INTO THE SPINAL CORD: INNATE IMMUNITY AND IMPLICATIONS FOR NEURAL TISSUE ENGINEERING

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[2] *Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia*
[3] *Neurology Department, IDIBAPS-Clinic Hospital, Barcelona, Spain*
[4] *School of Biological Sciences, University of Southampton, Southampton, United Kingdom*

Aim:
By reducing or modifying the inhibitory extracellular environment of the spinal cord, numerous studies show that regeneration of nerve fibres within TE scaffolds occurs. The capacity for a material to permit regeneration and neurite penetration has consequently been the major focus of histological assessment for such scaffolds within TE paradigms. Furthermore, most scaffolds are examined six weeks after injury where potential insights into the inflammatory reaction are missed. In this study, comparatively short time points are used to gather insights into the innate immune reaction associated with implanting a biocompatible scaffold material into the spinal cord.

Method:
Male Wistar rats (250 to 300g) underwent a partial laminectomy at T7, followed by dura incision/removal and a coronal incision into posterior horn with iris scissors. The scaffold was then placed in the lesion (Figure 1), while controls were left with an incision only. The animals were perfused after 1, 2, 3, 6 or 14 days, and used for histology or quantitative PCR. The surgeries and PCR were performed using random and blinded experiments, respectively.

Results:
The recruitment of neutrophils into the pHEMA was rapid and after 24 hours the scaffold was filled exclusively with these cells. Migration of activated macrophages into the pHEMA was apparent after 2 days, with many of these cells seen in the highly vascularised grey matter. The molecular environment suggests a different inflammatory response. Significantly greater gene expression of TGF-b after three and six days occurs with pHEMA implants. TGF-b is recognised as an important inflammatory mediator in this injury scenario, and is known to influence the remodelling of ECM after injury.

Conclusions:
We show that TE scaffolds not only provide a substrate for regeneration – they alter the inflammatory process and wound healing response within the CNS.

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A STUDY FOR CONSTRUCTION OF THREE-DIMENSIONAL NEURAL NETWORK IN VITRO

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Aim:
Three-dimensional (3-D) culture of neural stem/progenitor cells (NSPCs) can not only provide a large space for cells to expand, but also mimic in vivo-like 3-D microenvironment and allow the NSPCs to grow into tissue-like neural network, which can be applied for drug discovery, tissue transplantation and so on. An efficient method to construct the 3-D neural network using NSPCs as cell seeds is necessary.

Method:
NSPCs isolated from embryonic day 14 (E14) rat forebrain were immobilized in three-dimensional type Ⅰ collagen gels. Initially, the collagen-cells constructs were cultured in 96-well using medium consisting of DMEM/F12/RPMI1640 (1:1:1) supplemented with N2, EGF and bGFG at 37℃ 5%CO_2. Then, the initial medium was replaced with NB/B27 supplemented with bFGF and BDNF for up to 42 days. The NSPCs marker protein nestin and sox2 were detected by FlowCellect™ Rodent NSC Characterization Kit and the neural network was observed by confocal microscopy after stained with fluorescein labeled antibody and Live/Dead Viability-Cytotoxicity Kit.

Results:
The NSPCs cultured with this medium expanded in the collagen I scaffolds in “neurosphere” growth pattern firstly and then spread in the scaffold forming neural network structure. The culture conditions led to abundant neurons with astrocytes and oligodendrocytes as supportive cells, forming synaptically linked tissue-like neural networks. Cells maintained live cell rate around 82% in neural network pattern at least for 42 days.

Conclusions:
The constructed neural networks have a potential use for drug screening, detection of environment toxins and replacement therapy.

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GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR GENE TRANSFER EXERTS A PROTECTIVE EFFECT IN SCIATIC NERVE FOLLOWING CONSTRICTION-INDUCED PERIPHERAL NERVE INJURY

Guei-Sheung Liu[1,3], Gregory J Dusting[1,2] & Ming-Hong Tai[3]
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Aim:
Damage to peripheral nerves often results in various sensory and motor abnormalities. The loss of neurotrophic factor support has been proposed to contribute to the development of peripheral neuropathy. The main objective of this research is to investigate the role of GDNF deficiency and protective effect using peripheral gene delivery in a rat model of constriction-induced peripheral nerve injury (CCI).

Method:
We began by defining chronologically the molecular events and behavioral changes that occur after inducing CCI in a rodent model of peripheral nerve injury. This was followed by evaluation of the therapeutic effect of GDNF gene delivery via intramuscular route and measuring its outcome using immunohistological and molecular methods.

Results:
It was shown that the mechanical and thermal hypersensitivity increased on the injured side at day 7 after induced CCI. These neurological changes were found to correlate to the structural changes in the sciatic nerve after injury. GDNF gene delivery was effective in the restoration of GDNF content, and hence, downstream Akt signalling pathway, an important pro-survival signal for nerves. These, in turn, prevent the loss of Schwann cells and axon fibers in peripheral nerves after constriction-injury insults. Most importantly, histological improvements were accompanied by a significant improvement in behavioural assessment.

Conclusions:
Our study showed for the first time that a single IM injection of Ad-GDNF improved the histological features of axonal damage in the sciatic nerve after CCI. GDNF and down-stream molecules hold promising potential as therapeutic targets in peripheral nerve injury.

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DESIGN OF SMART ALIPHATIC POLYESTERS WITH CONTROLLED PARALLEL-CHANNEL ARCHITECTURES FOR NERVE REPAIR

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Aim:
We present a simple and unique technique to create pliable biodegradable scaffolds composed of aliphatic polyesters with a plurality of distinct channels running parallel along the length of the scaffolds for repair and regeneration of peripheral nerve defects.

Method:
Branched poly(ε-carprolactone-*co*-D,L-lactide) macromonomers (P(CL-LA)) with various LC/LA compositions were successfully synthesized using pentaerythriol which has four hydroxyl end groups.

Results:
The crosslinked P(CL-LA)s demonstrated temperature dependent changes in elasticity over the melting temperature (T_m). *i.e.*, they are rigid below the T_m, while they become elastic above the T_m. Although pure PCL has a T_m around 60°C, the branched P(CL-LA)s shows a lower T_m around body temperature (~37°C). Injection molding of the crosslinked P(CL-LA) with silica capillary fibers as the templates resulted in producing pliable scaffolds with a plurality of distinct channels running parallel along the length of the scaffolds. The scaffolds also showed the good compatibility for cell adhesion.

Conclusions:
They could serve as the basis for investigating the effects of the geometrical and dimensional properties on axonal outgrowth and regeneration of peripheral nerve defects.

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Session 5A: Stem Cells 2
Thursday, 16 September 2010: 1400 – 1430 hours

HUMAN AMNION EPITHELIAL CELL (HAEC) TREATMENT FOR REDUCING TISSUE INFLAMMATION AND FIBROSIS AND AUMENTING REGENERATION

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Aim:
hAEC transplantation reduces inflammation and collagen in mice with lung or liver fibrosis and enhances tissue regeneration. However, the mechanisms remain unclear. We investigated effects of hAEC on inflammatory cells and collagen depositing hepatic stellate cells (HSC) to explore potential mechanisms involved in ameliorating hepatic fibrosis.

Methods:
Murine T-cells were identified by CD3/FoxP3, Kupffer Cells (KC) by F4/80 and hAEC by IMM immunohistochemistry. Thymidine uptake by T-cells measured to assess proliferation. Cytokines measured by ELISA or multiplex assays. HSC proliferation and measured apoptosis by MTT YoPro/ToPro assays, respectively.

Results:
CD3+T-cells in close proximity to IMM+hAEC were localized in livers of C57/bl6 mice. T-cell numbers did not differ between ±hAEC injected mice, but $FoxP3^+$ regulatory T-cells were elevated in mice receiving cells. hAEC did not induce T-cell proliferation in xeno-reactive assays. hAEC also suppressed mitogen-induced T-cell proliferation consistent with *in vivo* findings. Profiling secreted cytokines/chemokines, identified TGFβ, VEGF and soluble HLA-G as factors that could suppress T-cell activation. hAEC reduced KC (hepatic macrophage) numbers. MIF-1 from hAEC may inhibit KC recruitment; however MCP-1 was also secreted by hAEC. hAEC did not induce HSC proliferation, apoptosis or necrosis despite secretion of PDGF and TNFα that induce HSC proliferation and apoptosis, respectively. However, collagen deposition was reduced with co-incident reversion into quiescent HSC with reduced vimentin and α-smooth muscle actin staining following stimulation with hAEC secreted factors.

Conclusions:
Factors secreted by hAEC modulate inflammatory cell responses and reduce fibrosis. hAEC may be useful for treating liver and lung fibrosis and augmenting tissue regeneration.

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Session 5A: Stem Cells 2
Thursday, 16 September 2010: 1430 – 1500 hours

TOWARDS PLACENTA-DERIVED STEM CELL TREATMENT FOR MYOCARDIAL INFARCTION - EARLY CLINICAL AND PRE-CLINICAL EXPERIENCE

Gary Brooke[1,3], Chris Blair[2], Rebecca Pelekanos[1], Nick Timmins[3], James Hudson[3], Justin Cooper-White[3], Faten Zaibek[4], Nick Matigan[5], Alistair Chalk[5], Christine Wells[5], Michael Feneley[2] & Kerry Atkinson[1]

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Introduction:
Mesenchymal stem cells (MSC) expanded in culture (now often termed mesenchymal multipotent progenitor cells (still MSC)) are a potential cellular therapy for a broad range of medical conditions. This not only includes regenerative therapies, but also treatment of drug refractile graft-versus-host-disease. The rationale behind the latter is due to their potent immune-inhibitory properties. This property also leads to a possible further advantage – that of transplantation into an unrelated individual without the need for immunosuppression. MSC are usually generated from bone marrow aspirates. However, we have been investigating an alternative and more readily available source, the placenta. With over 3000 births at the Mater Mothers hospital alone, this tissue could potentially be an important source of MSC for clinical trials. Here we show our production process for clinical grade placental-derived MSC and a thorough analysis of these cells. We are now moving onto fully enclosed, automated systems for placental MSC manufacture. We also present some of our pre-clinical data on the use of MSC to treat acute myocardial infarction (AMI).

Materials and Methods:
Placentas were mechanically and enzymatically digested and MSC prepared as previously described[1]. MSC were phenotyped and functionally analysed using standard differentiation assays. Cells for clinical trials were from P2-P5. For gene arrays studies, placental MSC from passage 4 were compared to bone marrow derived MSC, unrestricted somatic stem cells (USSC) and skin-derived fibroblasts. Illumina gene arrays were used. Non-reperfused (permanent ligation of descending artery) myocardial infarcts were generated in mice and GFP+ transgenic MSC were injected intramyocardially at four sites around the infarction. Heart morphology and function was determined at days 3, 7 and 21 post infarction. MSC engraftment was determined by quantitative PCR.

Results:
We have established protocols for the production of MSC from placenta under GMP conditions. These MSC have been characterised both phenotypically and functionally. By gene array, we could show that placental derived MSC showed highly similar global gene expression to bone marrow derived MSC and USSC, however, each of the three populations retained a unique signature that reflected its tissue of origin. Using this system we generated approximately $3x10^9$ MSC per placenta. These placental MSC are now being used in clinical trials. With respect to introduction and other relevant research findings. Limitations of project should be included and suggestions for future research may be provided.

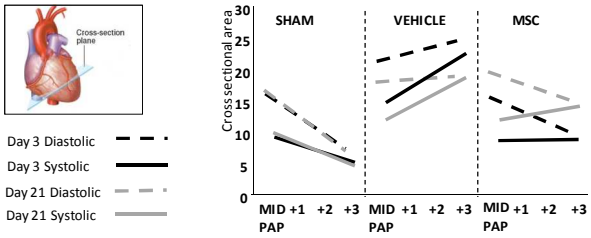

Fig. 1. Therapeutic effect of MSC on maintenance of LV dimensions and function after AMI. Sham groups had thoracotomies but no AMI. Inset, diagramatic representation of the cross sectionional virtual plane (inset) created by Visualsonics vivo 770. **A)** effect of IM MSC delivery on diastolic (dashed) and systolic (solid) LV cross-sectional area in short axis images acquired at mid papillary level, and at distal 1 mm intervals to +3 mm. MSC treated groups have reduced LV dilatation relative to vehicle controls.

Discussion and Conclusions:
MSC can be derived from placenta in a system designed for GMP standards and utilised in clinical trials. Placental derived MSC share many similarities with bone marrow-derived MSC, but are not identical and this may reflect in their therapeutic uses. MSC are excellent candidates for the treatment of AMI.

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1. Brooke, G., Tong, H., Levesque, J.P. & Atkinson, K. Molecular trafficking mechanisms of multipotent mesenchymal stem cells derived from human bone marrow and placenta. Stem Cells Dev (2008).

Funding:
Australian Stem Cell Centre, National Heart Foundation, Inner Wheel, Mater Foundation

ORAL PRESENTATION – ABSTRACT 182
Session 5A: Stem Cells 2
Thursday, 16 September 2010: 1500 – 1515 hours

THREE-DIMENSIONAL CELL ENGINEERING ENHANCES THE ANGIOGENIC EFFICACY OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

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Aim:
ECs grafted into ischemic tissue have limited angiogengic efficacy due to poor cell engraftment *in vivo*. We hypothesized that three-dimensionally engineered human umbilical vein endothelial cell (HUVEC) spheroids exhibit improved angiogenic efficacy following transplantation into mouse ischemic limbs when compared to HUVECs prepared by conventional two-dimensional monolayer culture.

Method:
HUVECs were placed in spinner flasks to generate spheroids. Hindlimb ischemia was induced in athymic mice. One day after ischemia induction surgery, trypsinized HUVECs cultured in monolayer or HUVEC spheroids were transplanted intramuscularly into ischemic limbs. 4 weeks after treatments, the physiological status, histological analysis, and immunohistochemical assays of ischemic limbs were followed.

Results:
HUVECs grafted as spheroids showed increased HIF-1α expression, reduced apoptosis and enhanced cell survival, resulting in enhanced HUVEC incorporation into the vasculature of ischemic tissue when compared to dissociated HUVECs. Transplantation of HUVEC spheroids also resulted in enhanced and prolonged secretion of paracrine factors as well as enhanced expression of factors involved in the recruitment of circulating angiogenic progenitor cells. As a result, limb survival was greatly improved by the HUVEC spheroid group.

Conclusions:
Following HUVEC transplantation into ischemic mouse hindlimbs, HUVEC spheroids showed improved cell survival, angiogenic factor secretion, neovascularization and limb survival compared to HUVECs grafted as dissociated cells. The enhanced cell survival and paracrine factor secretion was likely the result of hypoxia-adaptive signal cascades upregulated by the preconditioning of HUVECs to a hypoxic environment. Therefore, spheroid-based EC delivery could be a simple and effective strategy for improvement of the angiogenic efficacy in cells.

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Session 5A: Stem Cells 2
Thursday, 16 September 2010: 1515 – 1530 hours

FABRICATION OF JAGGED1-IMMOBILIZED SUBSTRATES FOR THE EX VIVO EXPANSION OF A CELL POPULATION CONTAINING HEMATOPOIETIC STEM CELLS

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Aim:
Notch signaling has been recognized as a key pathway to regulate the proliferation and differentiation of hematopoietic stem cells (HSC). In this study, the site-specific immobilization of a Notch ligand was designed to achieve the efficient Notch ligand-receptor binding based on the specific binding between protein A and Fc domain for the ex vivo expansion of a bone marrow cell population containing HSC.

Method:
A recombinant chimeric protein of Jagged1 and Fc domain was used for immobilization. Briefly, protein A was immobilized on N-hydroxysuccinimide ester-conjugated glass substrates. The protein A-immobilized glass substrate was exposed to the solution of Jagged1-Fc to fabricate Jagged1-immobilized substrates. As a control, Jagged1-Fc was coated non-specifically on fibronectin-coated glass substrates. Mouse lineage negative cells (Lin negative) were isolated from mouse bone marrow through the negative selection of CD3e, CD8a, NK1.1, Ly6G, and B220 and maintained in serum free X-VIVO media supplemented with stem cell factor and thrombopoietin.

Results:
Flow cytometric analyses showed that the cell population with c-kit positive, Sca-1 positive, Lin negative, and CD34 negative significantly increased on the Jagged1-immobilized substrates after 6 days of culture, whereas Jagged1-coated substrates and Jagged1-immobilized substrates with a Notch signaling inhibitor could not support the expansion.

Conclusions:
This result suggests that immobilization of Jagged1-Fc chimeric protein through a specific binding between proteinA and Fc domain enabled Jagged1 to increase the binding efficiency for the Notch receptors and consequently enhance the activation of Notch signaling, which leads to the promoted expansion of a cell population containing HSC ex vivo.

KEYNOTE ORAL PRESENTATION – ABSTRACT 405
Session 5B: Scaffold Design & Function 2
Thursday, 16 September 2010: 1400 – 1430 hours

APPLICATION OF NUMERICAL MODELLING IN THE DESIGN AND EVALUATION OF SCAFFOLDS FOR TISSUE ENGINEERING

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Aim:
The aim of this work was to show how numerical modeling could be used in developing of the proper scaffolds for the bone and cartilage tissue engineering. It was assumed that optimal scaffold must initially provide conditions for cells and new tissue growth and then decay with the kinetics adjusted to new tissue formation.

Method:
In this study, the several numerical tools were developed to design the optimal scaffolds, with honeycomb-like pattern, fully interconnected channel network, and controllable porosity, which can be fabricated using the Solid FreeForm Fabrication techniques. A novel numerical algorithm was proposed to design the scaffold with suitable stiffness/strength at each stage of new tissue formation. A new numerical method was also elaborated to simulate the biodegradation of the scaffolds in the case of complex, non-homogeneous scaffold architectures. Additionally, the mechano-regulation model was used to evaluate the developed scaffold with respect to tissue regeneration.

Results:
The results showed that application of different scaffold materials and architectures is an efficient method of tuning up scaffold properties with a significant influence on its degradation kinetics and new tissue formation. Such scaffold with complex geometry, consisted of several biodegradable materials was able to provide proper conditions for tissue regeneration.

Conclusions:
The application of numerical modeling helps in designing scaffolds for tissue engineering. The combining experiments and numerical systems might results in more efficient way to find an optimal scaffold for clinical application.

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Session 5B: Scaffold Design & Function 2
Thursday, 16 September 2010: 1430 – 1445 hours

GLASS FILLED POLYMERS FOR SCAFFOLDS FABRICATION – THE MODULATION OF CELL RESPONSE BY TAILORING FILLER CHEMISTRY

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Introduction:
It is hypothesised that with using glass as a component (filler) of polymer based medical devices it is possible to improve cells activity and trigger osteoblastic differentiation ensuring low inflammatory reaction for bone tissue regeneration devices. This improvement is a result of specific, tuneable chemistry and degradation of the filler.

Methods:
In the study PLDL (Purac) was used. Titanium doped phosphate glass powder with concentration 10, 20 and 40 vol.%, and β-tricalcium phosphate (TCP) 10 vol.% as a control filler were used. Advancing contact angle, ζ-potentials, mechanical (DMA) and thermal (DSC) properties, and human osteoblast cells responses (spreading, immunostaining – cytoskeleton, differentiation – osteocalcin, osteopontin) to the materials were evaluated.

Results & Discussion:
ζ-potentials drop, and wettability and stiffness improvement were observed with increasing glass filler loading. Cell cytoskeletons were very well developed on the samples with glass content up to 20 vol.%, and response was more favourable then observed for TCP filled samples (fig.1). Expression of osteocalcin and ostopontin was positive for all the samples including pure PLDL.
Conclusion: Improved wetting behaviour, lower ζ-potentials, and specific chemistry of the glass filled polymer enabled the modulation of cell response, and bioactivity improvement in comparison with β-TCP filler. Changes to the mechanical properties of the materials filled with the glass were less pronounced than for β-TCP filled materials. It was concluded that biochemical cues related to the glass filler composition were critical to improve cell response.

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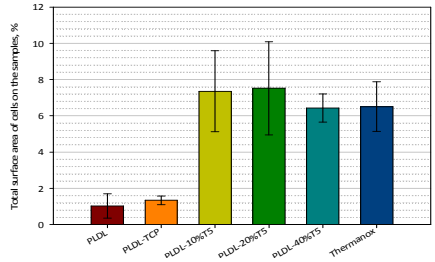

Fig. 1 *Total surface area (%) of Human primary osteoblasts growing on the surface of test samples after 3 days in culture. Cytoskeletons (average cell) of the cells grown on PLDL-TCP and PLDL-20vol%T5.*

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Session 5B: Scaffold Design & Function 2
Thursday, 16 September 2010: 1445 – 1500 hours

THE USE OF BIO-FUNCTIONALIZED ULTRATHIN POLYCAPROLACTONE MEMBRANE FOR DEVELOPMENT OF TISSUE ENGINEERED VASCULAR GRAFTS

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Anisotropic cellular structure is crucial for the maintenance of physiological function of native tissues. However, existing tissue-engineering scaffolds are typically isotropic. Cell sheet technologies provide a potential approach to address these deficiencies. Here, we describe the way to create tissue engineered vascular grafts using surface-modified biaxially-stretched polycaprolactone (μXPCL) membranes and co-culture of human endothelial progenitor cells (hEPC) and human fetal mesenchymal stem cells (hfMSC).

μXPCL membrane was fabricated from PCL pellets using bi-axial stretching technique. One side of membrane was modified with CD 34 antibody conjugation and cultured with hEPC to form the intima layer; while the other side of membrane was coated with electrospun collagen nanofibre and seeded with hfMSC as the perivascular layer. The TE vascular grafts were characterized for the viability, proliferation and functionality of hEPC and hfMSC.

Biaxial stretching processing resulted in ultrathin membranes with 10-15 micro thickness and five-fold increased mechanical strength. Modifications of CD34 conjugation resulted in improved haemocompatibility as demonstrated by the assessment of contact activation, platelet interactions and whole blood response and promoted hEPC specific cellular adhesion, rapid cellular proliferation and subsequently the formation of a layer of cells with endothelia characteristics. On the other hand, the surface coating of collagen nanofibre increased hydrophilicity with improved cellular adhesion, more rapid proliferation of hfMSC and upregulated expression of smooth muscle cell markers. Our study demonstrated the possibility to create a TE vascular graft with proper surface modifications and the co-culture of appropriate stem cells.

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Session 5B: Scaffold Design & Function 2
Thursday, 16 September 2010: 1500 – 1515 hours

ALIGNMENT OF MYOBLASTS THROUGH MICRO-SCALED TOPOGRAPHIC ENGINEERING OF ULTRATHIN, STRETCHED POLYCAPROLACTONE FILMS

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Aim:
Skeletal muscle tissue engineering provides an attractive solution to the significant tissue loss secondary to trauma or surgical ablation. However, the induction of myoblast alignment remains a significant challenge. Here, we proposed the induction of myoblast alignment through utilisation of micro-scaled topography generated in the ultrathin, stretched polycaprolactone films (S-PCL), a FDA approved bioresorbable material.

Method:
The S-PCL films were fabricated by proprietary stretching process. The S-PCL surface was treated with sodium hydroxide (NaOH) and coated with collagen prior to culturing the C2C12 cells for various timepoints. Surface characterization of the S-PCL films was done before and after NaOH treatment to ascertain that topographies have not been altered. Analyses to characterize cells in terms of its alignment, proliferation and differentiations were also performed.

Results:
Ultrathin thin S-PCL in the range of 4μm was produced successfully. From SEM and AFM images of the films prior to the seeding of cells, microchannels of width 10-20μm were observed. These micro-scaled topographies of the S-PCL films were shown to allow good cellular adhesion and proliferation with the inducement of alignment.

Conclusions:
This work showed possibility of overcoming the alignment issue with simple controlled stretching to produce microchanneles without the expensive laser processing methods. The S-PCL film can support skeletal muscle tissue engineering through a layer-by-layer approach using a bioresorbable substrate.

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Thursday, 16 September 2010: 1515 – 1530 hours

BIOMECHANICAL CHARACTERISATION OF A MICRO/MACROPOROUS POLYCAPROLACTONE TISSUE INTEGRATING VASCULAR GRAFT

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Aim:
The objective of the present study was to characterize the short-term biomechanical properties of cast micro/macroporous polycaprolcatone(PCL) tubes intended for application as tissue-integrating blood vessel substitutes.

Method:
Micro/macroporous PCL vascular grafts with defined macropore structures were produced by rapidly cooling PCL solutions containing dispersed gelatin particles in dry ice, followed by solvent and gelatin extraction. A Bose-Enduratec BioDynamic chamber configured for cardiovascular applications was used to measure the diametrical stability (dilation) of tubular samples under hydrodynamic flow conditions.

Results:
Microporous PCL tubes withstood the hydrodynamic stresses induced by short-duration flow rates up to 1000 ml/min, which resulted in estimated internal pressures in excess of arterial pressure (80-130 mm Hg). Macroporous PCL tubes accommodated the hydrodynamic stresses generated by short-duration, flow rates up to 1000 ml/min, which resulted in estimated internal pressures similar to venous pressure (30mm Hg).The dilation of microporous PCL tubes under short-term pulsatile flow conditions (1Hz) increased from 10 to 100 µm with increasing mean flow rate. Both microporous and macroporous tubes exhibited a burst strength higher than 900 mm Hg which is in excess of arterial pressure (80-130 mm Hg). Mouse fibroblasts efficiently colonized the external surface of macroporous PCL materials over 8 days in cell culture and cell numbers were higher by a factor of two compared with microporous PCL.

Conclusions:
These findings demonstrate that micro/macroporous PCL tubes designed for vascular tissue engineering can accommodate the hydrodynamic stresses generated by short-duration, simulated blood-flow conditions and exhibit good potential for integration with host tissue.

KEYNOTE ORAL PRESENTATION – ABSTRACT 392
Session 5C: Cancer Research 1
Thursday, 16 September 2010: 1400 – 1430 hours

CELL SIGNALING NETWORK LINKING OSTEOMIMICRY, OXIDATIVE STRESS AND CANCER METASTASES

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Cancer bone metastasis is lethal because it allows cancer cells to acquire increased migration, invasion and growth properties through interaction with the host microenvironments. The emphasis of this presentation will be in three areas: the appropriate models for studying the biology of cancer metastases, the limitations of animal models for clinical translation, and the novel hypotheses generated from model studies that can be further tested for therapeutic development.

In our laboratory, we have pursued the understanding of β2-microglobulin (β2-M) as a growth factor and a signaling molecule that supports lethal metastasis *in vivo* in human prostate, breast, lung and renal cancer cells. β2-M mediates this process by activating osteomimicry (i.e. the ability of prostate cancer cells to mimic the bone) and epithelial to mesenchymal transition (EMT, a fundamental process required by embryonic cells to gain the invasive and migratory potentials for organogenesis) to promote lethal bone and soft tissue metastases in host mice. β2-M interacts with its novel receptor, hemochromatosis protein (HFE), a major histocompatibility complex (MHC)-like protein to modulate iron responsive pleiotropic cell signaling pathways and the status of oxidative stress or redox balance of cancer cells. β2-M and its downstream signaling pathways are promising prognostic markers of cancer metastases and novel therapeutic targets for cancer therapy. The significance of our study is that cancer mortality and morbidity primarily result from invasion and metastasis. We demonstrate the dynamic functions of a "house-keeping" gene, β2-M, in inducing EMT in human prostate, breast, lung and renal cancer cells, and osteomimicry in prostate cancer cells; and promoting their migration, invasion and metastasis. β2-M overexpression also increased cancer lethality *in vivo*. Targeting β2-M complexing with its novel receptor, HFE, may improve the survival and morbidity of prostate cancer patients.

ORAL PRESENTATION – ABSTRACT 125
Session 5C: Cancer Research 1
Thursday, 16 September 2010: 1430 – 1445 hours

POLYELECTROLYE MULTILAYER FILMS MODULATE CELLULAR MARKERS OF ADHESION AND POLARITY IN NORMAL AND CANCEROUS PROSTATE CELLS

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Aim:
Our goal is to develop novel 3-Dimensional polyelectrolyte (PE) microstructures (microtubes) for cell culture to understand the microenvironment effect on cancer genesis by investigating *(i)* excocrine gland morphogenesis into cell differentiation, *(ii)* acini formation and disorganisation during cancer. This paper reports the influence of multilayered PE films on adhesion, proliferation and differentiation of prostatic cells.

Method:
PE films were assembled from poly(sodium 4-styrenesulfonate) (PSS) and poly(allylamine hydrochloride) (PAH) as described previously[1]. Initial attachment, adhesion and proliferation of normal and cancerous prostatic cells were studied where terminating surface charge and number of PE layers were varied. Fibronectin was a control film.

Phenotype of normal (PNT2-C2) and cancerous (PC3) prostatic cell was identified by immunostaining for structural differentiation markers including epithelial basal (cytokeratin CD44) and luminal markers (cytokeratin 18). Cellular junctions were also investigated (E-cadherin, Zo1).

Results:
Neither the PAH-terminated or PSS-terminated PE films were cytotoxic. However, while PAH-terminated PE films improved initial attachment and subsequent adhesion of both the PNT2 and PC3 cells, PSS-terminated film enhanced proliferation of the cells (at 24, 48 and 72 hours). These results are currently being exploited to optimise the 3D culture conditions on PE microspheres and tubes.

Conclusions:
These 2D PE film results guide engineering of the 3D cell culture scaffold system, which allows measurement of the functional cellular differentiation in lumen-containing tubular formats to provide new insights in the mechanisms controlling epithelial polarity and lumen formation.

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ORAL PRESENTATION – ABSTRACT 394
Session 5C: Cancer Research 1
Thursday, 16 September 2010: 1445 – 1500 hours

CALCIUM AND VITAMIN D NUTRITIONAL STATUS IMPACTS ON BONE METASTASIS IN BREAST AND PROSTATE CANCER

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Breast and prostate cancer have a high propensity to metastasise to bone with more than 70% of patients with end stage disease having bone metastases. When breast or prostate cancer cells are well established in bone they have the ability to hijack normal regulatory pathways for osteoclast and osteoblast differentiation, activation and survival to promote their own growth. At this stage they are known to induce a vicious cycle that can induce bone destruction, with or without increased bone formation, and by releasing growth factors from bone, further promote tumour growth. However this cycle can only realistically be generated when tumours reach a critical size. The initial establishment and growth of breast and prostate cancer cells in bone must be dependent on the microenvironment in bone itself, however important factors supporting early cancer establishment in bone are not well understood. In particular, the importance of the background bone remodelling activity in the early stages of breast and prostate cancer establishment in bone has not been systematically investigated. We have conducted studies in xenograft models of prostate and breast cancer growth in soft tissue and in bone to determine the effects of dietary restriction of calcium or vitamin D. In addition we have coupled these studies with pharmacologic inhibition of bone resorption using osteoprotegerin (OPG) to remove the contribution of bone resorption from observed effects. We have found that whereas breast and prostate cancer growth in soft tissue sites have not been impacted by calcium or vitamin D restriction, both of these interventions promote more rapid growth of breast cancer and prostate cancer cells within the bone. Blocking of bone resorption removes or greatly diminishes the effects of calcium restriction and vitamin D deficiency respectively. Thus nutritional status, through its effects on bone remodelling activity, may be an important influence on prostate and breast cancer progression to bone metastasis. Vitamin D deficiency and inadequate calcium intake are both common in older patients at a high risk of developing breast and prostate cancer and low vitamin D status has been associated with increased risk of cancer progression. Thus, detection and correction of nutritional deficits in calcium and vitamin D could be part of the overall strategy in the management of patients with these cancers.

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Session 5C: Cancer Research 1
Thursday, 16 September 2010: 1500 – 1515 hours

HUMAN PRIMARY OSTEOBLAST MATRIX AS MODEL TO INVESTIGATE INTERACTIONS OF BREAST CANCER CELLS WITH THE BONE MICROENVIRONMENT

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Aim:
Bone metastases frequently occur in advanced stages of breast cancer (BC). At this stage, the disease is incurable with current treatment options. So far the mechanisms leading to homing of BC cells to bone are poorly understood. This may be attributed to the lack of appropriate *in vitro* models that allow investigating the complex cancer cell-bone interactions. The aim of this study is the development and application of such a model.

Method/Results:
To mimick the bone microenvironment, human primary osteoblasts (hOBs) were cultured on *Thermanox* coverslips under conditions that promote matrix mineralisation. After five to six weeks of culture, hOBs were removed. SEM analysis indicated that this procedure yielded a dense, fibrillar matrix (OBM). Immunohistochemistry further revealed that the OBM contained calcium, osteopontin, osteonectin, osteocalcin and collagen-I, hence had a bone-like composition. Subsequently the invasive breast cancer cell lines MDA-MB-231, SUM1315, T47D and the non-tumorigenic breast cell line MCF10A were grown on OBM. Collagen-coated tissue culture plastic and standard tissue culture plastic were used as references. Proliferation assays revealed that MDA-MB-231 and T47D cells had similar proliferative capacities on all surfaces, whereas proliferation of MCF10A cells was reduced on OBM compared to references. Moreover, we found differences in cell spreading, whereupon the more aggressive BC cells displayed an aligned morphology on OBM. These cells furthermore invaded the OBM to a greater extent.

Conclusion:
Taken together, we conclude that the OBM represents a useful model system to study the complex interactions between BC cells and bone *in vitro*.

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Session 5C: Cancer Research 1
Thursday, 16 September 2010: 1515 – 1530 hours

STUDYING INTERACTIONS BETWEEN HUMAN PROSTATE CANCER CELLS AND OSTEOBLASTS USING A NOVEL 3D *IN VITRO* CO-CULTURE MODEL

Shirly Sieh[1], Amy A Lubik[1,2], Judith A Clements[1,2], Colleen C Nelson[1,2] & Dietmar W Hutmacher[1]
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[2] *Australian Prostate Cancer Research Centre - Queensland, Princess Alexandra Hospital, QLD, Australia*

Aim:
We aim to better address the complex intercellular interactions underlying prostate cancer (CaP) bone metastasis through in vitro three dimensional (3D) model. This model is used to better understand the response of CaP cells (LNCaP) to synthetic androgen in a bone microenvironment.

Method:
CaP cells were embedded in polyethelene glycol (PEG)-based hydrogels, and co-cultured with hOB in form of tissue engineered bone (TEB) constructs by 'glueing' the two constructs together using similar hydrogel components. After 26 days of culture, the co-cultures and controls (grown in absence of hOBs) were treated for two days with the synthetic androgen (R1881). Fluorescence imaging and quantitative Real-time PCR (qRT-PCR) were performed to analyse the co-cultures.

Results:
From the fluorescent images, the LNCaP colonies are in close proximity with the TEB construct, fostering a close cell-cell interaction. qRT-PCR revealed that there was an increase in PSA, FASN, VEGF, RUNX2 and TGFB1 expression in the LNCaP cells when co-cultured with hOBs compared to controls. These genes have been associated with CaP progression. Moreoever, LNCaPs treated with R1881 expressed lower level of Runx2 compared to non treated LNCaPs, implying that activation of androgen receptor suppresses the transcription of RUNX2.

Conclusions:
These findings suggest that the bone microenvironment is able to sustain growth of CaP cells and may render them more aggressive. We believe that the presented co-culture model will provide new insights into the molecular mechanisms of bone metastasis, and may ultimately offer a potential platform for drug screening.

KEYNOTE ORAL PRESENTATION – ABSTRACT 395
Session 5D: Bone 2
Thursday, 16 September 2010: 1400 – 1430 hours

PROSPECTS FOR SKELETAL REGENERATION USING INNOVATIVE SKELETAL STEM CELL BASED APPPROACHES – BRIDGING THE GAP

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The use of human stem cells, including skeletal stem cells and adult cell based therapies using appropriate scaffolds and growth factors is currently one of the most exciting and promising areas for disease treatment and reparative medicine. Skeletal stem cells (commonly referred to as Mesenchymal stem cells) or human bone marrow stromal stem cells are defined as multipotent progenitor cells with the ability to generate cartilage, bone, muscle, tendon, ligament and fat. However, to date, there remains a paucity of information both on the presence, development, activity, location, and distribution of skeletal stem cells in vivo, as well as a lack of unique skeletal stem cell markers, preventing the isolation of pure stem cell populations. Thus development of technologies to facilitate the identification and isolation of specific cell types to provide sufficient stem/progenitor cell populations for tissue regeneration as well as identification of optimal scaffolds to aid de novo tissue formation remains a significant clinical need. Cell isolation has traditionally centred on magnetically-activated and fluorescence activated cell sorting.

We have used cell sort as well as application of polymer microarray chemical approaches, to examine the efficacy of a large number of polymer blends, generated by blending different combinations and ratios of characterised polymers (binary and tertiary blends), to support selection, attachment, growth and osteogenic differentiation of human skeletal stem cells. Strategies include the use of polymer libraries comprising well characterised poly (urethanes) printed in a microarray format on aminoalkylsilane slides for selection of osteoprogenitors from human bone marrow stromal cell populations. In addition we have examined polymers provided using a combination of high-throughput chemistry for material fabrication for biological functionality from over 100 binary polymer blends. Cell-compatible biopolymers permissive for human skeletal stem cell attachment, growth and regeneration have been identified. To examine their bone regenerative potential, 3D scaffolds of polymer blends permissive for skeletal cell attachment were then seeded with human bone marrow STRO-1+ skeletal stem cells and implanted in critical-size segmental defects created in femora of immunocompromised mice. In parallel, we have examined the potential of tailored mono/dual growth factor containing polymer scaffolds to augment bone formation by providing 3D biomimetic scaffolds for skeletal cell growth, osteogenic differentiation and generation of mineralized bone tissue. Finally, translational studies aimed for clinical application, to examine the efficacy of human skeletal populations using impaction bone grafting as an exemplar, are in progress. The outcomes indicate the potential for collaborative approaches harnessing skeletal stem and progenitor cells together with innovative materials and tissue engineering approaches for de novo tissue formation. Interdisciplinary strategies across the life science and clinical science interface are set to play a vital role in the field of skeletal repair in an increasingly ageing population and offer exciting opportunities to improve the quality of life for many.

ORAL PRESENTATION – ABSTRACT 4
Session 5D: Bone 2
Thursday, 16 September 2010: 1430 – 1445 hours

CONSTRUCTION OF MICRO-NANO TWO-LEVEL 3-DIMENSIONAL FLUORESCENT CHITOSAN-HAP INTERCONNECTED NETWORKED BONE SCAFFOLD THROUGH A NANO-CRYSTALLON INDUCED BIOMINERALIZATION METHOD

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Aim:
The aim of this work is to demonstrate a method to prepare a nontoxic, high stress, intrinsic fluorescent, good biocompatibility Chitosan/ hydroxylapatite (HAp) composite scaffold with two level networked structure for bone tissue engineering application.

Method:
The nontoxic crosslinker, genipin, was used as a crosslinker to prepare chitosan framework. Through a nano-crystallon induced biomineralization method, was used to assemble HAp nanostructure on the channel wall of chitosan framework to prepare Chitosan/ hydroxylapatite composite scaffold. Rat bone marrow stem cells (rMSCs) were cultured on the scaffold to check its biocompatibility.

Results:
A two level networked structure of Chitosan/ hydroxylapatite composite obtained. The first level is of chitosan micro-prorous framework and the second level is of HAp nanostructure. The fluorescent property of the scaffold can be used for imaging of the scaffold and the cell at the same time. The HAp nano-network structure on the channel surface has influenced the morphology and cytoskeleton organization, and will further result in rMSCs lineage commitment. Most importantly, the rMSCs have a differentiation tendency to neuron.

Conclusions:
This kind of bone scaffold will have great potential applications in bone reparation.

ORAL PRESENTATION – ABSTRACT 32
Session 5D: Bone 2
Thursday, 16 September 2010: 1445 – 1500 hours

EFFECTS OF HYDROXYAPATITE NANOPARTICLE SHAPE ON MECHANICAL AND BIOLOGICAL BEHAVIOR OF NANOCOMPOSITE SCAFFOLDS

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Aim:
We aimed to develop a new class of composite biphasic calcium phosphate (BCP) scaffolds by coating a nano-composite layer, consisting of nano-hydroxyapatite (HA) particles and polycaprolactone (PCL), over the surface of BCP.

Methods:
The effects of HA particle size and shape in the coating layer on the mechanical and biological properties of the BCP scaffold were comprehensively examined.

Results:
Micro-computerized tomography (micro-CT) studies showed that the prepared scaffolds were highly porous (~91%) with an interconnected porous network (close to 100%) and large pore size (400-700 μm). The highest strength was obtained in the nano (needle shape)-composite coated scaffolds (2.1 ± 0.17MPa) , which was about twenty times higher than that of pure HA/β-TCP (0.1 ± 0.05 MPa) and about seven times higher than that of the micron HA- composite coated scaffolds (0.29 ± 0.07 MPa). Moreover, the nano-composite coated scaffolds showed an elastic behavior with a similar strain-stress profile to that of natural bone. These properties are essential for enhancing bone ingrowth in load-bearing applications. The nano-composite coated scaffolds showed superior in vitro bioactivity compared to the micron-composite and polymer coated scaffolds. Coating layers which contained needle shape HA particles and PCL showed strongest osteoblast differentiation profile with significantly higher osteogenic gene expression (Runx2, collagen type I, osteocalcin and bone sialoprotein) and alkaline phosphatase activity compared to the other groups.

Conclusion:
The developed composite scaffolds possessed superior physical, mechanical, elastic and biological properties rendering them potentially useful for bone tissue regeneration.

ORAL PRESENTATION – ABSTRACT 18
Session 5D: Bone 2
Thursday, 16 September 2010: 1500 – 1515 hours

A NEW SOL-GEL PROCESS FOR PRODUCING Na_2O-CONTAINING BIOACTIVE GLASS-CERAMICS

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Aim:
The sol-gel process of producing SiO_2-CaO bioactive glasses is well established, but problems remain with the poor mechanical properties of its amorphous form and the bioinertness of its crystalline counterpart. This may be improved by incorporating Na_2O into the bioactive glasses. However, the sol-gel production of Na_2O-containing bioactive glasses has been proved to be challenging. This work aims to develop a new sol gel process for the production of Na_2O-containing bioactive glass-ceramics, potentially for use as a medical implantation material.

Method:
45S5 (a Na_2O-containing composition) glass-ceramics have for the first time been successfully synthesized using the sol-gel technique in a water solution under ambient conditions.

Results:
A comparative study of sol-gel derived 45S5 and S70C30 (a Na_2O-free composition) materials revealed that the sol-gel derived 45S5 glass-ceramic material possesses a number of features that are desirable for biomaterials used in bone tissue engineering, including (1) the crystalline phase $Na_2Ca_2Si_3O_9$ that couples good mechanically strength with a satisfactory biodegradability, (2) formation of hydroxyapatite suggesting good bone-bonding, and (3) cytocompatibility. In contrast, the sol-gel derived S70C30 glass-ceramics contain a virtually inert crystalline phase $CaSiO_3$. Moreover, amorphous S70C30 largely transited to $CaCO_3$ with minor hydroxyapatite when immersed in simulated body fluid under the standard tissue culture conditions.

Conclusions:
sol-gel derived Na_2O-conatining glass-ceramics has advantages over other related Na_2O-free material, having an optimal combination of mechanical capability and biological absorbability.

ORAL PRESENTATION – ABSTRACT 202
Session 5D: Bone 2
Thursday, 16 September 2010: 1515 – 1530 hours

STRUCTURAL RESTORATION OF RABBIT CRITICAL-SIZED ULNA DEFECTS USING NOVEL HEPARAN SULFATES IN COMBINATION WITH β-TCP IMPLANTS

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Aim:
Improving the osteoinductive properties of synthetic bone substitutes used to treat large bone defects or fracture non-unions remains a priority. Typically, protein growth factors are used as exogenous agents to induce bone formation on these materials. Here, we present a novel heparan sulfate (HS) glycosaminoglycan sugar as an agent for the treatment of orthopedic trauma. An FDA-approved β–TCP [$Ca_3(PO_4)_2$] implant was selected as the delivery vehicle for HS.

Method:
In vivo efficacy was determined by treating bilateral 1.5 cm defects in the ulna of 20 male New Zealand White rabbits with β–TCP or β–TCP/HS combinations (at 3, 30 and 100 µg doses). Animals were sacrificed at weeks 4 and 8 post-operatively and subjected to X-ray, 3D micro-computed tomography (µ-CT), histology and mechanical testing for the assessment of bone regeneration.

Results:
Data at 4 and 8 weeks demonstrated clear evidence of accelerated new bone formation in defects treated with HS, irrespective of dose. Notably, by 8 weeks, structural restoration and complete regeneration of the cortical shell were evident following HS treatments. In all cases, treatment with β–TCP alone failed to induce bone formation. Importantly, functional integration was also enhanced, with ~two-fold increase in bone torsional stiffness between β–TCP/HS and β–TCP treatments. The mechanical properties of the defects (maximum torque: 0.6 +/- 0.1, stiffness: 0.004 +/- 0.003), however remained less than intact bone as the limited resorption of β–TCP implants impaired the infiltration of new bone.

Conclusions:
This study shows that HS is efficacious when combined with β–TCP implants for the regeneration of critical-sized bone defects and highlights the important role that HS plays in mediating tissue repair processes.

ORAL PRESENTATION – ABSTRACT 111
Session 6A: Stem Cells 3 - Adipose
Thursday, 16 September 2010: 1600 – 1615 hours

STRIKING A BALANCE: A ROLE FOR MACROPHAGES IN ADIPOSE TISSUE ENGINEERING

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Aim:
We have shown that inflammation induces adipogenesis in a tissue engineering (TE) chamber[1]. Our aim is to investigate the role of macrophages and inflammatory cytokines in adipogenesis using our *in vivo* TE model.

Method:
TE chambers were established in mice and harvested at timepoints ranging from 12hrs to 6 weeks. Contents were assayed for cytokines and transcription factors using gene expression and ELISA. Cell populations were cultured at early timepoints and identified using Immunohistochemistry. At later timepoints sections were stained for tissue components including macrophages. Chlodronate liposomes were used to kill macrophages locally in some experiments.

Results:
Macrophages were the first cells to enter the TE chambers at 12 hours post implantation. MCP-1 expression was high initially, increasing macrophage numbers and increasing inflammatory cytokine signaling with an increase in TNF-α, IL-1β and Lipocalin at day 7. These cytokines spiked again at 2 weeks when blood vessels preceded by macrophages were established in the chamber. Cell culture studies identified a population of progenitor cells expressing pref-1 or Stro-1. Local ablation of the macrophage population in the TE chamber prevented tissue formation.

Conclusions:
The macrophages that arrive first in the chamber secrete factors that induce more macrophages and progenitor cells to migrate along with angiogenesis. When the macrophages switch to an anti-inflammatory phenotype, adipogenesis is induced as measured by increases in PPARγ, FABP4 and leptin. The absence of any tissue when macrophages are removed confirms their critical roles in angiogenesis, adipogenesis and tissue engineering.

(1) Int J Obes 2008; 32 (2):239-48

ORAL PRESENTATION – ABSTRACT 114
Session 6A: Stem Cells 3 - Adipose
Thursday, 16 September 2010: 1615 – 1630 hours

SEPARATION AND DIFFERENTIATION OF ADIPOSE-DERIVED STEM CELLS BY MEMBRANE FILTRATION METHOD

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Aim:
Adipose tissue-derived stromal cells (ADSCs) can be induced into several mesenchymal cell lineages. Therefore, ADSCs are considered as a promising stem cell source for tissue engineering repair. Purification and isolation of specific mesenchymal stem cells from tissues are necessary to obtain adequate stem cells for use in clinical applications. In this study, we developed a novel isolation method to purify primary ADSCs by the membrane filtration method for rapid purification of ADSCs from adipose tissues.

Method:
The ADSC primary cell solution was permeated through the membrane (Nylon mesh and polyurethane foaming membranes) by batch-type filtration and perfusion permeation methods. After filtration of ADSC solution through the membranes, the recovery solution was permeated through the membranes to recover the adhered cells on the membranes. The cells in the permeate and recovery solutions were analyzed by flow cytometry from surface marker of MSCs (e.g., CD73 and CD90). The differentiation capability of cells separated by the membrane filtration method was evaluated by culture with induced medium of differentiation.

Results:
Polyurethane forming membranes having 12 μm could effectively separate ADSCs in the recovery solution by perfusion-type filtration method. Cells separated through polyurethane forming membranes by the perfusion method showed at least 1.5 times higher surface marker expression of MSCs and differentiation capability.

Conclusions:
We developed a novel isolation method to purify primary mADSCs by the membrane filtration method for rapid purification of ADSCs from adipose tissues.

ORAL PRESENTATION – ABSTRACT 159
Session 6A: Stem Cells 3 - Adipose
Thursday, 16 September 2010: 1630 – 1645 hours

PRESERVATION OF HEMATOPOIETIC STEM CELLS FROM UMBILICAL CORD BLOOD STORED IN A SURFACE DERIVATIZED WITH POLYMER NANOSEGMENTS

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Tissue culture flasks were prepared with immobilized amphiphilic nano-segments of Pluronic F68 and F127 - polyethylene oxide (PEO)-polypropylene oxide (PPO)-PEO triblock copolymers - on their surfaces. These so-called "Pluronic-immobilized flasks" were used for the preservation of hematopoietic stem and progenitor cells from umbilical cord blood. The expression ratio of surface markers (CD34) on hematopoietic stem and progenitor cells stored in Pluronic-immobilized flasks was significantly higher than that in polystyrene tissue culture flasks or commercially available bio-inert flasks (i.e., low cell-binding cultureware). This was due to the presence of flexible brush-like segments of Pluronic on the Pluronic-immobilized flask. A good correlation was found between the number of CD34+ cells and the ratio of viable CD34+ cells from cord blood in several flasks after five days of storage. Therefore, the high number of CD34+ cells was thought to have originated from the high viability of these cells stored in Pluronicimmobilized flasks. It was found that there was an optimal surface concentration of Pluronic on the Pluronic-immobilized flask surfaces for the preservation (high number and survival) of these stem and progenitor cells. The foregoing results were attributable to the high density of Pluronic nano-segments on the flask surface, limiting the movement of these flexible segments.

ORAL PRESENTATION – ABSTRACT 98
Session 6A: Stem Cells 3 - Adipose
Thursday, 16 September 2010: 1645 – 1700 hours

DIFFERENTIATION OF ADIPOSE DERIVED STEM CELLS INTO CONTRACTILE SMOOTH MUSCLE CELLS INDUCED BY TGF-B1 AND BMP4

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[2] *National Tissue Engineering Center of China, Shanghai, China*

Aim:
The feasibility of differentiation of human ASCs (hASCs) into cells with phenotypic and functional properties of SMCs was explored.

Method:
hASCs isolated from human lipoaspirate were expanded to passage 5 and then induced with administration of transforming growth factor-β1 (TGF-β1) and bone morphogenetic protein-4 (BMP4) either alone or in combination in culture medium. Expression of SMC-related markers including smooth muscle alpha actin (α-SMA), SM22α, calponin, and smooth muscle myosin heavy chain (SM-MHC) were detected by immunofluorescent staining, RT-PCR and Western blot analysis.

Results:
It was found that only under the circumstance of a combined stimulation with TGF-β1 and BMP4, both early and mid markers (α-SMA, SM22α, calponin) as well as a late marker (SM-MHC) of SMC differentiation were identified to similar levels as those in human umbilical artery smooth muscle cells (hUASMC). More importantly, these smooth muscle differentiated cells showed the function of contracting collagen matrix lattice when they were entrapped inside. The contractile function of differentiated hASCs was further enhanced by direct exposure to carbachol, consistent with what occurred in hUASMC.

Conclusions:
These results provide evidence that ASCs possess the potential to differentiate into contractile smooth muscle-like cells when stimulated by TGF-β1 and BMP4 together. SMCs differentiated from hASCs may provide an abundant source as seed cells for blood vessel engineering.

ORAL PRESENTATION – ABSTRACT 99
Session 6A: Stem Cells 3 - Adipose
Thursday, 16 September 2010: 1700 – 1715 hours

THE EFFECT OF IONIC PRODUCTS FROM AKERMANITE DISSOLUTION ON PROLIFERATION AND OSTEOGENIC DIFFERENTIATION OF HUMAN ADIPOSE-DERIVED STEM CELLS

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[2] *National Tissue Engineering Center of China, Shanghai, China*

Aim:
To elucidate the underlying mechanism of the effect of the extract from akermanite, containing Ca, Mg and Si ions, on proliferation and osteogenic differentiation of hASCs.

Method:
The original extract was obtained at 200 mg akermanite/ml LG-DMEM and further diluted with LG-DMEM. The final extracts were denoted as 1/2, 1/4, 1/8, 1/16, and 1/32 extracts based on the concentrations of the original extract. The LDH assay and live/dead stain were used to reveal the cytotoxicity of the different extracts on hASCs, while the MTT assay was carried out to quantitatively evaluate the proliferation of cells after being cultured with the extracts for 1, 3 and 7 days. Osteogenic differentiation of hASCs cultured in the extracts was detected by ALP expression and calcium deposition, and further confirmed by real-time PCR analysis.

Results:
It was shown that Ca, Mg and Si ions in the extract could suppress the LDH release and proliferation of hASCs, whereas promote their osteogenic differentiation. Such effects were concentration-dependent with the 1/4 extract (Ca, Mg and Si ions were 2.36, 1.11, 1.03mM, respectively) being the optimum in promoting the osteogenic differentiation of hASCs. An immediate increase in ERK was observed in cells cultured in the 1/4 extract, but no difference in JNK and P38 phosphorylation. Such osteogenic differentiation of hASCs promoted by released ions could be blocked by MEK1-specific inhibitor, PD98059.

Conclusions:
Ca, Mg and Si ions extracted from akermanite in the concentrations of 2.36, 1.11, 1.03mM, respectively, could facilitate the osteogenic differentiation of hASCs via an ERK pathway, and suppress the proliferation of hASCs without significant cytotoxicity.

ORAL PRESENTATION – ABSTRACT 62
Session 6A: Stem Cells 3 - Adipose
Thursday, 16 September 2010: 1715 – 1730 hours

CELL CYCLE ANALYSIS FOR HUMAN ADIPOSE-DERIVED SEM CELLS AT DIFFERENT CULTURE TIME

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Aim:
Human adipose-derived stem cells (hADSCs), as an attractive stem cell source for tissue regeneration, have shown their long term capability of proliferation, can be continuous cultured at least one month without passage and still maintain their stemness. During one month culturing, it is needed to know the cell growth status and cell cycle at different culture time for further research.

Method:
In this paper, we investigated the cell growth status using live and dead kit and examined the cell cycle by flow cytometry. Moreover, the expressions of cyclin D1, P27 were also detected by western blot.

Results:
Fluorescence staining showed that hADSCs cultured at different time during one month culturing had a very high survival rate, scarcely showed dead cells even at the 32nd day. It was also shown that most of cells were at G_0/G_1 phase by detection of high cyclin D1 expression and little P27 expression, which indicated there wasn't contact inhibition during the long term culture. At the same time, the percentage of cells at G_2/M phase showed a periodical changing trend, which could explain the reason why there was more than one plateau phase in growth curves of ADSCs.

Conclusions:
In summary, these results suggest that hADSCs still maintain high survival rate and vigorous proliferation capacity without contact inhibition during long term culture without passage.

ORAL PRESENTATION – ABSTRACT 97
Session 6A: Stem Cells 3 - Adipose
Thursday, 16 September 2010: 1730 – 1745 hours

A SMALL DIAMETER ELASTIC BLOOD VESSEL WALL PREPARED UNDER PULSATILE CONDITIONS FROM POLYGLYCOLIC ACID MESH AND SMOOTH MUSCLE CELLS DIFFERENTIATED FROM ADIPOSE-DERIVED STEM CELLS

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Aim:
To investigate the feasibility of constructing an elastic vessel wall in small diameter (less than 6mm) using smooth muscle cells (SMCs) differentiated from human adipose derived stem cells (hASCs) under pulsatile stimulation in a bioreactor.

Method:
The isolation and culture of human adipose-derived stem cells (hASCs) in vitro, then the differentiated hASCs were detected by immunofluorescent staining and collagen gel lattice contraction assay. Histological staining, Western blot analysis, collagen quantification and biomechanical assessment were performed to evaluate the constructed vessels. A paired t-test (Student's t-test) was performed and $p<0.05$ was considered statistically significant.

Results:
With the induction of TGF-β1 and BMP4 in combination for 7 days, hASCs were found to acquire a SMC phenotype characterized by the expression of SMC-related markers including α-SMA, calponin, and SM-MHC. The differentiated hASCs with dynamic loading were found to maintain their SMC phenotype within 3-dimensional PGA scaffold with a high level of collagen deposition close to that of native ones. More importantly, the engineered vessel under pulsatile stimulation exhibited significant improvement in biomechanical properties over that generated from static conditions.

Conclusions:
An elastic small diameter vessel wall (4 mm in diameter) with improved biomechanical strength could be engineered by *in vitro* culture of SMC-differentiated hASCs on the PGA scaffold in a blood vessel bioreactor.

ORAL PRESENTATION – ABSTRACT 101
Session 6A: Stem Cells 3 - Adipose
Thursday, 16 September 2010: 1745 – 1800 hours

EFFECTS OF STATIC MAGNETIC FIELD ON VIABILITY AND FUNCTION OF ADIPOSE-DERIVED STEM CELLS

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Aim:
The present study was to determine whether static magnetic field (SMF) affects viability, proliferation, cell-surface marker profile, cell-factor secretion, trans-differentiation, apoptotic risk, and DNA integrity of superparamagnetic-iron-oxide (SPIO)-labeled adipose-derived stem cells (ASCs).

Methods:
ASCs were divided three groups. Cells in the group 1 were loaded with SPIO and then subjected to SMF (0.6 Tesla). Cells in the group 2 were not loaded with SPIO, but still subjected to SMF. Cells in groups 3 were not loaded with SPIO nor subjected to SMF.

Results:
Following a 2-days exposure to SMF, ASCs in groups 1 and 2 had a similar viability, proliferation, cell-surface marker profile, cell factors, relative to ASCs in group 3. Efficacy of adipogenic and osteogenic differentiations were not significantly different among the three groups of ASCs.

After a 7-days exposure to SMF, ASCs in groups 1 and 2 showed a small, but significant reduction in proliferation rate, relative to group 3. Profile of cell-surface marker in groups 1 and 2 differed from that of group 3. Moreover, ASCs in groups 1 and 2 showed a significant reduction in secretion of cell factors (VEGF, HGF, and IGF-1) and adipogenic differentiation. TUNEL and Comet assays did not show an evident increase in apoptotic cells and DNA damage.

Conclusion:
Short-term (2 days) exposure to SMF does not affect viability and biological properties of both SPIO-labeled and unlabeled ASCs. Long-term (7 days) exposure to SMF significantly reduces proliferation and differentiation of ASCs and alters their phenotype.

KEYNOTE ORAL PRESENTATION – ABSTRACT 286
Session 6B: Tissue Engineering from a Clinical Point of View in the Orthopaedic Arena
Thursday, 16 September 2010: 1600 – 1630 hours

THE WIDE GAP BETWEEN BENCH AND BEDSIDE: FAILURES IN TRANSLATING RESEARCH INTO PRACTICE

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Laboratory-based research is often seen as research in its purest form. However, laboratory researchers may overestimate the proportion of their research that will make it into clinical practice, and underestimate the difficulties that can arise when attempting to achieve this goal.

Fundamentally, any laboratory research is based on a model for what is expected to happen in the clinic, or it represents a small link in a clinically important process. Consequently, flaws associated with extrapolating and applying the results can occur. This is aside from flaws based on assumptions, methods and interpretation of results that can occur with any scientific experiment. This presentation explores the reasons why laboratory research so often fails to result in benefits in the clinic, and illustrates this with examples of failures in getting research findings from the bench to the bedside.

Detailing the pitfalls in getting research into practice will highlight the areas that need to be addressed to make laboratory research more clinically useful.

KEYNOTE ORAL PRESENTATION – ABSTRACT 382
Session 6B: Tissue Engineering from a Clinical Point of View in the Orthopaedic Arena
Thursday, 16 September 2010: 1630 – 1700 hours

ATTACK ON BACK PAIN – A TISSUE ENGINEERED RESPONSE

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Chronic Back Pain that is lower back or leg pain of more than 3 month duration costs the Australian health system $1.2b in direct health related costs and nearly $8B in indirect losses including lost productivity. While non-operative treatments are the main stay of treatment, invasive surgery by way of spinal fusion and disc replacement are the last choice options. The inter-vertebral disc may not be the cause of all types of back pain, however being a viscoelastic organ and the largest moving component of a spinal motion segment it is very easily prone to wear and tear. There are no treatment or management options that either heal or repair such wear, tear and injuries to the disc. Our team at UNSW has worked in the area of tissue engineering of the intervertebral disc for the past one decade. I will review the underlying principles, some of the basic sciences involved, discuss strategies and address some of the challenges. The paper will focus on signalling molecules and cellular strategies (Biological Therapies) and not on scaffolds.

Unlike using a drug or a device, biological therapy proposes to use naturally existing or derived or mimicing substances as a formulation to either repair or regenerate tissues or to combat the detrimental effects of injury and inflammation. The biologically derived or bio-mimetic (Synthetic but similar to the natural) substances could be a gene or part of a gene, a protein or its component(s) or a cell. Over the years all three strategies have been used in laboratory conditions or in animals for healing or augmenting the function of the inter-vertebral disc.

The role of active genes is to produce proteins which could either be enzyme (functional) or other proteins that contribute to make the outside of the cell environment (extracellular matrix). The extracellular matrix proteins give the disc nucleus tissue, consisting of large proteo-glycans, its ability to hold water molecules, which in turn maintains some of the disc's load bearing properties and helps maintain annulus structural integrity. Using either viral or non viral vectors, a gene which either decrease inflammation, or increases good quality extracellular matrix or alternatively improves cellular longetivity; many a researchers have shown in preclinical studies that a favourable outcome for the disc is possible. The general feeling is that viral based gene therapy treatments will not make the regulatory hurdle to reach clinic, as the target disease condition is low back pain which is non-lethal, non-malignant and non-paralytic condition. My team has shown that using a non-viral delivery system using a gene to combat the shortening of the tip of chromosomes (telomeres) that is associated with aging, it is possible to slow down aging of disc cells and improve population doubling.

Recombinant human (rh) proteins are widely used for a range of conditions including diabetes and anaemia of chronic renal failure. rhBMP-2 and rhBMP-7 are in clinical use for augmenting spinal fusion and fracture repair. Howard An's group from Chicago has demonstrated in multiple rabbit experiments that BMP-7 can rehydrate an intervertebral disc nucleus that has been chemically degenerated and that viscoelastic properties of the disc can be restored. Unlike the rhBMP-7 (OP-1) used for spinal fusion where it is closely bound to Collagen type-1 as a carrier, pure unbound protein formulation of rhBMP-7 as a single dose treatment for disc degeneration is being evaluated in a phase II safety and dose escalation study under a FDA approved protocol as an Investigational New Drug (IND). Similarly rhBMP-14 (aka rhGDF-5) is undergoing an IND evaluation with our unit as one of the participating centres.

The rational of using the "factory" or the Cells that makes these molecules makes logical sense. One can use either cartilaginous tissue or adult bone marrow derived cells. The source of cells ideally should be from another person (allogenic) or from an animal (Xenogenic). Other sources for stem cells including fat have been used. In a randomised clinical trial Prof Meisel's team for Halle Germany has been able to demonstrate early clinical efficacy of using autologous disc cells for transplant into degenerate discs when compared to a control arm. The challenges of having the cells processed in a GMP facility and the short shelf live remains an issue along with the need for two procedures in the same patient. With the risk of teratoma formation, as cells in the disc will be forced to stay in one location for a long time, it is not felt that embryonal stem cells may ever be directly implanted.

Similar to diabetes, hypertension and obesity, the approach of managing risk factors associated with any multi-factorial chronic disease appears to be gaining strength amongst spinal health professionals for chronic back and neck pain. Degeneration of the disc may not always be a/the cause of back pain; however it is an important risk factor. Targeting the disc when symptoms are moderate while chronic, and the level of disability is low makes excellent public health sense. The immediate research challenge is to develop instruments that can discriminate and demonstrate changes in such population, as we have traditionally studied patients with more severe pain scores and severe disability. The theoretical discussion that the disc environment is a challenging one, has been successfully undermined by experimental demonstration of some of these molecules – specifically the BMP's working in that challenging environment. The recombinant human BMP's are truly leading the field of biological regeneration of the intervertebral disc.

Our group has first discovered that a mutation of the BMP13 gene is partly responsible for the congenital condition in humans were discs do not form in the neck region. We went on to demonstrate that rhBMP13 in vitro is anti-osteogenic and pro-chondrogeneic. Further, in a sheep model we demonstrated the protective role of rhBMP13 in preventing degeneration of the disc. We believe that if and when rhBMP-13 reaches a translational outcome it will be the only regenerative reparative strategy in musculoskeletal medicine based on a disease target.

KEYNOTE ORAL PRESENTATION – ABSTRACT 396
Session 6B: Tissue Engineering from a Clinical Point of View in the Orthopaedic Arena
Thursday, 16 September 2010: 1700 – 1730 hours

CHANCES AND CHALLENGES IN BONE REGENERATION – LEARNING FROM ENDOGENOUS REGENERATION PROCESSES

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For a majority of the population, musculoskeletal injury and disease represent a significant threat to quality of live and the largest health care burden. Musculoskeletal healing requires a certain amount of biomechanical stimulation as guide and support for healing to take place. A too intense stimulus or a lack of stimulus results in a delay of healing processes.

The basic understanding of the biological response to physical stimulation appears to be the key to a) characterize healing potential, b) optimize clinical treatment protocols and c) give guidelines to the design of implants. The general aim is to promote specific biological cascades while avoiding e.g. excessive inflammatory processes that could hinder regeneration healing. This concept aims at strengthening the endogenous healing cascades and supports regeneration even in cases where it would not occur naturally.

We employ stimuli that mimic the natural cascades of tissue formation by combining biotechnological approaches with conventional implant concepts. We target tissue regeneration over a number of different scales (cell, tissue, organ, extremity). In a joint effort of partners we were able to transfer these mechanisms into implant technologies for bone, cartilage and muscle regeneration. Even though such advanced therapies become now readily available, they frequently lack specificity: Little is known about the biological healing potential in patient at need. Further, most advanced therapies require rather complex treatment processes and tend to be less efficient compared to state of the art treatments. As a consequence, research planning now tries to include from early on health-economic aspects and risk assessment to allow a specific and effective design of future technologies with a faster track access towards clinical applications.

KEYNOTE ORAL PRESENTATION – ABSTRACT 397
Session 6B: Tissue Engineering from a Clinical Point of View in the Orthopaedic Arena
Thursday, 16 September 2010: 1730 – 1800 hours

REGENERATIVE APPROACHES IN OSTEOCHONDRAL, CARTILAGE AND TENDON REPAIR

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The last two decades have witnessed the development of cell-based therapy as a major biomedical research area, including the repair of musculoskeletal tissues, such as articular cartilage, tendon and ligament. Both differentiated and undifferentiated stem cells have been used as starting cell sources. In particular, the use of multipotent adult mesenchymal stem cells holds great promise for future therapeutic strategies. In addition to the cell type used, the cell delivery system is of critical importance. Cell delivery may be achieved by direct cell injection or by grafting engineered constructs derived by cell seeding into natural or synthetic biomaterial scaffolds. While direct injection is the most direct and convenient means of cell delivery, the latter approach is capable of producing three-dimensional engineered tissues with mechanical properties compatible with those of cartilage, ligament and tendon. So far, only cell-based strategies for articular cartilage repair using autologous chondrocytes (ACI) have been used routinely in the clinic. This review will focus on the current status and clinical needs of cell-based therapies for articular cartilage, ligament and tendon repair.

ORAL PRESENTATION – ABSTRACT 30
Session 6C: Cancer Research 2
Thursday, 16 September 2010: 1600 – 1615 hours

MODELING OF CANCER SPHEROIDS IN BIOENGINEERED 3D MICRO-ENVIRONMENTS

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Aim:
The objectives of our study were to develop and validate bioengineered microenvironments to culture ovarian cancer cells in a 3D platform and a computational model simulating the formation of multi-cellular spheroids.

Method:
Our 3D approach involves the use of synthetic hydrogels formed from peptide-functionalized multi-arm polyethylene glycol macromolecules via the factor XIII-catalyzed reaction. In contrast to naturally-derived matrices, the biological (e.g. incorporation of target biomolecules, like RGD integrin-binding motifs), biochemical (e.g. sensitivity to proteolytic degradation), and physical (e.g. material stiffness) properties of these biomimetic hydrogels are independently modulated. Epithelial ovarian cancer (EOC) cells were embedded within hydrogels and cultured for up to 4 weeks. Quantitative analyses were performed to determine mRNA/protein expression, survival and proliferation upon 3D culture and in presence of the chemoreagent paclitaxel. Our computational modeling approach investigated mechanical stresses associated with the growth of EOC spheroids within a bioengineered microenvironment.

Results:
EOC cells formed multi-cellular spheroids within hydrogels and revealed higher resistance towards paclitaxel than 2D monolayers. The functionalisation of hydrogels with RGD integrin-binding motifs, sensitivity to cell-secreted proteases and decreased matrix stiffness enhanced multi-cellular spheroid formation, proliferation, and survival after exposure to paclitaxel of EOC cells cultured within this 3D system. Mathematical analyses predicted how the growth of spheroids was influenced by biochemical stimuli including matrix stiffness.

Conclusions:
The design features render this biomimetic 3D concept one of the most versatile culture tools described so far to our knowledge. Bioengineered 3D matrices provide innovative avenues to explore fundamental cell-extracellular matrix interactions promoting tumorigenesis and chemoresistance.

ORAL PRESENTATION – ABSTRACT 366
Session 6C: Cancer Research 2
Thursday, 16 September 2010: 1615 – 1630 hours

TOWARDS RNAI THERAPY FOR CANCER – SOLVING THE CRITICAL ISSUES

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RNA Interference holds great promise as a therapy for viral diseases via its ability to directly kill infected cells via down-regulation of critical target genes. However, to be effective it must overcome the issues of delivery and efficacy that currently limit its clinical adoption. We have been addressing these two issues over the last few years. In vivo delivery is clearly holding up adoption with large, positively-charged and unstable siRNA molecules difficult to work with in the whole animal setting. I will present a range of delivery technologies we have been investigating including nanopatches, dendrimers, and liposomes. We have developed a fast, reliable and easy method to make siRNA-loaded liposomes for in vivo delivery and these particles are highly potent, have low toxicity and have excellent pharmacokinetic properties (Wu et al Pharm Res, 26:512-22, 2009) . We have also investigated ways to improve the efficacy of RNAi and have developed siRNAs and shRNAs that are able to not only silencing target genes but are also able to evoke innate and adaptive immune responses against targeted virally infected cells (Gu et al PNAS, 106(20):8314-19. 2009). This new class of "bifunctional" and "trifunctional" siRNAs should be much more effective at treating viral diseases via the induction of Interferon and inflammatory cytokines. Finally, we are currently screening large human siRNA libraries in an effort to identify novel cellular targets whose loss will result in the death of only virally-infected cells. This synthetic-lethal screen will yield a new class of antiviral siRNAs that target cellular genes rather than viral genes.

A number of other technologies including nanopatches, dendrimers and dendrisomes for delivery of siRNA. In terms of efficacy we have been addressing the issue of more potent siRNAs that evoke both innate (bi-functional) and adaptive (tri-functional) immune responses as well as being potent at gene silencing. Our work shows that enhancing immune responses to target genes give excellent results in animal models.

ORAL PRESENTATION – ABSTRACT 393
Session 6C: Cancer Research 2
Thursday, 16 September 2010: 1630 – 1645 hours

QUANTUM DOT-BASED PROBING OF CELL SIGNALING IN PROSTATE CANCER CELLS PREDICTS PATIENTS' SURVIVAL

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Purpose:
Conventional immunohistochemistry (IHC) color imaging coupled with pathological evaluation of tissue specimens have been the standard practice in pathology for cancer diagnosis and detection. IHC true color imaging cannot detect multiple biomarkers at a time on the same tissue slide due to the inability of unmixing the visible colors. We developed a multiplexed quantum dot-immunohistochemical (mQD-IHC) protocol using bright semiconductor QD light-emitting nanoparticles with composition-dependent tunable emission from visible to near infrared to 1) detect the expression/activation of critical cell signaling proteins at the single cell level; 2) image the plasticity of prostate cancer (PCa), such as their ability to undergo epithelial to mesenchymal transition (EMT), and 3) examine the utility of this technology in clinical PCa specimens to determine its ability to invade and to predict the metastatic capability of human PCa. Our goal is to expand the pathology from a primarily diagnostic- to prognostic-based discipline, capable of predicting the lethal progression of prostate cancer *prior to* clinical manifestation of distant metastases.

Materials and Methods:
We developed a multiplexed quantum dot-IHC (mQD-IHC) protocol in conjunction with the newly released Vectra Slide Analysis System with the inForm software by Cambridge Research & Instrumentations, Inc.. We assessed cell signaling mediators downstream from β2-Microglobulin (β2-M) that participated in the osteomimicry of human PCa cells. The co-expression or activation of β2-M (cytosolic β2M), phosphorylated cyclic AMP responsive element binding protein (nuclear pCREB) and androgen receptor (nuclear AR), assigned as a triple-positive, was examined in 10 prostate cancer specimens from patients with known metastatic status. The same cellular signaling network components in 11 PCa bone metastatic specimens were also examined.

Results:
The overall median % of triple positive for β2-M-pCREB-androgen receptor cells was 51.5%. When stratified by metastasis, there is a significant difference in the % of triple positive for the samples with metastatic potential (median = 61%) vs. those without metastatic potential (median = 0%); p=0.01 by a *Wilcoxon rank sum test*. The results were confirmed in 11 PCa bone metastatic specimens.

Conclusion:
Additional tissue specimens with confirmed survival data will be analyzed and the cell-signaling-network-based mQD-IHC will be automated by Vectra System in a high throughput manner. By defining the expression/activation of the β2-M/p-CREB and AR cell signaling components using the new mQD-IHC detection and image analysis protocol, one could expand the pathology from a primarily diagnostic- to prognostic-based discipline, capable of predicting the lethal progression of prostate cancer *prior to* clinical manifestation of distant metastases.

ORAL PRESENTATION – ABSTRACT 398
Session 6C: Cancer Research 2
Thursday, 16 September 2010: 1645 – 1700 hours

INCLUSION OF INDUCTIVE STROMAL CELLS ENHANCES THE EFFICIENCY OF XENOGRAFT MODELS OF HUMAN LOCALISED PROSTATE CANCER IN MICE

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Prostate cancer (PCa) research has been hindered by the inability to reliably xenograft localised human prostate cancer. Previous studies have demonstrated survival of these tumours *in vivo*, however the host mouse environment fails to facilitate active growth and proliferation of human prostate tissues, significantly reducing the utility of this model. We hypothesised that providing an inductive stromal niche to localised PCa xenografts will enhance survival and growth of human cancer cells in mice because it would enhance androgen-receptor mediated signalling. To test this hypothesis, we grafted localised PCa specimens into the kidney capsule site into adult NOD-SCID male host mice supplemented with testosterone, with supportive embryonic mouse prostatic mesenchyme. Our results demonstrated that providing an inductive stromal microenvironment enhanced the survival and proliferation of human prostate cancer xenografts. Specifically, this was induced by increased the vascularity of the tumours and enhanced the stromal androgen receptor signalling. Importantly, the Gleason Grade of the original patient specimen was maintained in the xenografted tissue even in the presence of mouse stromal cells. Our data showed that providing the human PCa xenografts with an inductive stromal niche significantly increased the efficiency and reliability of the model system. This significant advance will lead to a greater understanding of the factors that influence PCa progression, and could be utilised as a translatable pre-clinical model or allow personalised medicine for men with PCa.

ORAL PRESENTATION – ABSTRACT 190
Session 6C: Cancer Research 2
Thursday, 16 September 2010: 1700 – 1715 hours

PURIFICATION AND CHARACTERIZATION OF CANCER STEM CELLS IN COLON CANCER CELLS TREATED BY ANTI-CANCER DRUGS

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Aim:
Colorectal carcinoma is the second leading cause of cancer death in the world. A subpopulation of tumor cells, termed caner stem cells (CSCs) are able to initiate tumor growth. The characterization of CSCs will help to devise novel diagnostic and therapy procedure. We investigated the population of $CD133^{+}$ (identified as CSCs) in human colon cancer cells (LoVo) treated with chemotherapy drugs cultivated with and without serum, and investigated the pluripotent gene expression (Oct4, Nanog, Sox2) of the cells.

Method:
LoVo cells were treated with several anti-cancer drugs (Aspirin, 5-FU, Oxaliplatin, Cisplatin, Mayoclinic, RoswellPark, FLOX) in serum and serum-free conditions. The concentration of CEA (carcinoembryonic antigen) in the sample media was analyzed every two days. CD133 ratio of LoVo cells was analyzed using flow cytometry. Pluripotent gene expression of LoVo cells was analyzed by RT-PCR method.

Results:
Cells treated with anti-cancer drugs changed to be sphere-like morphologies. The specific CEA productivity secreated by cells increased with the increase in anti-cancer drug concentration. The ratio of $CD133^{+}$ cells decreased with drug concentration increased in serum and serum-free conditions. Cells expressing $CD133^{++}$ (high expression of CD133 cells) increased with the drug concentration in serum condition, but no $CD133^{++}$ expressed cells were observed in serum-free condition.

Conclusions:
Colon cancer cells treated with anti-cancer drugs in both serum and serum-free condition secreated higher production of CEA per cell. $CD133^{++}$ cells were only found in serum condition. The $CD133^{+}$/$CD133^{++}$ expression analyzed by flow cytometry suggested that $CD133^{++}$ cells have smaller size and should be CSCs.

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Thursday, 16 September 2010: 1715 – 1730 hours

ESTABLISHMENT OF A HUMAN BONE ENGINEERED PLATFORM MODEL TO INVESTIGATE PROSTATE CANCER-BONE INTERACTION IN NOD/SCID MICE

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Aim:
Prostate cancer (PC) has a predilection to form bone metastases. Several *in vivo* models therefore have been developed to investigate PC bone interactions. The standard human bone chip model in mice is limited by donated tissues variability and poor vascularisation, which we aim to overcome by using Tissue Engineered Human Bone Construct (hTEBC)*.

Method:
Polymer scaffolds were seeded with human osteoblasts and wrapped with osteoblast sheets (hTEBC). hTEBC and human bone chips from proximal tibia were implanted subcutaneously in the back of male NOD/SCID mice. 7 or 12 weeks after implantation human PC cells (PC3 and LNCaP) were injected directly into the implants. 8 weeks later mice were euthanized and X-ray images were taken. Tissues of interest were excised, and analysed using Micro-computed tomography, histology, histomorphometry and immunohistochemistry.

Results:
We found a 100% success rate of tumour formation for both hTEBCs and bone chips injected with LNCaP cells at 7 weeks. The frequency of tumour formation was lower for PC3 cells, with 78% and 67% in bone chips and hTEBCs, respectively. For both cell types, tumour volume was significantly higher using hTEBCs compared to bone chips. Advantageously hTEBC tumours were more symmetrical distributed around the implant compared to bone chips. PC3 tumours were light-coloured while LNCaP tumours had a dark red appearance, indicating high angiogenesis.

Conclusion:
Our novel *in vivo* model to study the PC bone-interaction is efficient in forming tumours and may be a valuable alternative to the bone chip model. Future studies will further validate this model.

*Zhou, Y *et al.* (2007) Biomaterial 28, 814-24

KEYNOTE ORAL PRESENTATION – ABSTRACT 361
Session 6D: Bone 3
Thursday, 16 September 2010: 1600 – 1630 hours

TAMING THE FRONTIERS: ADVANCES IN SURFACE MICROENVIRONMENTS FOR REGULATING CELL AND TISSUE GROWTH

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In cell therapies, control of transplanted-cell fate remains a significant challenge. Material-based transplantation systems offer a promising means to control cell fate for regeneration of functional tissue. My seminar will focus on recent developments in 3D scaffolds and nanostructured surfaces that provide a mean to control cell proliferation and differentiation.

As for biomaterials, I will focus on polyelectrolytes and 3D micropatterning. Then, I will give recent examples of published work showing how micro and nanodevices spatially control 3D cell cultures. At the end, I will go further showing the promising supply of microfluidics and lab-on-chip devices. Whether for tissue engineering or for drug screening, cell biologists have started to explore 3D cell culture approaches. This symposium focuses on recent developments in 3-dimensional scaffolds and the nanostructuring of the surfaces for those scaffolds.

The microenvironment, and substrate nanostructure in particular, provide an extrinsic influence on the cellular mechanisms that control proliferation and differentiation. For cell and tissue engineering it is important to "tame that frontier" and to utilise such nanostructuring advances in the microenvironment to (i) create appropriate assay systems to develop therapies and (ii) create appropriate scaffolds for implants.

The CEA in France is one of the largest centres in Europe for nanoscience research and has fostered the development of many novel lab-on-chip applications. Main activities of the CEA will be overviewed with a focus on the Biochip lab activities and the use of microtechnologies to analyse the part of microenvironment factors in carcinogenesis and more particularly in the control of proliferation/differentiation balance.

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Session 6D: Bone 3
Thursday, 16 September 2010: 1630 – 1700 hours

3-DIMENSIONAL STRUCTURES FOR MEASURING CELL RESPONSES

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Aim:
The microenvironment, and substrate nanostructure in particular, provide an extrinsic influence on the cellular mechanisms that control proliferation and differentiation. For developing appropriate surfaces and scaffolds for cell and tissue engineering it is important to create appropriate assay systems to measure cellular responses to the microenvironment. A significant cellular response is the mechanical attachment to the underlying substrate or scaffold and 3-dimensional assay systems offer advantages in simulating the natural microenvironment for cells. We describe a 3-dimensional scaffold system to assay the mechanical responses of cells.

Method:
Hollow tubular scaffolds were constructed from polyelectrolytes. Cells (e.g. 3T3-L1) were grown to cover the tubular scaffolds. Cellular function was altered by exposure to forskolin and cytochalasin D, and the resultant change in the mechanical response of the cells was assayed from the deformation of the tubular structure.

Results:
Confocal and electron microscopy showed that the cells attached to the tubular structures. The actin cytoskeleton was disrupted by the addition of either forskolin or cytochalasin D, which was confirmed by confocal microscopy. Those compounds also reduced the mechanical force applied by the cells to the tubular structures.

Conclusions:
The 3-dimensional tubular structures provided a convenient real-time assay of the effect of compounds that targeted the disruption of the cytoskeleton. This assay system is adaptable to measuring the influence of other intracellular processes that have an endpoint to alter a physical response of cells.

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Session 6D: Bone 3
Thursday, 16 September 2010: 1700 – 1715 hours

BIOMIMETIC COMPOSITE COATING ON POLY (ε-CAPROLACTONE) AND SILANE MODIFIED TRICALCIUM PHOSPHATE SCAFFOLDS FOR BONE TISSUE ENGINEERING

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Aim:
This study aimed to demonstrate the feasibility of carbonated hydroxyapatite (CHA)-gelatin composite coating on poly (ε-caprolactone)/silane modified tricalcium phosphate (PCL/TCP(si)) scaffolds for bone tissue engineering.

Method:
To enhance mechanical property of the PCL/TCP scaffolds, TCP was modified with 3-glycidoxypropyl-trimethoxyasilane (GPTMS). TCP was then incorporated into PCL via a homogenizing process and the scaffolds were fabricated through a screw extrusion system (SES). To enhance osteogenic properties of the fabricated scaffolds, CHA-gelatin was coated onto the scaffolds through a developed biomimetic co-precipitation process. In vitro studies were performed using bone marrow stromal cells (BMSCs) to assess cell-scaffold interaction, proliferation and bone related gene and protein expressions.

Results:
The GPTMS modification of TCP and CHA-gelatin composite coating on the scaffolds were successfully carried out as confirmed by both XPS and FTIR. Compression tests revealed a 342% compressive stiffness increment of PCL/TCP(si) scaffolds compared to PCL/TCP scaffolds. After 7 days, cellular distribution was found to be most uniform on CHA-gelatin composite coated PCL/TCP(si) compared to all other scaffolds as seen in confocal microscopy and SEM. After 10 days, cell numbers were 1.82, 1.96 and 1.4 times higher on CHA-gelatin composite coated PCL/TCP(si) scaffolds compared to PCL/TCP, PCL/TCP(si) and CHA coated PCL/TCP(si) scaffolds, respectively. Furthermore, RT-PCR and Western blot analyses revealed that CHA-gelatin composite coated PCL/TCP(si) scaffolds stimulate osteogenic differentiation of BMSCs the most compared to the other scaffolds.

Conclusion:
These results demonstrate that the two way modified CHA-gelatin composite coated PCL/TCP(si) scaffolds with significantly improved structural and functional performance could be a potential candidate for bone tissue engineering.

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Thursday, 16 September 2010: 1715 – 1730 hours

GLYCOSAMINOGLYCAN HYDROGELS FOR SUSTAINED DELIVERY OF BONE MORPHOGENETIC PROTEIN-2

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Aim:
Bone morphogenetic protein-2 (BMP-2) is a potent osteoinductive factor however its clinical use is limited by a short biological half-life, localized action and rapid local clearance. Heparin (HP), a highly sulfated glycosaminoglycan (GAG) has suitable properties to address these limitations. HP binds and sequesters BMP-2 and protects it from proteolysis. In this study, we co-crosslink a small amount of native or chemically modified HP into a hyaluronan (HA) hydrogel to investigate the suitability of these hydrogels to deliver BMP-2.

Method:
We used thiol-modified hyaluronan (Glycosil™) to form Glycosil hydrogels. In addition, 0.3% of thiol-modified HP was mixed with Glycosil™ to fabricate Heprasil™ hydrogels while 0.3% unmodified HP was mixed with Glycosil™ to form Glycosil-HP hydrogels. BMP-2 was incorporated into these macromonomers before co-crosslinking the thiol components with poly (ethylene glycol) diacrylate. We studied the release of BMP-2 and evaluated its bioactivity *in vitro* by determining alkaline phosphatase (ALP) activity and phosphorylation of SMAD 1/5/8. The bone-forming efficacy of different hydrogels was tested in rat intramuscular sites and evaluated at 4 and 8 weeks via 2D radiography, 3D μ-CT and histological analyses.

Results:
BMP-2 release from the hydrogels was less than 35% over a period of 35 days. Incorporation of HP substantially controlled BMP-2 release, in contrast to hydrogels without HP. Hydrogels sustained the bioactivity of BMP-2 as demonstrated by enhanced ALP activity and SMAD 1/5/8 phosphorylation. In the *in vivo* model, regions of mineralization were apparent as early as 4 weeks post-implantation for hydrogels incorporated with BMP-2. Samples harvested at week 8 also showed significant amounts of new bone in all hydrogels containing BMP-2. Notably, Heprasil, Glycosil and Glycosil-HP treatments without BMP-2 only resulted in soft tissue formation.

Conclusions:
Heprasil and Glycosil hydrogels show promise as delivery systems for BMP-2 and this strategy needs to be tested further in relevant clinical models.

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Thursday, 16 September 2010: 1730 – 1735 hours

NANOFIBROUS PLGA SCAFFOLDS LOADED WITH NANODIAMOND AS SUBSTRATES FOR ADHESION AND GROWTH OF HUMAN BONE-DERIVED CELLS

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Aim:
Electrospinning is a promising technique for creating advanced nanostructured biomaterials for tissue engineering. In this study, composite nanofibrous membranes containing a copolymer of L-lactide and glycolide (PLGA) and diamond nanoparticles were fabricated and used as substrates for the adhesion and growth of human osteoblast-like MG 63 cells.

Method:
For electrospinning, PLGA was dissolved in a mixture of methylene chloride and dimethyl formamide (2:3) at a concentration of 2.3 wt.%, and the nanodiamond powder was added at a concentration of 0.7 wt.% (after evaporation of the solvents, this concentration in pure PLGA reached about 23 wt.%). The composite nanofibrous membranes were then prepared using Nanospider™ equipment (Elmarco Ltd., Czech Republic), seeded with MG 63 cells and incubated in a medium DMEM with 10% of fetal bovine serum.

Results:
Both PLGA and PLGA-nanodiamond scaffolds enabled attachment, spreading and subsequent proliferation of MG 63 cells, although the cell numbers on nanodiamond-containing meshes, obtained on days 1, 3 and 7 after seeding (from 11,300±1,600 to 200,300±10,300 cells/cm^2) were significantly lower than the values on cells on pure PLGA meshes (from 48,500±4,000 to 268,600±12,200 cells/cm^2). This cell behaviour might be attributed to the presence of bigger and more numerous material clusters in the composite PLGA-nanodiamond meshes. Nevertheless, the cell spreading area on PLGA-ND membranes (428 ± 19 μm^2) was similar to that on pure PLGA meshes (475 ± 14 μm^2).

Conclusions:
Composite PLGA-ND meshes gave relatively good support for the adhesion and growth of human bone-derived cells. Thus, after some improvements, this material has potential for the use in bone tissue engineering.

Supported by the Acad. Sci. CR (Grants No. KAN400480701 and IAAX00100902).

ORAL PRESENTATION – ABSTRACT 302
Session 6D: Bone 3
Thursday, 16 September 2010: 1735 – 1740 hours

BENCHMARKING AUTOLOGOUS VS. ALLOGENIC MESENCHYMAL PROGENITOR CELLS FOR THE RECONSTRUCTION OF CRITICAL-SIZED SEGMENTAL TIBIAL BONE DEFECTS IN A LARGE ANIMAL MODEL

Arne Bemer[1], Johannes Reichert[1], Siamak Saifzadeh[1], Michael Schuetz[1] & Dietmar Hutmacher[1]
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Aim:
The reconstruction of large bone defects remains a major challenge for orthopaedic surgeons and among the different bone engineering concepts, scaffold/cell-based strategies are a common approach. Mesenchymal progenitor cells (MPCs) represent an attractive cell population for the concept of bone engineering. Their special immunological characteristics suggest that MPCs could be used in a allogenic application. In our study, we assessed the regenerative potential of autologous vs. allogenic MPCs in an ovine critical sized segmental defect model.

Methods:
Ovine MPCs were isolated from bone marrow aspirates, expanded and cultured with osteogenic media for two weeks before implantation. Cells were seeded onto medical grade polycaprolactone-tricalcium phosphate (mPCL-TCP) scaffolds and fixed with platelet riched plasma (PRP). Autologous and allogenic transplantation was performed by using the cell seeded scaffolds (n=8). Unloaded scaffolds with PRP served as a control. Bone healing was assessed twelve weeks after surgery by radiology, micro computed tomography, biomechanical testing and histology.

Results:
No local or systemic rejection was observed after transplantation of allogenic cells. Radiology, biomechanical testing and histology showed no significant difference in the bone formation of the autologous vs. allogenic group. Both cell-groups showed more bone formation than the scaffold with PRP alone.

Conclusion:
Allogenic transplantation of mesenchymal progenitor cells leads to statistically similar amount of bone regeneration.

ORAL PRESENTATION – ABSTRACT 157
Session 6D: Bone 3
Thursday, 16 September 2010: 1740 – 1745 hours

DRUG RELEASE FROM A MACROPOROUS 25HEMA HYDROGEL UNDER RHEOLOGICAL STIMULATIONS

Chao Li, Kah Hoong Eric Low & Xia Lou
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Aim:
The study aims to investigate the effects of rheological stimulations on controlled drug delivery from a macroporous poly (2-hydroxyethyl methacrylate) (PHEMA) hydrogel (25HEMA).

Method:
Methylene blue (MB) is used as a model drug. Controlled dynamic stimulations were applied to MB-loaded 25HEMA hydrogel using a HAAKE MARS II Rheometer (Fig. 1).

Results:
Figure 2 shows the release of MB from 25HEMA hydrogel under oscillatory stimulations (f=1Hz, γ=0.5, 5%). A burst release during the first hour was observed, followed by a continuous slow release. At 0.5% shear strain, 25HEMA hydrogel achieved 35% relative release after 7 hours and the percentage was increased to 42% when the shear strain was changed to 5%. The initial release was attributed to the eluting of MB through edge and boundary area of the hydrogel. The quicker release in the later stage under 5% was attributed to their larger recoverable and longer distance forced flow in comparison to that from 0.5% shear strain. Increasing frequency from 1Hz to 15 Hz had also increased the amounts of drug released (Fig. 3).

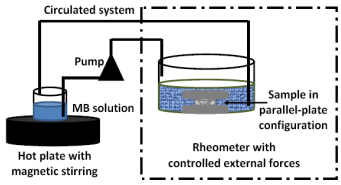

Figure 1 Schematic illustration of the set-up for drug delivery study under rheological stimulations

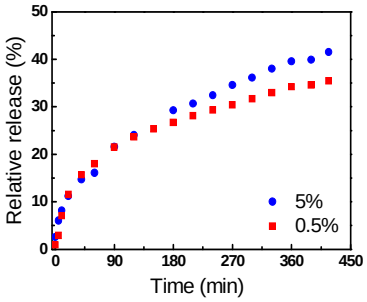

Figure 2 Effect of shear strains (0.5% and 5%) on relative MB release

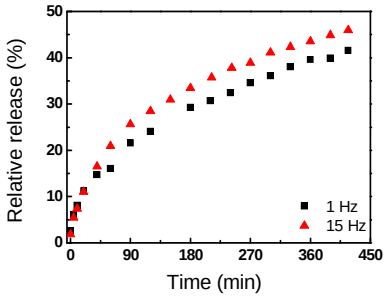

Figure 3 Effect of frequency levels (1Hz and 15Hz) on relative MB release

Conclusions:
Macroporous 25HEMA hydrogel was used to delivery MB under the influence of rheological stimulations. It was found that increasing shear strain and frequency accelerated the release of MB from the macroporous 25HEMA hydrogel.

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Session 6D: Bone 3
Thursday, 16 September 2010: 1745 – 1750 hours

RHEOLOGICAL AND STRESS RELAXATION PROPERTIES OF PHEMA AND PHEMA-TiO_2 COMPOSITE HYDROGELS

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Aim:
The study aims to characterize the viscoelastic properties of poly (2-hydroxyethyl methacrylate) (PHEMA) and PHEMA-TiO_2 hydrogels. The effect of shear stresses on the stress relaxation behavior of PHEMA and PHEMA-TiO_2 hydrogels was also investigated.

Method:
The viscoelastic and stress relaxation properties were examined using a HAAKE MARS II Rheometer (HAAkE, Germany). The geometry is demonstrated in Figure 1.

Results:
The complex modulus, $|G^*|$ is dependent on the HEMA contents in the hydrogels (Fig.2). The influence of TiO_2 nanoparticles on viscoelastic properties of the hydrogels was less significant in comparison to that of HEMA contents. The difference of $|G^*|$ was due to the changes of morphological structure of the hydrogels.

A rapid recovery was found in the hydrogels after the stress was released (Fig.3). The hydrogels containing a high HEMA content presented higher shear stress resistance, attributed to their less porous structure. 40HEMA and 40HEMA-7.5TiO_2 hydrogels can completely recover at a stress of 250Pa, while the permanent deformation was noticed for both 30HEMA (2%), and 30HEMA-7.5TiO_2 (3%) hydrogels.

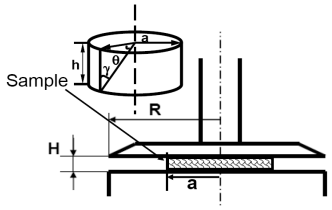

Figure 1 Experimental set-up for rheological test

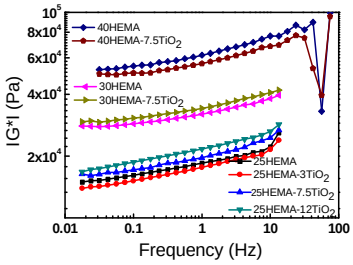

Figure 2 The complex modulus $|G^*|$ of the hydrogels

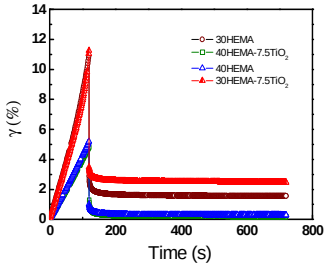

Figure3 Creep-recovery of hydrogels at τ=250Pa

Conclusions:
The HEMA contents have shown a great impact on $|G^*|$ values and the stress resistance. TiO_2 nanoparticles addition had little effect on the $|G^*|$, but it caused more permanent deformation for the hydrogel at a same stress. Our findings suggest that the dynamic mechanical responses of PHEMA hydrogels can be optimized by altering the composition of the materials.

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Thursday, 16 September 2010: 1750 – 1755 hours

A MULTILAYERED POLYMERIC-POLYELECTROLYTE MICROCARRIER SYSTEM POTENTIATED BMP-2 ACTIVITIES IN A RAT MODEL OF POSTEROLATERAL SPINAL FUSION

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[1] *Yong Loo Lin School of Medicine, National University of Singapore, Singapore*
[2] *Division of Bioengineering, National University of Singapore, Singapore*

Despite overwhelming evidence underlining the osteoinductive potency of bone morphogenetic protein 2 (BMP-2), large doses continue to be required with approved collagen carriers in contemporary clinical applications. Here we describe a collagen-free, multilayered, polymeric-polyelectrolyte microbead system incorporating heparin as a carrier for low dose rhBMP-2.

Sodium alginate microbeads were coated alternately with 0.5% poly(allylamine) and 0.1% heparin (4-layers) followed by rhBMP-2 adsorption (1μg). Beads were subsequently loaded into porous PCL/TCP scaffolds and implanted posterolaterally at L4-L5 lumbar spinal segments in SD rats (n=8). Results were compared with collagen coated PCL/TCP (positive control) and uncoated alginate beads (negative control).

Confocal microscopy, histochemistry and molecular methods demonstrated rhBMP-2 tethering unto heparin which modulated its release kinetics and *in vitro* bioactivity. Plain x-ray, micro-CT, histology and fluorochrome bone labeling revealed new bone formation in animals implanted with coated-beads and collagen carriers while uncoated-beads failed to induce new bone formation. Additionally, bone volume and biomechanical stability were significantly higher in coated-beads group compared with collagen carriers ($p<0.05$).

In summary, this microcarrier system successfully amplified rhBMP-2 bioactivity *in vitro* and *in vivo*. The relative ease of ionic binding between heparin (a polyanaion) and poly(allylamine) (a polycation) eliminates the need for organic solvents. The carrier design also ensures that rhBMP-2 is tethered to the surface of the microbeads and readily available to the cells rather than encapsulated/confined within alginate capsules.

These results indicate that the alginate microbead system could further be developed as efficient, collagen-free, alternative vehicle for BMP-2 in spine fusion and bone defect healing.

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Session 6D: Bone 3
Thursday, 16 September 2010: 1755 – 1800 hours

THE COMBINATION OF STROMAL CELL-DERIVED FACTOR-1 AND BONE MORPHOGENETIC PROTEIN-2 FOR BONE TISSUE ENGINEERING

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Aim:
The aim of this study is to investigate the osteogenic properties of combined stromal cell-derived factor-1 (SDF-1) and bone morphogenetic protein-2 (BMP-2) for bone tissue engineering.

Method:
Expression of CXCR4 (a cell-surface receptor of SDF-1) and runx2 (early marker of osteogenesis) genes in rat MSC treated with SDF-1, BMP-2, and mixed SDF-1/BMP-2 was evaluated by real time RT-PCR. Then, SDF-1, BMP-2, and mixed SDF-1/BMP-2 were incorporated into gelatin hydrogel as the release carrier, and cultured with MSC *in vitro* in an osteogenic medium to assess the CXCR4 and runx2 expression. The gelatin hydrogel incorporating SDF-1, BMP-2, and mixed SDF-1/BMP-2 was further studied *in vivo* in a critical ulna defect of Wistar rat to observe bone regeneration.

Results:
MSC treated with BMP-2 and mixed SDF-1/BMP-2 showed up-regulated CXCR4 expression while those treated with SDF-1 expressed CXCR4 gene as equally as non-treated cells. This indicates that BMP-2 and SDF-1/BMP-2 can activate cell recruitment via a receptor of SDF-1. Up-regulation of runx2 gene was also found in MSC treated with BMP-2 and SDF-1/BMP-2. The similar result of MSC cultured in the gelatin hydrogel incorporating SDF-1, BMP-2, and mixed SDF-1/BMP-2 was also observed. In addition, the gelatin hydrogel incorporating mixed SDF-1/BMP-2 showed a significantly higher runx2 expression of MSC and bone regeneration 4 weeks post-operatively, compared with that incorporating either SDF-1 or BMP-2.

Conclusions:
The combination of SDF-1/BMP-2 had a novel osteoinductive activity for bone tissue engineering.

KEYNOTE ORAL PRESENTATION – ABSTRACT 399
Session 7A: Craniofacial 1
Friday, 17 September 2010: 0900 – 0930 hours

REGENERATIVE MEDICINE TO ADDRESS CONVENTIONAL TREATMENT LIMITATIONS OF CRANIOMAXILLOFACIAL BATTLE INJURIES

Robert G Hale
US Army Dental Trauma Research Detachment, Taxes, USA

This presentation will focus on currently uncorrectable craniomaxillofacial (CMF) battle injuries (BI) seen in Iraq and Afghanistan. Open wounds and facial fractures predominate, which are often further complicated by avulsions and burns: A study analyzed Joint Theatre Trauma Registry database for maxillofacial battle-injuries experienced by U.S. Soldiers in Iraq/Afghanistan conflict to describe type, distribution and mechanism of injury. Methods and Materials: JTTR was queried from October 19, 2001 to December 12, 2007 for maxillofacial BI entered in the database using ICD-9 codes; the data was compiled for BI Soldiers. Results: 7,770 BI were identified. Approximately 26% had maxillofacial BI. There were 4,783 maxillofacial BI among the 2,014 BI (2.4 injuries per Soldier). Majority of maxillofacial BI were male (98%). Average age was 26 years old. Penetrating soft tissue injuries and fractures were 58% and 27%, respectively. 76% of fractures were open. Frequency of facial fractures was mandible 36%, maxilla/zygoma 19%, nasal 14%, and orbit 11%. Remaining 20% not otherwise specified. Primary mechanism of injury was explosive devices (84%).

Another study of 142 combat injured burn casualties demonstrated that 77% had face burns. The outcomes of four severely injured service members were also studied: despite multiple, extensive surgical reconstructions these service members, representative of approximately 10% of all CMF battle injured service members, end up with poor facial form and function.

Regenerative medicine and tissue engineering offers promise to the severely wounded service member by leveraging the latest advances in stem cell biology with bioengineering and surgery to reconstruct currently uncorrectable deformities with fewer surgeries, less invasive surgeries and with better results. The aim of the Dental and Trauma Research Detachment is to develop solutions by collaborating with scientists and clinicians of civilian research institutions to return wounded service members back to function as soon as possible. Examples of current research initiatives will be presented: regenerative medicine bone putty to potentially give us a biomaterial to regenerate CMF bone reliably and with better control than even autologous sources and a “Biomask” to serve as an in situ bioreactor to regenerate facial skin on burn casualties.

KEYNOTE ORAL PRESENTATION – ABSTRACT 364
Session 7A: Craniofacial 1
Friday, 17 September 2010: 0930 – 1000 hours

ROLE OF ORAL MUCOSA IN SOFT TISSUE ENGINEERING IN CRANIOMAXILLOFACIAL SURGERY

Stephen E Feinberg[1], Antonio Peramo[1], Kenji Izumi[2] & Cynthia L Marcelo[1]
[1] *University of Michigan, Ann Arbor, Michigan, USA*
[2] *Niigata University, Niigata, Japan*

Regeneration of oral mucosal tissue has not been previously addressed in craniomaxillofacial (CMF) soft tissue injuries. Development of an **e**x **v**ivo **p**roduced **o**ral **m**ucosal **e**quivalent (EVPOME) has lagged behind that for skin. The lack of available tissue engineered/regenerative medicine (TE/RM) produced oral mucosa has limited the ability of surgeons to reconstruct the oral cavity and other functional units that contain a muco-cutaneous (M/C) junction, i.e. lips, eyelids, nares. In this presentation we will discuss the fabrication of a human tissue engineered oral mucosa suitable for intraoral grafting, (2) show results from our Phase I clinical trial, and (3) present preliminary data on the manufacture of M/C constructs that could be used to form human lips for facial reconstruction.

KEYNOTE ORAL PRESENTATION – ABSTRACT 400
Session 7A: Craniofacial 1
Friday, 17 September 2010: 1000 – 1030 hours

IMMUNOMODULATION OF WOUND RESPONSE

Anh Le
University of Southern California, Los Angeles, CA, USA

The goals of tissue engineering (TE) and regenerative medicine are to successfully regulate inflammation resolution to limit further tissue injury and to prevent development of chronic immune-mediated inflammation, to achieve not only a scarless wound healing, but also to enhance the limited regenerative capability of mammalian tissues. Similar to normal physiology of wound healing, success of tissue engineering (TE) relies on the temporal and spatial control of the inflammatory wound microenvironment, and thus modulates subsequent healing and scar formation. Our recent studies demonstrated that cell-based therapy with human mesenchymal stem cells derived from the gingival tissues (GMSC) significantly resolved mucosal ulceration, promoted epithelial proliferation, and regenerated damaged epithelial linings in compromised mucosal wounds such as experimental colitis and mucositis models. These findings demonstrated that GMSC possess dual functions as an immunomodulator to suppress inflammation-related tissue injury and a regenerative role to restore epithelial lining (Zhang Q et al, 2010). In another model of excisional skin wounds, we showed that GMSCs are capable of switching polarization of macrophage toward M2, also known as wound healing macrophage, characterized by enhanced anti-inflammatory and phagocytic activities, and a marked acceleration of wound healing. Likewise, studies in keloid, a fibrotic dermal scar, have also supported a strong link between inflammatory responses and wound healing (Zhang Q et al, 2009). In this model of pathological wounds, we demonstrated that the altered niche in keloids, predominantly inflammatory, contributes to the acquirement of the scar phenotype and in vivo modification of the immediate scar niche, can suppress skin fibrosis.

ORAL PRESENTATION – ABSTRACT 401
Session 7A: Craniofacial 1
Friday, 17 September 2010: 1030 – 1045 hours

FACIAL RECONSTRUCTION WITH CELL-ASSISTED LIPOTRANSFER (ADIPOSE PROGENITOR-ENRICHED MICROFAT GRAFTING)

Kentaro Doi & Kotaro Yoshimura
Department of Plastic Surgery, University of Tokyo, Tokyo, Japan

Aim:
To repair facial lipoatrophy, which is a disfiguring and socially disabling problem that accompanies several inherited and acquired diseases such as lupus erythematosus profundus.

Method:
Through preclinical studies, we developed a novel strategy called cell-assisted lipotransfer (CAL), which is grafting of adipose stem/progenitor cell (ASC)-enriched micro-fat tissue; progenitor number deficit of aspirated fat tissue was compensated for by supplementation with ASCs isolated from anothervolume of aspirated fat tissue. Our clinical study of CAL began in 2003. More than 80% of a total of nearly 500patients received CAL for breast augmentation or reconstruction, while 18 patients underwent it for reconstruction of facial lipoatrophy. We also have done a number of animal studies on cellular events seen in adipose remodeling such as lipografting.

Results:
Clinical results suggest that postoperative atrophy of transplanted fat tissue was prevented by the improved ASC/adipocyte ratio achieved in the CAL technique.Animal studies showed that many adipocytes in grafted fat died after transplantation and gradually replaced with new adipocytes derived from activated ASCs.

Conclusions:
Although further studies are necessary, clinical resultswere pretty encouraging and promising; patients hadlong-term sustained soft tissue augmentation without leaving any scars.

ORAL PRESENTATION – ABSTRACT 169
Session 7A: Craniofacial 1
Friday, 17 September 2010: 1045 – 1100 hours

THE ELASTIC PROPERTIES AND EFFECT OF UNIAXIAL TENSION ON ADIPOSE TISSUE EXPLANTS

Kieran Rowe[1], Silvia Leo[2], Andrea O'Connor[2], Anthony Penington[1,3], Wayne Morrison[1,3] & Keren Abberton[1,3]
[1] *The O'Brien Institute; Fitzroy, VIC, Australia*
[2] *The University of Melbourne Department of Chemical and Biomolecular Engineering, VIC, Australia*
[3] *The University of Melbourne Department of Surgery, St Vincent's Hospital Campus, Melbourne, VIC, Australia*

Aim:
Mimicking the mechanical milieu of native tissue can encourage growth of this tissue. Stretch can also increase tissue growth. Our aims are to determine the elastic modulus of explanted rat adipose tissue, and the effect on gene expression of cyclic uniaxial tensile strain.

Method:
Adipose explants were harvested from rat abdominal subcutaneous tissue. Samples were subjected to uniaxial strain in an Instron 5848 MicroTester, to determine the elastic modulus of the tissue. Further samples were subjected to cyclic 25% strain at 1Hz for 20 minutes with or without a prestrain to 50% and samples processed to extract RNA for gene array analysis.

Results:
We have demonstrated a modulus for rat subcutaneous adipose tissue of 3.3±0.2kPa, less than that measured parallel to the skin plane[1], likely explained by the presence of fascial sheets in that direction. The shape of the stress-strain curve we obtained does not fit current simplistic models of extracellular matrix behavior under tension, requiring a more complex model to describe this behavior. We show a decrease in modulus for subsequent cycles for 50% and higher strains, but not 25% strain. This suggests some form of irreversible change at strains of 50% and above.

Conclusions:
Knowledge of the elastic properties of adipose tissue may aid adipose tissue engineering by mimicking this mechanical environment. Tension is known to increase growth of many tissues and elucidation of gene expression changes in response to stretch may help us understand this mechanism.

KEYNOTE ORAL PRESENTATION – ABSTRACT 377
Session 7B: Biological Scaffolds 1
Friday, 17 September 2010: 0900 – 0930 hours

EXTRACELLULAR MATRIX-DIRECTED REGENERATION OF SOFT TISSUES

David J McQuillan, PhD[1,2] & Hui Xu[1]
[1] LifeCell Corporation and [2] Center for Advanced Research and Technology, Kinetic Concepts Inc., Branchburg, NJ, USA

Clinical performance of biologic-derived extracellular matrix scaffolds are dependent on the method of processing to remove cellular elements and other xenogeneic structures that can elicit a negative immune response. The ability to sterilize and preserve the scaffold while not inducing damage to the complex extracellular matrix (ECM) structure is equally critical in allowing for integration and remodeling into host tissue, and driving a positive clinical outcome. We have developed methods to process and preserve a porcine-derived dermal scaffold that retains critical ECM structural elements while eliminating specific structures that are recognized in xeno-transplantation. ECM composition and integrity was assessed by in vitro analysis, and capacity to drive soft tissue repair and regeneration demonstrated in a non-human primate model of abdominal wall repair. The performance of the scaffold was compared and contrasted to other biologic-derived scaffolds and a hypothesis has developed to describe the dependence of the mechanism of action on the tissue processing method. Our approach to mitigate negative immune response allows host tissue regeneration, and has been validated in clinical studies that will be described.

ORAL PRESENTATION – ABSTRACT 252
Session 7B: Biological Scaffolds 1
Friday, 17 September 2010: 0930 – 0945 hours

BLADDER AUGMENTATION WITH A NOVEL DECELLULARIZED MATRIX IN A SWINE MODEL

Gilad E Amiel, Deandra Ramsey, Tung Shu, Weigo Jian, Seth P Lerner
Scott Department of Urology, Baylor College of Medicine, Houston, Texas, USA

Aim:
To evaluate a bladder augmentation model utilizing porcine derived, decellularized aorta in swine.

Method:
Six mini-swine females underwent laparoscopic bladder augmentation with a 4x10cm decellularized aorta patch. Blood tests were taken at baseline, 3,6,9,12 months. At 3-month laparoscopic biopsy was performed. At 3, 6 and 12 months a cystogram, and at 12-month, after a urodynamic study, animals were sacrificed. Kidneys, ureters and bladder specimens were obtained for evaluation.

Results:
All 6 animals maintained full health up to the one-year time-point. Blood tests, including Hb, WBC and renal function tests were all normal at all time-points. Cystograms demonstrated no extravasation or vesicoureteral reflux. Urodynamic studies demonstrated high capacity, up-to 700cc, with low pressure and high compliance. Biopsy at 3 months showed cell migration and neovascularization onto the matrix on H&E. At the one-year time point, only native tissue was found without the matrix, and only non-absorbable stay-sutures indicated the location of the matrix.

Conclusions:
We show successful reconstruction and integration of an unseeded, decellularized blood vessal matrix into native bladders, which can ultimately serve as an off-the-shelf product. Prior research using seeded and unseeded allograft has shown mixed results. This highly translational and easily reproducible model for bladder augmentation could benefit many spinal cord injury and other patients, while obviating the need for costly prior biopsy and cell harvesting, cell separation and seeding, decrease morbidity, and be done in a minimally-invasive approach.

ORAL PRESENTATION – ABSTRACT 24
Session 7B: Biological Scaffolds 1
Friday, 17 September 2010: 0945 – 1000 hours

A RAPID PHOTOCHEMICAL CROSSLINKING METHOD FOR FABRICATION OF COVALENTLY CROSSLINKED PROTEIN-BASED BIOMATERIALS

Christopher M Elvin[1], Tony Vuocolo[1], Alan G Brownlee[1], Mickey Huson[3], Misook Kim[1], Russell E Lyons[1], Lillian Sando[1], Glenn A Edwards[4], John AM Ramshaw[2] & Jerome A Werkmeister[2]
[1] *CSIRO Livestock Industries, St. Lucia, QLD, Australia*
[2] *CSIRO Molecular & Health Technologies, Clayton, VIC, Australia*
[3] *CSIRO Materials Science & Engineering, Geelong, VIC, Australia*
[4] *Faculty of Veterinary Science, University of Melbourne, Werribee, VIC, Australia*

Aim:
To develop a rapid photochemical method for fabrication of effective and non-toxic protein-based biomaterials.

Method:
The photochemical process uses tris(2,2'-bipyridyl)dichlororuthenium(II) hexahydrate ($[RuII(bpy)_3]^{2+}$) and sodium persulphate (SPS) in the presence of white light to rapidly and efficiently catalyse intermolecular protein dityrosine formation. Dityrosine crosslinks were readily formed by photochemical curing of resilin, fibrinogen and gelatin as shown by the presence of high molecular weight protein polymers formed after crosslinking by SDS-PAGE analysis. Resilience of macroscale strips was measured using tensile testing, or, at the nanoscale, using AFM. The presence of dityrosine was shown by its fluorescence and by LC/MS analysis following acid hydrolysis. The adhesive strength of gelatin was determined by photo-crosslinking of 2 bovine amnion membranes.

Results:
We originally used this photochemistry to fabricate a dityrosine-crosslinked recombinant protein-based rubber material (Resilin), which is found in the jumping mechanism of fleas and the flight systems of insects. Resilin is the most elastic protein material known and shows remarkably high resilience (rubber efficiency >97%) and fatigue lifetime (>300 million cycles). We have also used this photochemistry to develop a rapidly-curing, high adhesive strength and elastic surgical tissue sealant derived from fibrinogen or gelatin (PhotoSeal™) for wound closure following surgical procedures.

Conclusions:
We envisage development of high fatigue lifetime spinal disc prostheses (PhotoDisc™) derived from crosslinked resilin mimics (that are resistant to proteolysis). We have also produced a highly adhesive and elastic protein-based surgical tissue sealant based on gelatin (PhotoSeal™). This material would be very attractive for a number of applications in surgery or tissue engineering. In particular, use of this gelatin tissue sealant would be advantageous in surgical procedures where high elasticity and adhesion are required, for example in gastrointestinal or lung tissue repair.

ORAL PRESENTATION – ABSTRACT 126
Session 7B: Biological Scaffolds 1
Friday, 17 September 2010: 1000 – 1015 hours

ENGINEERING CARTILAGE USING ACELLULAR CARTILAGE SHEET

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Department of Plastic and Reconstructive Surgery, Shanghai 9th People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Key Laboratory of Tissue Engineering, Shanghai, China

Aim:
Acellular cartilage could provide native extracellular matrix for cartilage engineering. However, cells are difficult to migrate into decellularized cartilage due to it's nonporous structure. The aim of this study is to make acellular cartilage sheets, and use the sheets as scaffold to engineer cartilage in vitro.

Method:
Cartilage from pig ear was cut into a circular cylinder with diameter about 6 mm. Cartilage sheets were then achieved by frozen section of the shaped cartilage at thickness of 10 μm-30 μm. The sheets were then decellularized by SDS, and lyophilized. Chondrocytes were isolated from pig ear by enzymic digestion and expanded in culture. Cells at passage 2 were collected and seeded on decellularized sheets. The sheets were then loaded layer by layer as a "Sandwich", and cultivated in the presence of fetal bovine serum for 4 weeks.

Results:
Histological analysis showed that cells were completely removed from cartilage sheets after 24 hours of SDS treatment. After re-seeding cells and loading 20 layers of sheets together, a cylinder shaped cell-scaffold construct was achieved. Cartilage-like tissues were formed after 4 weeks of culture. Histological analyses showed the formation of mature cartilage with typical lacuna structure, which was further confirmed by anti-type II collagen antibody staining. Cartilage formation using 10 μm-thickness sheets was better than using 30 μm-thickness sheets. Moreover, a designed shape of cartilage could be achieved by pre-shaping the sheets.

Conclusion:
Aceelular cartilage sheet could be ideal scaffold for engineering cartilage with designed shape.

ORAL PRESENTATION – ABSTRACT 318
Session 7B: Biological Scaffolds 1
Friday, 17 September 2010: 1015 – 1030 hours

THE CAPABILITY OF BIOFUNCTIONAL MATRICES TO INFLUENCE EMBRYONIC KIDNEY DEVELOPMENT – A POSSIBLE CONTRIBUTION TO TREATMENTS BASED ON REGENERATION

David DR Sebinger[1], Andreas Ofenbauer[1,2], Mathieu Unbekandt[3], Mikhail Tsurkan[1], Jamie A. Davies[3] & Carsten Werner[1]
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[2] *University of Vienna, Austria*
[3] *Centre for Integrative Physiology, University of Edinburgh, Scotland*

As tool, organoculture of murine, embryonic kidneys plays an important role in research towards kidney regeneration. Using basic principles of interfacial science, we have now struck a new path in culturing embryonic kidney rudiments and embryonic organs/tissue in general, which requires just a low volume of medium, on a (optionally functionalized) solid support, localized by surface tension. Obvious benefits are the visibility and reduction of the culture medium, which is especially interesting if expensive additives are used. Moreover it allows long-term cultivation and influences the development of the cultured embryonic kidneys positively, resembling the organ embryogenesis in vivo.

Our novel culture system opens up new vistas for building regeneration promoting matrix environments for kidney tissue. One focus is the impact of extracellular matrix (ECM) components on kidney development and repair: several single ECM proteins and glycosaminoglycans such as collagen types, hyaluronic acid and heparin have been reconstituted on surfaces by utilizing thin layers of reactive maleic-anhydride copolymers as pre-coatings.

Additionally investigating and applying the natural structure/composition of the whole ECM of the murine adult kidney has been enabled by decellularization techniques.

Another field of interest are the biophysical influences such as stiffness or viscosity on kidney development. Therefore, starPEG-heparin hydrogels have been applied, which can be independently tuned with respect to biomolecular composition and elasticity, and permits to present a plethora of bioactive molecules.

Eventually our work should contribute to artificial biomaterials that mimic the regulatory features of the natural environment of renal tissue for therapeutical and basic biological purposes.

ORAL PRESENTATION – ABSTRACT 260
Session 7B: Biological Scaffolds 1
Friday, 17 September 2010: 1030 – 1045 hours

CHARACTERISATION OF ISLET EXTRACELLULAR MATRIX

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Aim:
Extracellular matrix (ECM) is essential for regulating intercellular communication and maintaining cell function. The survival rate of islets post-isolation is low, purportedly due to degradation of ECM. Therefore, this study aimed to identify the ECM components of the islet with the ultimate aim of improving their survival in an encapsulated system via presentation of appropriate ECM molecules.

Method:
ECM of adult rabbit and rat pancreas was characterised using immunohistochemistry to determine the native matrix proteins suitable for islet encapsulation. Formalin-fixed pancreatic sections were probed for collagens I & IV, laminin, fibronectin, perlecan, heparan sulfate (HS) & chondroitin sulfate (CS).

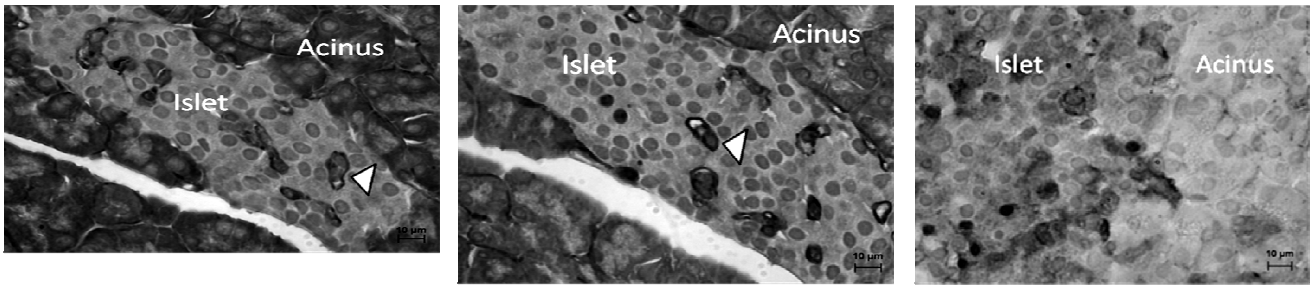

Figure 1. Localisation of laminin (a), collagen IV (b) and HS (c) in adult rat (a,b) and rabbit (c) pancreas. (◁) intra-islet microvascular basement membrane

Results:
All probed matrix components were localised to the pancreatic acinus. Laminin and collagen IV were significantly localised to the intra-islet microvascular basement membranes (Figure 1a and b), while perlecan was only localised to the general islet region. HS was significantly greater in the islet region compared to the acinus (Figure 1c).

Conclusions:
Laminin and collagen IV localizations are consistent with literature as they are major constituents of the basement membrane. However, this is among the first studies to demonstrate significant HS localization within the islet by immunohistochemistry. HS has been shown to modulate pancreatic endocrine cell differentiation during early development and a high content of HS in adult islet matrix may help to maintain islet health and function. Future studies include the optimization of antibody and fixative conditions to identify the proteoglycans to which the HS is attached.

Acknowledgements:
This research was supported under Australian Research Council's *Discovery Projects* funding scheme (project number DP0986447).

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Friday, 17 September 2010: 1045 – 1100 hours

DEVELOPMENT OF WOUND DRESSING COMPOSED OF HYALURONIC ACID AND COLLAGEN SPONGE WITH EPIDERMAL GROWTH FACTOR

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Aim:
This study is designed to investigate the efficacy of wound dressing composed of hyaluronic acid (HA) and collagen sponge containing epidermal growth factor (EGF), focusing on the wound healing in deep dermal burn of rats.

Method:
Two types of wound dressings were prepared. Group 1 is a spongy wound dressing composed of high-molecular weight HA, hydrolyzed low-molecular weight HA, and collagen. Group 2 is a spongy wound dressing composed of high-molecular weight HA, hydrolyzed low-molecular weight HA, and collagen containing EGF. The effect of EGF on fibroblast proliferation and its cytokines production was investigated in the culture system. In addition, the effect of EGF on wound healing was investigated in animal test using rats. The abdomen skin of SD rat was shaved and then contacted with boiling water for 3 sec to result in a deep dermal burn, measuring 30mm in diameter. Three days later, the necrotic tissue was excised, and covered with each dressing. The wound conditions were observed 1 week and 2 weeks later. Control group is a commercially available wound dressing composed of alginate.

Results:
EGF released from wound dressing (Group 2) promote fibroblasts proliferation and its cytokines production that are essential for wound healing, especially angiogenesis. Wound size and granulation tissue formation associated with epithelialization were evaluated macroscopic and histologic appearances. The wound healing was promoted more effectively in group 2, compared with group 1, and control.

Conclusions:
HA and collagen sponge including EGF is a promising wound dressing for burn injury and skin ulcers.

KEYNOTE ORAL PRESENTATION – ABSTRACT 311
Session 7C: Biomaterials & Surfaces 1
Friday, 17 September 2010: 0900 – 0930 hours

COATINGS FOR THE CONTROL OF BIOINTERFACIAL INTERACTIONS

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Aim:
The effective control of biointerfacial interactions is the key to many biomedical applications, such as cell culture tools, biosensors and implantable medical devices. Whilst a number of coating strategies have been used to achieve this goal, many are limited with respect to their function and transferability between different substrate materials. Here, our aim was to develop a robust coating platform that leads to highly effective control of biomolecule- and cell-surface interactions.

Method:
We have used surface-initiated graft polymerisation methods for the homo- and copolymerisation of monomers that provide low non-specific interactions, functional groups and switchable surface properties. Subsequently immobilised compounds included bioactive peptides as well as small molecule labels. Coatings were characterized using XPS and AFM. Cell culture experiments were carried out using L929 and HeLa cells as well as mesenchymal stem cells.

Results:
We have developed a robust coating platform that can be applied to a wide range of substrate materials. These coatings can achieve superior control over biointerfacial interactions such as protein adsorption and cellular responses. Sophisticated control over the coating architecture has been achieved in particular with controlled free radical polymerisation (ATRP, RAFT, Iniferter) macro-initiators.

Conclusions:
We expect that this coating platform will be translated into a variety of biomedical applications, including applications in tissue engineering and regenerative medicine.

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Friday, 17 September 2010: 0930 – 0945 hours

ENHANCEMENT OF *IN VITRO* BIOCOMPATIBILITY OF CROSSLINKED GELATIN FILMS BY AC 50HZ NITROGEN GLOW DISCHARGE

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Aim:
To investigate the effects of AC 50Hz nitrogen glow discharge on the enhancement of biocompatibility of crosslinked gelatin films.

Method:
Preparation of crosslinked gelatin film:
The 0.05% w/v of gelatin solution was coated onto glass cover slips and air dry overnight to obtain gelatin film. The gelatin film was dehydrothermal crosslinked in a vacuum oven at 140ºC for 48 hr.

Plasma treatment of crosslinked gelatin films and their characterization:
The glow discharge system was set up using AC 50Hz power supply. The crosslinked gelatin films were treated with plasma using nitrogen gas. The treatment time was varied from 3 to 30 sec. The plasma-treated and untreated crosslinked gelatin films were characterized, including water contact angle by a contact angle meter, surface roughness by Atomic Force Microscopy (AFM), surface chemistry by XPS, and biocompatibility by *in vitro* cell culture using rat bone marrow derived stem cells (rMSC).

Results:
The result showed that the water contact angle of gelatin films was decreased with increasing plasma treatment time. The treatment of nitrogen plasma for 3-30 sec had no effect on surface roughness of gelatin film as revealed by AFM results. The XPS analysis showed that the N-containing functional groups generated by nitrogen plasma were incorporated onto the film surface and they were increased with increasing treatment time. *In vitro* test using rMSC revealed that on the number of cells attached on nitrogen plasma-treated gelatin films was significantly promoted, compared to untreated samples. The greatest enhancement of cell attachment was noticed when the films was plasma-treated for 15 sec. The water contact angle and nitrogen to carbon (N/C) ratio of this treated film were 27-34º, and 25-34%, respectively. This could suggest the suitable surface properties of gelatin film for best rMSC attachment.

Conclusions:
The introduction of nitrogen plasma treatment could enhance cell attachment on the surface of crosslinked gelatin films.

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Friday, 17 September 2010: 0945 – 1000 hours

PREPARATION OF POLYDIMETHYLSILOXANE (PDMS) STIFFNESS GRADIENTS FOR MESENCHYMAL STEM CELL CULTURE AND DIFFERENTIATION

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Aim:
Substrate stiffness appears to play an important role in stem cell differentiation. Different tissues display characteristic particular matrix elasticity *in vivo* and this elasticity appears to direct stem cells into the different cell lineages. This effect has also been reported for discrete samples of different stiffness substrate stiffness *in vitro*. Polydimethylsiloxane, PDMS, is a widely used biocompatible polymer in biomedical area. In this study, we aimed create a lateral stiffness gradient using PDMS and study mesenchymal stem cells behaviour on this material.

Method:
The stiffness gradient PDMS was fabricated based on the generation of temperature gradient across the polymer during heat-induced crosslinking. The elastic modulus across the gradient PDMS was examined by means of nanoindentation with a spherical diamond tip. Rat mesenchymal stem cells, rMSCs, were seeded on protein-coated PDMS gradients and non-coated gradients were used as the control. Cell morphology, osteogenesis, and adipogenesis of rMSCs were analysed.

Results:
The elastic modulus and hardness across the gel was 1.3-2.9 MPa and 0.11 - 0.2 MPa, respectively. Changes in the percentage of curing agent allowed tuning of the range of elastic modulus. Cell behavior was dependent on both stiffness and protein coating. rMSCs showed different osteogenic and adipogenic potential across the protein-coated PDMS gradients.

Conclusions:
PDMS with a lateral stiffness gradient was fabricated using a temperature gradient during PDMS crosslinking. This study shows potential for application of PDMS gradients in cell mechanotransduction research.

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Session 7C: Biomaterials & Surfaces 1
Friday, 17 September 2010: 1000 – 1015 hours

THE EFFECT OF GEOMETRICAL CONSTRAINTS ON CELL MIGRATION

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The internal architecture of three dimensional (3D) polymeric scaffolds plays a critical role in tissue engineering. Previous studies have shown that it can control the extent of cell colonisation, scaffold vascularisation and tissue in-growth. However, identifying which individual aspects of the scaffold architecture affects cell infiltration within a 3D environment is complex as it can be difficult to separate and analyse the importance of individual scaffold properties such as pore size, tortuosity, interconnectivity and porosity.

We have developed a microfludic platform, which allows us to analyse the effect of individual geometries, such as those seen within tissue engineering constructs, on cell migration in a controlled 2D environment. The device consists of a main chamber with migration channels protruding perpendicularly off this chamber. In operation, the channel dimension and fluid surface tension prevent fluid from exiting the main chamber into the migration microchannel. Thus it is possible to establish a confluent cell monolayer in the main chamber without any cell migration or media flow into the adjacent microchannel. Migration is then initiated by backfilling the microchannels with media, establishing a connection between the main chamber and the microchannel. By varying the migration channel conformation, it is therefore possible to analyse individually the effect of geometric variables such as channel size, contraction and expansion of channel width, tortuosity and channel junctions

Using this microfluidic platform, we have studied the migration characteristics of NIH-3T3 mouse fibroblasts and human bone marrow mesenchymal stem cells (hMSCs). Migration rates and characteristics were analysed using real time microscopy and cell tracking. We demonstrate that for NIH-3T3 mouse fibroblast geometrical constraints such as channel size, contractions and expansions, and channel junctions significantly influence the cell wave migration speed and cell colonisation of space. In contrast, only channel size affected the migration rates of hMSCs, other geometrical constraints did not modify migration characteristics.

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Friday, 17 September 2010: 1015 – 1030 hours

BEHAVIOR OF PATTERNED ENDOTHELIAL CELLS IN THREE-DIMENSIONAL TISSUE ENGINEERED BY CELL SHEET TECHNOLOGY

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Aim:
Some studies have showed that the insertion of network-formed endothelial cells in the tissue improve the vascularization of regenerated tissue. In this study, we examined the influence of micropatterning of endothelial cells in three-dimensional tissue on their behavior in angiogenesis.

Method:
Poly-acrylamide (PAAm) micropattern was fabricated on temperature responsive cell culture dishes (TRCDs) for making patterned TRCDs. The patterned TRCDs was utilized to harvest patterned human umbilical vein endothelial cells (HUVEC) by reducing the temperature to 20 □C. The patterned HUVECs were then layered on or in normal human dermal fibroblast (NHDF) sheet(s) for creating three-dimensional tissue. Effect of micropattern designs on the morphological change of HUVECs in the three-dimensional tissue was observed by confocal microscopy.

Results:
In NHDF sheets, patterned HUVECs formed the networks on 100 □m interval stripe patterned dishes, unlike 1000 □m interval dishes after 1 day culture. The network formation was observed rapidly compared with the non-patterned HUVECs. On the other hand, patterned HUVECs put on NHDF sheets grew to cobblestone shape and increased their width. But the patterned endothelial cells between two NHDF sheets, they changed their three-dimensional morphology and decreased their width.

Conclusions:
Patterned HUVECs changed their morphology and network forming behavior by changing three-dimensional layout or the designs of micropattern in the tissues. By this technology we may fabricate desirable blood vessel networks in the regenerated tissues.

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Friday, 17 September 2010: 1030 – 1045 hours

CULTIVATION OF HEMATOPOIETIC STEM CELLS ON THE SURFACE-MODIFIED MATERIALS HAVING NANO-SEGMENTS AND EXTRACELLULAR MATRIX PROTEINS

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Aim:
Umbilical cord blood (UCB) is a promising source of stem cells for hematopoietic stem cell (HSC) transplantation. However, the low number of HSCs that can be obtained from a single UCB donor limits the transplantation of UCB for patients. We performed two culture methods of HSCs, 2D and 3D culture. We investigated how to create and mimic the bone marrow niche for the expansion of HSCs by using nano-segments and extracellular matrix (ECM) on the surface of the materials.

Method:
HSCs were cultured on the surface-modified dishes immobilized nano-segments or ECMs (2D-culture). A direct *ex vivo* HSC expansion involving filtration of UCB through polyurethane foaming (PU) membranes having nano-segment was also performed, and was followed by 3D-culture of HSCs on the membranes.

Results:
PU membranes having nano-segments of carboxylic acid showed higher expansion fold of HSCs among surface-modified PU membranes investigated in this study. The rinsing solution using in direct ex vivo expansion in 3D-culture should contain platelet-poor plasma more than 15%. In 2D-culture of HSCs, high surface density of amino group on the culture dishes was not favor, and the optimal surface density of amino group was found for HSC expansion.

Conclusions:
The direct *ex vivo* expansion of HSCs from UCB filtered through membranes can provide a simple 3-D culture method of HSCs and a high *ex vivo* expansion fold of HSCs with a simple operation. There found optimal functional groups and ECMs with optimal surface density for high expansion fold of HSCs in 2-D culture.

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Friday, 17 September 2010: 1045 – 1100 hours

CREATION OF POROUS CHITOSAN HYDROGEL USING DENSE GAS CO_2

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[3] *Center for Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, USA*

Aim:
The aim of this study was to investigate the feasibility of fabricating porous chitosan hydrogel using dense gas CO_2 as a pore-foaming agent during crosslinking of chitosan in an aqueous phase and to eliminate the use of surfactant or other toxic reagents.

Method:
Dense gas CO_2 was dissolved and saturated in chitosan aqueous solution during genipin crosslinking. Upon depressurization, CO_2 was released from the aqueous phase and to create in the hydrogel. Scanning electron microscopy was used to measure the pore size. The equilibrium swelling ratio (ESR) and mechanical properties of the fabricated hydrogels were assessed to determine the effect of porosity on hydrogel performance.

Results:
A three-dimensional (3D) porous and rigid chitosan hydrogel was produced with various thicknesses using the dense gas CO_2 as a pore-foaming agent. The average pore diameter was 43.8 ± 11.6 μm, when using CO_2 at 100 bar and 37 ºC for crosslinking reaction of genipin and chitosan. No porous structure was produced in chitosan that was crosslinked at atmospheric condition. Chitosan hydrogels produced at dense gas CO_2 condition displayed a two-fold higher ESR (17.2 ± 0.86) and slightly lower compressive modulus (73.9 ± 6.8 kPa) than samples produced at atmospheric conditions due to the presence of porosity.

Conclusions:
Dense gas CO_2 was efficient as a pore-foaming agent to create 3D and homogenous porous chitosan hydrogels during genipin crosslinking. This process eliminates the use of solvent and surfactant. This hydrogel scaffold can be potentially used in soft tissue regeneration such as cartilage or skin.

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Session 7D: General Session
Friday, 17 September 2010: 0900 – 0915 hours

CURRENT PROGRESS AND CHALLENGES IN CRYOPRESERVATION OF TISSUE ENGINEERED CONSTRUCTS

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Aim:
Cryobiology is of immense importance to the viable preservation of a broad range of cells and both native and engineered tissues, which in turn leads to applications in regenerative medicine. Development of platform technologies in cryopreservation is required for the successful preservation of a variety of tissue-engineered constructs (TECs).

Key challenges in cryopreservation of TECs are maintaining:

- viability
- integrity of constructs
- cellular functions
- attachment to scaffolds (for cell-scaffold systems)
- proliferation and differentiation (for stem cells (SCs))
- cell-cell junctions (for cell aggregates)

We have undertaken to develop cryopreservation strategies to address these issues.

Method:
We applied vitrification (solidification of liquid into an amorphous or glassy state) as opposed to freezing (solidification into a crystalline state).

Results:
We established vitrification of mesenchymal and neuronal SCs 2D/3D cultures and hepatocytes seeded on collagen coated films as a part of bioartificial liver device. The formulation of improved cryopreservation solutions is an integral part in developing this platform technology. To achieve this goal, microencapsulated hepatocytes and hepatocyte spheroids were used which were preserved without damage to their integrity. Application to mesenchymal SCs growing on scaffold/matrix maintained the microstructure of the scaffold while completely preserving the ability of the SCs to proliferate and subsequently differentiate.

Conclusions:
We established a foundation for the development of a platform technology that will allow cryogenically preserve TECs and adult SC cultures to be distributed to patients in need. This will have high impact on clinical application of tissue-engineered implants.

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Session 7D: General Session
Friday, 17 September 2010: 0915 – 0930 hours

SILICONE-PEG COPOLYMERS AS A TREATMENT OF HYPERTROPHIC SCARS

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Aim:
A frequent outcome of wound repair is the formation of hypertrophic scars, characterised by the overproduction of collagen. A common treatment of these scars includes silicone therapy. This study aims to examine the proliferation of human dermal fibroblasts exposed to a range of commercially available and synthesised silicone-PEG copolymers, with the overall aim of reducing collagen production.

Method:
Fibroblasts analysed were isolated from human dermal skin, along with hypertrophic scar derived fibroblasts (HSF) and normal fibroblasts (nHSF) isolated from the same patient. Cells were cultured in DMEM containing FBS, penicillin, streptomycin, and L-glutamine and maintained in a humidified atmosphere (5% CO_2 at 37°C). Fibroblasts were exposed to a range of silicone-PEG copolymers, at various dilutions, and proliferation was examined using a Cyquant direct cell proliferation assay.

Results:
Cell proliferation was assessed after exposing human dermal fibroblasts to a variety of silicone-PEG copolymers. Results showed fibroblast origin (scar or non scar derived) did not impact on the degree of proliferation when exposed to the copolymers; however, variation in copolymer molecular weight did impact on proliferation. It was shown that the smaller molecular weight copolymers resulted in a higher reduction in fibroblast proliferation.

Conclusions:
Silicone-PEG copolymers show potential for the treatment of hypertrophic scars where, the mechanism of fibroblast internalisation of the copolymer is currently under investigation.

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Friday, 17 September 2010: 0930 – 0945 hours

INNOVATIVE REGENERATIVE TREATMENT FOR THE TYMPANIC MEMBRANE PERFORATION-CLINICAL APPLICATION-

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Objective:
To establish the new treatment for regeneration of the tympanic membrane (TM) without conventional surgical therapy.

Study Design:
Clinical pilot study

Setting:
3 General hospitals

Patients and methods:
The patients with chronic TM perforation without active inflammation were randomly selected from 53 outpatients. Their ages ranged from 21 to 84 (average 56). Materials for the TM repair were a gelatin sponge with basic fibroblast growth factor (b-FGF) and a fibrin glue.

The effectiveness of this novel therapy for the TM repair was estimated 3 weeks after the procedures. After creating a mechanical disruption of the TM perforation edge under the microscope, a gelatin sponge with b-FGF was placed over the perforation in contact with the residual TM. Fibrin glue was dripped over it. In case complete closure of the TM perforation was not achieved, the above same treatment was performed repeatedly. The final estimation was performed 3 months after the treatment.

Results:
Complete closure of the TM perforation was achieved in 98.1%(52/53) patients within 4 time treatments. The number/rate of the cases whose the TM perforation were able to close completely by 1, 2, 3 and 4 times were 38 (72%), 10 (19%) and 2 (4%) and 2 (4%), respectively. Average hearing levels of all patients were improved. No sequela were observed in all patients.

Conclusions:
The study demonstrated that the combination of a gelatin sponge and a bFGF was effective for regeneration of the TM. This regenerative therapy is easy, simple, cost-effective and non-invasive method for outpatients.

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Session 7D: General Session
Friday, 17 September 2010: 0945 – 1000 hours

LONG-TERM EFFECTS OF HYDROGEL PROPERTIES ON HUMAN CHONDROCYTE BEHAVIOUR

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Aim:
Nature-derived and synthetic hydrogels are promising for cartilage tissue engineering but their relative effectiveness in promoting and maintaining chondrogenesis remains to be seen. In this study, we compared the effects of different hydrogels (alginate and Factor XIIIa-cross-linked MMP-sensitive PEG at two stiffness levels) on the behaviour of human chondrocytes and the development of construct properties over 12 weeks *in vitro*.

Method:
Human chondrocytes from macroscopically normal regions of an osteoarthritic joint were expanded to passage 3, and encapsulated in alginate (1% and 2% w/V) or MMP-degradable Factor XIIIa-cross-linked PEG multi-arm PEG (2.5% and 3.5%) hydrogels. Constructs were cultured for up to 12 weeks in serum-free chondrogenic media and assessed for cell viability and morphology, DNA content, mRNA expression, matrix localisation and mechanical properties.

Results:
Chondrocytes remained viable throughout culture, and were characteristically round in alginate but mostly spread in PEG gels at both concentrations. Chondrogenic gene (COL2A1, aggrecan) expression increased in all hydrogels, but alginate constructs had higher expression levels of these genes (up to 90-fold for COL2A1) and more prominent staining of the protein by immunohistochemistry. Chondrocytes continually expressed COL1A1 and COL10A1, indicative of de-differentiation and hypertrophy. After 12 weeks, constructs with lower polymer content were stiffer than similar constructs with higher polymer content, with the highest compressive modulus measured in 2.5% PEG gels.

Conclusions:
While synthetic hydrogels offer many advantages over natural hydrogels such as alginate, they must be further optimized to elicit desired chondrocyte responses for use as cartilage models and development of functional tissue-engineered articular cartilage.

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Friday, 17 September 2010: 1000 – 1015 hours

BFGF DOWN-REGULATE SMA TO ANTAGONIZE CONTRACTION OF BMSC ENGINEERED CARTILAGE

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Aim:
Bone marrow stem cells (BMSCs) have been proved an ideal cell source for cartilage engineering, however, contraction and deformation of cell-scaffold constructs during chondrogenic induction limits its wide application. Smooth muscle action (SMA) is known as a temperate cytoskeleton for cell contraction. Whether SMA is related to contraction of the construct and whether basic fibroblast growth factor (bFGF) can down-regulate SMA to antagonize contraction of BMSC engineered cartilage were explored in this study.

Method:
Porcine BMSCs were seeded into a polyglycolic acid (PGA) sheet and in vitro cultured for 6 weeks. The media were divided into four groups: A. regular medium (DMEM + 10%FBS), B. chondrogenic medium (regular medium+10ng/ml TGFβ1, 50ng/ml IGF, 40ng/ml dexamethasone), C. chondrogenic medium + 50ng/ml bFGF, D. regular medium + 50ng/ml bFGF. Shape changes of the constructs were recorded every week until 6 weeks, and the expression of SMA was also investigated.

Results:
All the constructs began to contract dissimilarly since the third week after culture. At the end of sixth week, the constructs in group C achieved the slightest contraction with 60.9% of original size and the highest wet weight, while the constructs in group B obtained the largest contraction with only 12.4% of original size. RT-PCR and western blotting demonstrated that bFGF remarkably decreased expression of SMA and enhanced deposition of collagen II and glycosaminoglycan.

Conclusions:
These results indicated that bFGF could down-regulate SMA expression to suppress the contraction of BMSC engineered cartilage and no negative influence on cartilage formation.

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Session 7D: General Session
Friday, 17 September 2010: 1015 – 1030 hours

PROTOCATECHUIC ACID COMBINED WITH BDNF INCREASE IN PRODUCTION OF RAT DOPAMINERGIC NEURONS FROM CULTURED MESENCEPHALIC NEURAL STEM/PROGENITOR CELLS

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Parkinson's disease (PD) is gradual progressive neurodegenerative disorder with clinical symptoms manifesting after at least 60% of the dopaminergic neurons in the midbrain have degenerated. Several attempts have been made to generate dopaminergic neurons from freshly isolated fetal midbrain neural stem/progenitor cells (NS/PCs) in vitro for potential use in cell replacement therapy for PD. However, it is unclear whether expanding NS/PCs from midbrain do have the capacity to produce functional dopaminergic neurons after long-term culture. In this study, we describe the isolation, expansion, and in vitro characterization of fetal rat (E13.5) midbrain NS/PCs and their differentiation into functional nerve cells including dopaminergic neurons. Protocatechuic acid (PCA), an efficient antioxidant and neuroprotective property [1-2], was tested for its ability to induce dopaminergic phenotype in cultures from epidermal growth factor and basic fibroblast growth factor -derived mesencephalic NS/PCs. When these cells were incubated in media containing serum, the dopaminergic phenotype was rarely expressed. The addition of PCA increased the number of dopaminergic neurons (up to 0.5% of total cells as compared to controls). Treatment with brain-derived neurotrophic factor (BDNF) also induced a significant increase (about 2%) in the number of tyrosine hydroxylase-immunoreactive neurons. Notably, the mixture of the most effective doses of these compounds induced a further increase in the number of DA neurons (up to 4% of total cells). These results suggest that PCA may contribute to efficient production of dopaminergic neurons for transplantation therapies in PD under the appropriate culture conditions and without genetic manipulations.

Acknowledgements:
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ORAL PRESENTATION – ABSTRACT 219
Session 7D: General Session
Friday, 17 September 2010: 1030 – 1045 hours

DEVELOPMENT OF NOVEL SCAFFOLD MATERIAL BASED ON ALGINATE-MODIFIED ATELOCOLLAGEN

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Regenerative tissue with a certain volume cannot survive without receiving a blood supply. Therefore, construction of a vascular network in regenerative tissue is a key technology in tissue engineering.

As a system to construct a vascular network, we developed a scaffold material that induces neovascularization from the recipient bed to inside of the material. The material was designed to mimic the function of extracellular matrix. Desirable functions are considered to be: (1) adequate mechanical strength of the scaffold, (2) protease degradability, (3) cell adhesiveness, and (4) a reservoir property for an angiogenic growth factor such as bFGF. In order to build these functions into the material, we selected atelocollagen as a protease-degradable polymer and alginate as a reservoir for growth factor. This material is based on a covalently-bonded structure of atelocollagen and alginate, and promptly forms hydrogel following addition of calcium ions.

The mechanical strength of the obtained gel was controllable by adjusting the concentration of atelocollagen or alginate, or the degree of covalent bonds between alginate and atelocollagen. A cell-adhesion study indicated that this material has cell adhesiveness comparable to that of collagen. In addition, we confirmed the binding ability of the gel with bFGF by an enzyme-liked immunoassay.

This material with these functions is expected to induce effective neovascularization *in vivo*. Such a material will be a useful tool to fabricate three-dimensional regenerative tissue with a suitable volume for transplantation.

ORAL PRESENTATION – ABSTRACT 103
Session 7D: General Session
Friday, 17 September 2010: 1045 – 1100 hours

ALIGNED MYOTUBES ON POLVINYL ALCOHOL MICRO PATTERNED PLATES IN VITRO

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Aim:
Artificially manufactured skeletal muscle has been sought after in the clinical medical field; however *in vitro*, myoblasts fuse into random alignments and make a myoblast sheet with scattered myotubes. Here we show a versatile method for making aligned myotubes *in vitro* involving aligned 50 and 200μm width groove patterned plates.

Method:
A photomask, containing 50 or 200μm parallel shadow lines between slits, was set on the Azidophenyl polyvinyl alcohol (AzPhPVA) coated polystyrene plate and exposed to UV light. AzPhPVA changed to PVA by UV and attached to the plate, and remaining AzPhPVA was washed off. On each patterned plate, 1×10^4cells/ml P2 L6 myoblasts were seeded and then cultured in DMEM F-12 Ham with 2% horse serum and 1% penicillin streptomycin.

Results:
Aligned myotubes are necessary to make skeletal muscle. For this purpose, we contrived aligned 50 or 200μm width grooves and seeded L6 myoblasts. In both patterned plates, aligned myotubes started appearing after one week and were confirmed by multinucleated shapes and anti-myosin immunofluorescent staining. In comparison with mainly one myotube in a 50μm groove, there were fascicles of aligned myotubes in a 200μm groove. However in both patterns, skeletal myofibers having sarcomere were not observed.

Conclusions:
In both 50 and 200μm width groove patterns, aligned myotubes were assembled, and there were fascicles of aligned myotubes in 200μm patterns. This suggests PVA micro patterns succeeded to make aligned myotubes, and they are useful to make single myotubes in 50μm patterns and myofibers in 200μm patterns.

KEYNOTE ORAL PRESENTATION – ABSTRACT 243
Session 8A: Craniofacial 2
Friday, 17 September 2010: 1130 – 1200 hours

BIOLOGICAL AND SYNTHETIC SCAFFOLDS FOR DENTAL TISSUE ENGINEERING

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Objective:
Our long term objective is to establish reliable methods to bioengineer full sized, functional teeth and supporting alveolar bone tissues. A current challenge for tooth regeneration is to devise methods to regenerate teeth of predetermined shape and size, using cells derived from postnatal tissues. For this purpose, we are investigating the use of biological and synthetic scaffold materials to facilitate dental epithelial (DE) and dental mesenchymal (DM) cell interactions for subsequent mineralized dental tissue formation. Here we present our studies of adult dental progenitor cells seeded onto natural porcine tooth ECM scaffolds, and synthetic hydrogel materials.

Methods:
Second and third molars harvested from 5 month old pigs were decellularized by repeated cycling in graded detergents. Decellularization efficiencies were quantified and compared using PicoGreen DNA analysis, morphological, and histological analyses. Natural porcine molar tooth ECM composition was characterized using Masson's Trichrome, Movat's Pentachrome, immunohistochemical (IHC) analyses of ECM components, and two photon fluorescent excitation (TPFE) analysis. Harvested and cultured porcine DE and DM cells were statically seeded onto natural decellularized porcine scaffolds, and monitored for up to 4 wks in culture. TPFE was used to examine cellular ingrowth into the scaffolds. Synthetic hydrogels were used to fabricate three-dimensional (3D) DE-DM cell constructs. Human dental pulp cell seeded Collagen gel was co-cultured with porcine DE cells suspended in Growth Factor Reduced (GFR) Matrigel. The resulting 3D cell layers were cultured *in vitro* and harvested at weekly intervals, or implanted subcutaneously into nude rats and harvested after one or four weeks, for subsequent molecular and histological analyses.

Summary of Results:
Porcine tooth decellularization in 5% SDS/1% Triton-X detergent treatment cycles resulted in efficient cell removal, and preservation of ECM proteins including collagens I and IV, fibronectin, and laminin. Preliminary data revealed that optimized decellularization treatment resulted in a 4-fold decrease in dental cell nuclei as compared to (non-decellularized) controls, and that dental cell ingrowth was observed after 7 days in culture, indicative of dental cell repopulation. IHC analyses of 3D synthetic hydrogel constructs revealed the expression of dental tissue-specific markers including Amelogenin (AM), Dentin Sialophosphoprotein (DSPP), and the expression of Laminin 5 and collagen IV in the basal membrane at the DE-DM border. Analyses of 3D DE-DM in vivo implants revealed the formation of irregular hard tissues after 4 weeks.

Conclusions:
Porcine molar tooth buds can be decellularized efficiently, while maintaining the integrity of the tooth bud ECM, and repopulated with DE and DM cells. Identified natural tooth ECM components and gradients instructing tooth tissue differentiation will be used to guide biomimetic scaffold fabrication. Our 3D hydrogel DE/DM constructs revealed promise in generating organized dental tissue formation. We anticipate that these approaches will facilitate the eventual establishment of reliable methods to elaborate dental tissues of specified size and shape.

This work is supported by NIH/NIDCR ROI DE 016132 and R01OD006058 (PCY).

ORAL PRESENTATION – ABSTRACT 324
Session 8A: Craniofacial 2
Friday, 17 September 2010: 1200 – 1215 hours

SOLID FREEFORM FABRICATION OF HYDROGEL STRUCTURES AND CELL ENCAPSULATION

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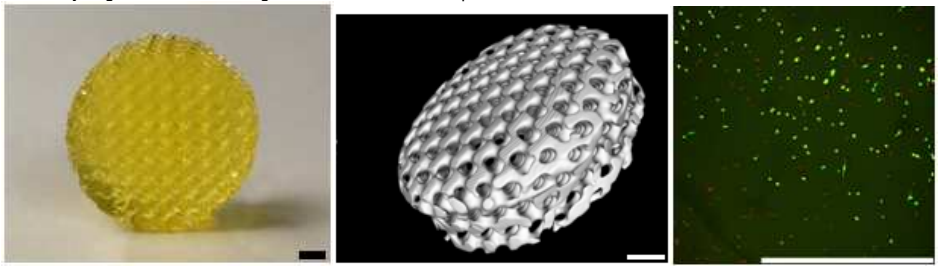
We aim to fabricate computer-controlled hydrogel structures containing viable encapsulated cells to overcome the low seeding densities which are inherent to most pre-fabricated scaffold systems.

We developed a resin based on poly(ethylene)glycol (PEG) and poly(D,L-lactide), and utilised it in the fabrication of computer-designed hydrogel structures by stereolithography. Furthermore, we synthesised methacrylated gelatine and encapsulated chondrocytes inside gel scaffolds using photo-polymerisation.

PEG-based hydrogel structures were successfully prepared using stereolithography. The structures closely resembled the initial designs as ascertained using micro-computed tomography. The continuous gyroid pore network allowed for perfusion of the gel and deformation up to 70 % strain without permanent damage. Using a solution of 10 % gelatine-methacrylate and 0.1 % Irgacure 2959 photo-initiator in cell culture medium, chondrocytes suspended at 0.5×10^6 cells/mL were encapsulated with 30 s of UV exposure. After 24 h, cell morphologies were consistent with viable cells shown with confocal microscopy and were actively metabolising demonstrated using the WST1 assay.

Three-dimensional biodegradable hydrogel structures were designed and prepared for the first time by stereolithography, at high resolution. Porous and non-porous hydrogels with well-defined architectures and good mechanical properties were produced. Gelatine-methacrylate is a suitable artificial extracellular matrix, which can be employed for cell encapsulation using photo-polymerisation. It is anticipated that the use of gelatine-methacrylate and solid freeform fabrication techniques will result in well-defined hydrogel structures with high densities of viable encapsulated cells.



Left, middle: PEG-based hydrogel structures prepared by stereolithography. Right: chondrocytes encapsulated in photo-polymerised gelatine. Scale bars are 1 mm.

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Session 8A: Craniofacial 2
Friday, 17 September 2010: 1215 – 1230 hours

INJECTABLE SCAFFOLD FOR BONE TISSUE ENGINEERING APPLICATIONS

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Aim:
The aim of this work was to develop an injectable scaffold for growth factor and/or cell delivery in bone regeneration applications.

Method:
PLGA/PEG particles were fabricated by high temperature blending of PLGA (85:15 53kDa) and PEG 400 polymers. Polymer pieces were ground into particles to obtain the 100-200µm size fraction. Cylindrical scaffolds of 12mm length and 6mm diameter were prepared in moulds. Compressive strength of composite scaffolds was tested using a TA.HD+ texture analyser (Stable Microsystems). Young's Modulus of Elasticity was computed by determining the slope of the stress-strain curve along the elastic portion of deformation.

Results:
The PLGA/PEG formulation has a maximum compressive strength at fracture point of approximately 4MPa after 2 hours at 37°C, with a Young's Modulus of Elasticity close to 80MPa. These values exceed those achieved by many non-injectable scaffolds in clinical use. The material has porosities of between 40% and 60% and pore diameters exceeding 50 microns.

By manipulating certain variables in the formulation we have fabricated scaffolds with suitable rheological properties for use in an injectable formulation. Following injection, the scaffold hardens in 15 minutes, with full strength developing over 24 hours. Viability and proliferation of MSCs within the scaffold has also been demonstrated.

Conclusions:
Porous PLGA/PEG injectable scaffolds have been developed that harden at body temperature. The PLGA/PEG scaffolds have unconstrained compressive strength in the region of cancellous bone. Viability and proliferation of cells grown on the scaffolds has been demonstrated. Future work will assess bone regeneration in *in vivo* models.

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Session 8A: Craniofacial 2
Friday, 17 September 2010: 1230 – 1245 hours

MECHANICAL LOADING ENHANCES SEGMENTAL BONE DEFECT REPAIR BY ALTERING BONE FORMATION AND DISTRIBUTION

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Aim:
This study evaluated the effects of mechanical stimulation on large bone defect repair by allowing transfer of compressive ambulatory loads to the regenerating bone.

Method:
Bilateral six mm defects were surgically created in rat femora. Each defect was implanted with a nanofiber mesh tube filled with alginate hydrogel containing 5 µg bone morphogenetic protein-2 (BMP-2). Limbs were stabilized by either stiff fixation plates for the duration of the study or compliant plates that allowed transfer of compressive ambulatory loads upon actuation at week 4 post implantation. Healing was assessed by digital radiographs, microcomputed tomography (microCT), mechanical testing, histology, and finite element (FE) modeling.

Results:
Loading significantly increased regenerate bone volume and average polar moment of inertia. The response to loading was location-dependent with the polar moment of inertia increased at the proximal end of the defect but not the distal end. As a result, torsional stiffness was 58% higher in the compliant plate group, but failure torque was not altered. Histology demonstrated a greater amount of cartilage and a lesser degree of remodeling to lamellar bone in the compliant plate group compared to the stiff plate group. Finally, principal strain histograms, calculated by FE modeling, revealed that the compliant plate samples had adapted to more efficiently distribute loads in the defects.

Conclusions:
Together, these data demonstrate that functional transfer of axial loads via modulation of fixation plate stiffness enhances BMP-induced large bone defect repair by altering the amount and distribution of bone formed within the defect.

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Session 8A: Craniofacial 2
Friday, 17 September 2010: 1245 – 1300 hours

CELL-ADHESIVE ADVANTAGE OF OSTEOBLASTS ON TITANIUM IN THE MODEL OF IN VITRO OSSEOINTEGRATION

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Aim:
Titanium is empirically known and used as an excellent implantable material for osseointegration. However, the reason why titanium is such adequate material for osseointegration is still vague. We hereby focused on the initial adhesion and made a hypothesis that the competition between the osteoblast and the fibroblast is the dominant factor. This study was aimed to compare the cell adherent potential on several materials.

Method:
NIH3T3, a fibroblast cell line, and MC3T3-E1, an osteoblast-like cell line, were seeded together on each glass substrate vapor-deposited with titanium, gold, alumina and zirconia respectively. NIH3T3 and MC3T3-E1 were introduced GFP and DsRed expression vectors, respectively. The number of cells adhering on each substrate was counted by the quantification of the each fluorescent signals after three hours seeding.

Results:
Osteoblasts and fibroblasts shared the same ratio on each substrate, when they were seeded together at normal condition. However, when the cells received shear stress, the osteoblast ratio on the titanium substrate increased, but the ratio on the zirconia was almost the same. Alumina and gold showed low cell adherent potential at this condition.

Conclusions:
The results showed that initial cell attachment is due to the type of material and the trend of cell-adhesive potential materials was similar to the difference of osseointegration property in clinical field. It suggested that the cell type which firstly adheres on material have an influence to the success of osseointegration. The great performance of titanium in osseointegration is due to its high cell-adhesive effect for osteoblasts.

ORAL PRESENTATION – ABSTRACT 15
Session 8A: Craniofacial 2
Friday, 17 September 2010: 1300 – 1315 hours

THE EFFICACY OF DERMAL EXTRACTED – BONE POWDER SCAFFOLD ON THE HEALING OF RAT 'S CALVARIAL BONE DEFECTS

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Aim:
This study aimed to study the properties of the human dermal-extracted solution mixed with bone powder shaped into scaffold on the healing of rat's calvarial bone defects which are determined by radiopaque area in computed tomography (CT scan) and new bone formation in histological study.

Method:
Dermal-extracted solution, dermal-extracted solution mixed with bone powder, bovine collagen type I, and bovine collagen type I mixed with bone powder were structured into scaffolds and embedded into 24 female, aged between 12-14 weeks, wistar rats' calvarial bone defects. The rats were divided into 6 groups which were dermal-extracted solution, dermal-extracted solution mixed with bone powder, bovine collagen type I, bovine collagen type I mixed with bone powder, commercial product (CollaPlug®) and sham group. The rats were sacrificed 12 weeks after embedded. The calvarial bone were cut and examined with CT scan and H&E staining.

Results:
CT scans showed no statistically differences in both periphery and center of bone defects between dermal-extracted solution and the other groups. However, from histological study, the bone formation of dermal-extracted solution group was significantly more than the other groups except the dermal-extracted solution mixed with bone powder. The bone formation of dermal-extracted solution group was not statistically different from the dermal-extracted solution mixed with bone powder group. No complete bone bridge was found in any defect.

Conclusions:
Scaffolds from dermal-extracted solution and dermal-extracted solution mixed with bone powder both have osteoinductive property. However, bone powder did not improve the property of scaffolds.

ORAL PRESENTATION – ABSTRACT 2
Session 8A: Craniofacial 2
Friday, 17 September 2010: 1315 – 1330 hours

A REVIEW OF THE BIOMEDICAL APPLICATIONS OF NANOTECHNOLOGY IN BONE TISSUE ENGINEERING

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There is a growing need for bone tissue replacement due to an increasing aging population, accidents, sports injuries, ongoing wars, etc. To meet this demand, artificial scaffold biomaterials are being engineered to have the appropriate mechanical properties, surface chemistry, and surface topography to enhance cell attachment, growth, and tissue formation at such defect sites.

Nanomaterials (defined as those materials with constituent dimensions of less than 100 nm) have been recently introduced to the tissue engineering field as a new tool to improve the biocompatibility of the surface structure of bone implants. Engineers use techniques which create an environment conducive for bone regeneration by mimicking the natural organization of bone tissues, thus promoting bone tissue growth. Current research shows that the use of nanotechnology to create nanomaterials is a good option for these techniques, since these materials can mimic the surface properties of natural tissues. Therefore, recently, nanomaterials have been deemed promising candidates for improving traditional tissue engineering materials.

The surface properties of biomaterials are a key parameter because the interaction between the cells and tissue takes place on the surface of the implant. There is a large demand for implants which have surface properties that facilitate biological responses, while having proper mechanical properties throughout. Therefore, a great push has been made towards the development of nanostructure Ca-P-based surface coatings on various metallic and nonmetallic substrates for load-bearing implant applications such as hip joint prosthesis, knee joint prosthesis, and dental implants. Researchers are trying to find different methods to create nanostructure coatings. Typical coating methodologies like ion beam assisted deposition, plasma spray deposition, sol–gel derived coatings, electrodeposition, laser deposition, etc., are being studied at a laboratory scale. Some have already been introduced to industry.

In this review paper, the promise of nanomaterials for bone tissue engineering applications will be reviewed. First, different methods for coating the surface of the bone implants will be studied. Then, two sets of experiments will be investigated, in vivo and in vitro, and the results in each section will be compared. Lastly, the effects of different nanocoatings and parameters on biological responses will be reported. The results show that nanomaterials have preferred properties compared to conventional (or microstructured) materials in bone tissue engineering.

KEYNOTE ORAL PRESENTATION – ABSTRACT 384
Session 8B: Biological Scaffolds 2
Friday, 17 September 2010: 1130 – 1200 hours

RECONSTRUCTING THE STEM CELL MICROENVIRONMENT BY DELEGATION – THE LIQUID AND THE SOLID WAY

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The role of the microenvironment surrounding stem cells is increasingly acknowledged as an important biological driver of stemness and differentiation. Major efforts are directed towards the reconstruction of these microenvironments *in vitro* by modifying surface properties of cell culture dishes, or utilizing semi-artificial or fully synthetic polymer structures. What is often forgotten is the huge potential of cells, including stem cells, to create their own matrices and remodel them as they differentiate into certain lineages. We show here, how macromolecular crowding, a biophysical principle that governs the intra and extracellular millieu in multicellular organisms can be reversely bioengineered in a simplistic manner using carbohydrate-based macromolecules of defined hydrodynamic radius. Macromolecular crowding creates an excluded volume effect in the liquid phase (culture medium), this in turn drives enzymatic reactions and supramolecular assembly and this effect can be exploited to efficiently deposit extracellular matrices onto the cell culture plates (solid phase). Extending this to stem cells, we have effectively delegated the construction of the microenvironment entirely to the cells themselves as they either stay undifferentiated or move into different lineages upon induction. Furthermore, we demonstrate the efficiency of this system by driving MSCs into osteogenic and the adipogenic lineages with a higher efficiency and speed than hitherto known.

ORAL PRESENTATION – ABSTRACT 263
Session 8B: Biological Scaffolds 2
Friday, 17 September 2010: 1200 – 1215 hours

A SYNTHETIC BIOELASTOMER WITH TUNABLE MODULUS FOR TISSUE ENGINEERING APPLICATIONS

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Aim:
In many tissue engineering strategies, synthetic biomaterial substrates are oftenused to support cell growth and differentiation. Recently, through both the use of functionalised non-degradable poly acrylamide substrates of varying elastic moduli and photolithographically produced adhesive islands of varying size and shape, various researchers have shown that cytoskeletal tension can be highly deterministic of cell fate decisions particularly in human mesenchymal stem/stromal cells (hMSCs). Currently, few synthetic degradable biomaterials when presented as a tissue engineered construct are capable of modulating the cytoskeletal tension due to them lacking the required a.) low elastic moduli values (for example, 1-100 kPa); b.) biocompatibility, and c.) processability. In this presentation we will describe a novel biocompatible polymer gel system based on acrylated polypropylene glycol triol (aPPGT). This new bioelastomer system has tunable elastic moduli, can be easily surface modified and manufactured into porous three dimensional scaffolds or micro-patterned substrates.

Results:
We demonstrate that the PPGT gels can modulate hMSC: growth, transcription factor profiles, and differentiation capabilities as shown in previous studies using a polyacrylamide system as the substrate. We have then developed and characterised functional myocardial patches using heart digests as a cell source. This has resulted low modulus flexible patches for potential delivery of hMSC or functional myocardial tissue to myocardial infarcts.

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Session 8B: Biological Scaffolds 2
Friday, 17 September 2010: 1215 – 1230 hours

EXAMINATION OF EXTRACELLULAR MATRIX COMPOSITIONS OF TISSUE AND CELL-DERIVED BASEMENT MEMBRANES

Veronica Glattauer[1,2,3], Kellie Cartledge[1,2], Leanne Dyksterhuis[1], Julie Nigro[1,3], John Ramshaw[1,2], Aditya Vashi[1] & Jerome Werkmeister[1,3]
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Aim:
To examine the variation in the extracellular matrix (ECM) compositions of natural and cell-derived basement membranes (BM) to develop materials for use in tissue engineering.

Methods:
Natural BMs were seminiferous tubules and lens capsules; cell-derived BMs were EHS tumour (Matrigel™), myoid and Sertoli cells. BMs were also characterised using immunohistochemistry, lectin binding and SDS-PAGE. Identification and quantitation of glycosaminoglycan (GAG) components was achieved using Fluorophore-Assisted Carbohydrate Electrophoresis on samples solubilised using protease-K, and digested with hydrolases and eliminases. .

Results:
Protein and GAG analyses showed differences between samples, which were most marked for the different cell lines. Tissue BM particles are suitable for cells in spinner culture, with good attachment and growth. Tissue derived BMs typically did not have any hyaluronic acid (HA) component, and chondroitin sulfate (CS) components were in low abundance. The ECM deposited by each of the cell lines consisted of the major components that are found in BMs, including collagen IV, laminin, nidogen and perlecan. GAG from Sertoli cells contained HA and various CS disaccharides; myoid cells were similar, but lacked C-6S. Sertoli cells were limited to a heparin-6-sulphate BM, whereas myoid cells were rich in various heparin-6-sulphate components.

Conclusions:
It is difficult to isolate BM in large amounts from natural tissues, for example to use in a bioreactor. The present data suggest that combinations of the ECM secreted by different cell lines will enable materials to be developed in larger quantities that can mimic natural BMs, for example to allow stem cell expansion.

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Session 8B: Biological Scaffolds 2
Friday, 17 September 2010: 1230 – 1245 hours

CELL DETACHMENT ALONG WITH ELECTRICAL CLEAVAGE OF A ZWITTERIONIC OLIGOPEPTIDE LAYER

Takahiro Kakegawa, Hiroaki Suzuki & Juniji Fukuda*
Graduate School of Pure and Applied Sciences, University of Tsukuba, Japan

Aim:
We propose a culture surface to which cells are preferably attached and then readily detached by means of an electrical stimulus.

Method:
A self-assembled monolayer of the oligopeptide CAAAEKEKEKEKGRGDSP was fabricated on a gold surface via a gold-thiolate bond and the electrostatic force between the alternating charged glutamic acid (E) and lysine (K) sequence. Owing to the ionic salvation in the alternating sequence, the modified surface was resistant to the nonspecific adsorption of proteins, while cells adhered to this surface via the RGD sequence. The application of a negative potential to the gold surface resulted in the subsequent detachment of the attached cells along with the cleaving of the gold-thiolate bond.

Results:
Quartz crystal microbalance measurements revealed that the nonspecific adsorption of proteins on the peptide-modified surface was significantly reduced as compared to that to a surface without the modification. Cells were preferably attached on the surface, and then they were completely detached by applying a negative potential. In our previous study, which involved the use of a non-self-assembly peptide, ~10% of the cells remained attached even after 5 min of the potential application. In this study, probably owing to the nonfouling layer, almost all of the cells were completely detached within the 2 min of the potential application.

Conclusions:
The proposed approach could be used to completely and rapidly detach cells from a surface. This cell detachment approach appears to be a useful tool in building-block-based tissue engineering.

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Session 8B: Biological Scaffolds 2
Friday, 17 September 2010: 1245 – 1300 hours

NEUTROPHIL ACTIVATION INDUCED BY SILK SERICIN

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Aim:
Sericin, an adhesive silk protein, is being investigated for the design of biosynthetic hydrogels. However, sericin has been implicated in inflammatory responses in suture applications in previous literature. This study aims to examine activation of neutrophils in response to sericin as an indicator for potential inflammation.

Method:
Sericin from cocoons of *Bombyx mori,* (B), and *Antherea mylitta,* (A), was isolated and functionalised with methacrylate groups, B-MA and A-MA, to form poly(vinyl alcohol)/silk hydrogels. Sericin samples were exposed to blood from 3 human donors. Neutrophil activation indicated by integrin CD11b/CD18 expression was quantified via flow cytometry. Lipopolysaccharide (LPS, 5µg/mL) was the positive control and phosphate buffered saline (DPBS) was the negative control. Data was analysed using two-way analysis of variance with a value of $p<0.05$ taken as significant.

Results:
Neutrophil activation by LPS resulted in an eight-fold upregulation of integrin expression (Figure 1). Sericin proteins showed no significant neutrophil activation in blood from different donors when compared to negative controls ($p<0.05$). There was also no significant difference in integrin expression either between species or with the addition of methacrylate groups.

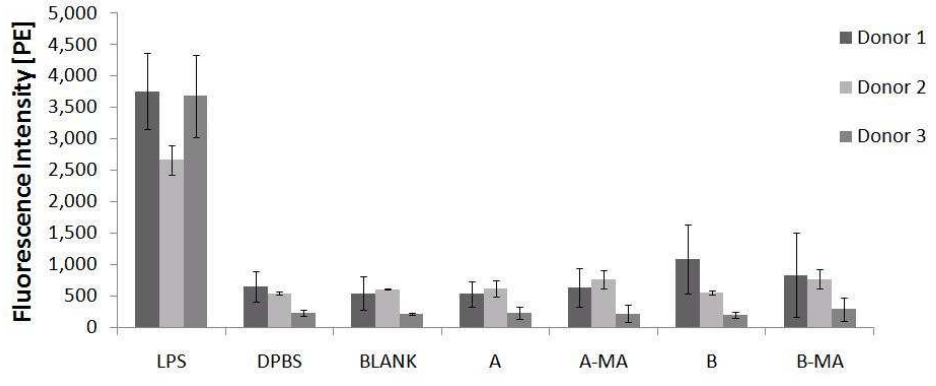

Figure 1: Effect of sericin on the expression of CD11b/CD18 in human neutrophils. Data represent geometric mean of fluorescence intensity of anti-CD11b/CD18 ± SD (n=3)

Conclusions:
Based on this study, unmodified and modified sericin proteins from both silkworm species studied, did not illicit significant neutrophil activation in blood sampled from 3 different donors. Further studies on production of proinflammatory mediators (e.g. TNF-α) and *in vivo* studies are required to confirm this finding.

Acknowledgements:
This research was supported under Australia India Strategic Research Fund (AISRF BF010049).

ORAL PRESENTATION – ABSTRACT 72
Session 8B: Biological Scaffolds 2
Friday, 17 September 2010: 1300 – 1315 hours

INFLUENCE OF CO-CULTURING MONOCYTES WITH SMOOTH MUSCLE CELLS: EFFECTS ON CELL ATTACHMENT, PHENOTYPE, METABOLISM AND MIGRATION INTO A DEGRADABLE POLYURETHANE SCAFFOLD FOR VASCULAR TISSUE REGENERATION

Joanne McBane[1], Soroor Sharifpoor[1], Kuihua Cai[1], Rosalind Labow[2] & J Paul Santerre[1]
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Aim:
Monocytes cultured on a degradable, polar-hydrophobic-ionic polyurethane (D-PHI) adopted an anti-inflammatory phenotype[1]. It was hypothesized that co-culturing the anti-inflammatory monocyte with human vascular smooth muscle cells (hVSMC) would promote hVSMC attachment, migration into the porous D-PHI scaffolds while maintaining a contractile hVSMC phenotype, ideal conditions for promoting tissue formation in vascular grafts.

Methods:
D-PHI scaffolds were synthesized[2] using 75% porogen (polyethylene glycol and $NaHCO_3$). hVSMCs and human monocytes isolated from whole blood[1] were seeded alone or in co-culture at a 1:2 ratio on the 6mm diameter porous scaffold disks (24h-28d). Cells were analyzed for viability (WST), cell attachment (DNA), cell migration (H&E) and lysates were analyzed for hVSMC contractile phenotype marker calponin (immunoblotting).

Results:
hVSMCs alone have higher metabolic activity (WST) than the co-cultured cells, suggesting a modulating effect from the monocytes (14d; $p<0.05$). Co-culture samples had DNA levels equivalent to an additive effect, except at 14d where there were more cells in the co-culture than the mono-cultures. hVSMCs alone remained around the perimeter of the scaffold (H&E) whereas co-culture samples showed good cell distribution throughout the scaffold and increased cell attachment at d14 ($p<0.05$). Contractile marker calponin, linked to cell motility, was high in early co-culture lysates but went down with time; however, hVSMCs alone maintained calponin levels out to 28d.

Conclusions:
hVSMCs attached to and maintained a contractile phenotype on D-PHI scaffolds. Co-culturing of monocytes and hVSMCs increased total cell attachment and infiltration while modulating metabolic activity of the hVSMCs. Future work is looking at more VSMC markers and tissue formation.

References:
1. McBane JE, *et al.* Biomaterials. 2009;30(29):5497.
2. Sharifpoor S, *et al.* Biomacromolecules. 2009;10(10):2729.

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Session 8B: Biological Scaffolds 2
Friday, 17 September 2010: 1315 – 1330 hours

POLY(VINYL ALCOHOL) HYDROGEL MICROSPHERES FOR CELL MICROENCAPSULATION PRODUCED BY SUBMERGED ELECTROSPRAYING

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Aim:
Encapsulation of cells within hydrogel microspheres is a promising therapy for diseases such as diabetes. This study investigated the morphology, size and encapsulated cell viability of poly(vinyl alcohol) (PVA) hydrogel microspheres prepared by submerged electrospraying with UV photopolymerisation.

Method:
PVA (16kDa, modified with 7 methacrylate groups/chain), dissolved in water at 20 wt%, was dispersed as fine droplets in cottonseed oil and 0.5% Span 80 by submerged electrospraying at voltages from 0 to 8kV. Dispersed droplets were UV photopolymerised to form hydrogel microspheres. Morphology and size distribution were determined from light microscopy and laser scattering respectively. L929 fibroblasts were encapsulated within microspheres produced at 4kV, and viability measured using a Live/Dead assay after 3 and 24 hours incubation in media.

Results:
Spherical hydrogel microspheres were produced with no agglomeration (Fig. 1). The mean diameter decreased from 3140±100µm at 0kV to 67±36µm at 8kV (Fig. 2). Cell viability 3 and 24 hours after encapsulation was 88±5% and 91±2% respectively, indicating the minimal short term effects of the electrospray technique.

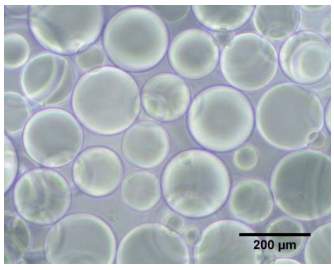

Figure 1: PVA hydrogel microspheres at 4kV

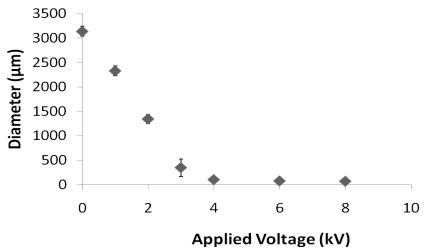

Figure 2: Microsphere diameter as a function of applied voltage

Conclusions:
This technique successfully produced PVA hydrogel microspheres and encapsulated cells with high viability. Further work will include longer term cell viability studies and mechanical characterization of microspheres.

Acknowledgements:
This research was supported under the ARC *Discovery Projects* scheme (DP0986447).

KEYNOTE ORAL PRESENTATION – ABSTRACT 367
Session 8C: Synthetic Scaffolds & Hydrogels
Friday, 17 September 2010: 1130 – 1200 hours

ENGINEERING SCAFFOLDS FOR SOFT TISSUE REGENERATION

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The composition and mechanical properties of the biodegradable polymers used and the macro-structural characteristics of the three dimensional scaffold generated, strongly affect the ability of the system to actively promote the regeneration of functional autologous tissue.

In general terms, Tissue Engineering can be classified into *in vitro* and *in vivo* types. While the former concentrates on the *ex vivo* generation of tissues using a pre-formed scaffold and donor cells, the latter aims at regenerating functional tissue at the site of implantation following the *in situ* generation of the scaffold.

Due to their stiffness, the scaffolds based on the widely used biodegradable aliphatic polyesters are not optimal for soft tissue applications. It is, therefore, apparent that a new generation of pre-formed scaffolds consisting of biodegradable elastomers, is called for.

In recent years, the *in vivo* generation of scaffolds has received increasing attention. This strategy aims at generating functional tissue at the site of implantation, by the *in situ* generation of the scaffold and the combined action of biomolecules and cells.

The first part of the talk will focus on the development of biodegradable elastomers for pre-formed scaffolds, while the second part will devoted to *in situ* generated scaffolds. After reviewing recent developments in the field on these two fronts, this presentation will focus on work done by our group, aiming at developing novel biodegradable polymers and scaffolding constructs.

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Session 8C: Synthetic Scaffolds & Hydrogels
Friday, 17 September 2010: 1200 – 1230 hours

COMBINATORIAL SCREENING OF CELL PROLIFERATION ON NANOFIBER GRADIENT LIBRARIES

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Aim:
To develop a combinatorial approach for screening the effect of nanofiber scaffold composition and properties on cell proliferation.

Method:
We introduce a two spinneret electrospinning method for the production of gradient nanofiber scaffold libraries, which consisted of non-woven mats of nanofibers containing a gradient in material composition and properties, suitable for studying cell responses such as adhesion, proliferation and differentiation. We electrospun polycaprolactone (PCL) nanofibers in combination with hydroxyapatite nanoparticles (nHA) to obtain a linear gradient of nHA libraries, as a single scaffold specimen, which facilitated us to screen the effect of nHA composition on *mouse MC3T3*-E1 cell adhesion and proliferation.

Results:
PCL nanofiber scaffold libraries with gradient of nHA composition were fabricated by utilizing two spinneret electrospinning. The uniform distribution of nHA, without any beaded formation, throughout the scaffold was achieved by controlling spinning parameters. SEM was employed to confirm the formation of nanofibers. TGA was used to demonstrate that the nanofiber mats contained linear gradients in nHA composition. The percent mass of the nHA present in each pre-determined positions across the scaffold libraries were determined in order to quantify the scaffold composition. Cell attachment (SYTOX green staining) and proliferation (dsDNA quantification) were also examined. The cell culture data showed presence of nHA enhanced the cell adhesion and proliferation compared to pure PCL. In addition, an increased cellular response has observed on the nHA rich side compared to other end of the scaffold specimen (nHA absence side), which indicate that the cells respond to scaffold composition and properties. We are further utilizing the combinatorial libraries to determine the optimum scaffold composition and properties required for osteogenic differentiation of stem cells.

Conclusions:
Two spinneret electrospinning is a straightforward method to produce nHA gradient libraries. This method is also capable to fabricate cross-gradient libraries of other biomaterials. The gradient libraries enable screening of cellular responses of many nanofiber scaffold composition with a single scaffold specimen. The combinatorial screening approach can be utilized to systematically identify scaffold composition and properties that optimize cell responses for tissue engineering applications.

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Friday, 17 September 2010: 1230 – 1245 hours

A NEW FAMILY OF ELASTOMERIC BIOCOMPOSITES WITH A POTENTIAL OF WIDE APPLICATIONS IN TISSUE ENGINEERING

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Aim:
Biodegradable elastomeric biomaterials have gained much attention in the field of tissue engineering. The purpose of this work was to develop a new family of elastomeric composites that have remarkable mechanical functions with a potential of wide applications in soft tissue engineering.

Method:
The elastomeric composites were fabricated from a group of elastomers, poly(polyol sebacate) (PPS) and Bioglass®, using the extrusion technique.

Results:
The newly developed elastomeric composite showed significantly improved cytocompatibility compared to the pure polymeric counterpart, evidenced by the comparable cell proliferation to clinically applied polymers. More importantly, the composites demonstrated remarkable mechanical features. (1) Unlike previous reports, the addition of microsized Bioglass® increases elongation at break from 160 to 550%, while tuning up the Young modulus of the composites. (2) The mechanical strength of PPS-Bioglass® composite drops abruptly when in contact with an aqueous solution, and the level of drop can be tuned by the percentage of Bioglass® in the composite such that the desired compliance of biomaterials by soft tissue engineering is maintained. (3) The degradation of the composites has a mechanically stable state over an extended period after one day soaking. This is another important feature of the new composites, as the stable mechanical strength provides reliable mechanical support to damaged tissues in the lag phase of healing process.

Conclusions:
These unique mechanical properties, together with improved biocompatibility, make this family of composites better candidates than plastics and related composite biomaterials for the applications of tissue engineering.

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Session 8C: Synthetic Scaffolds & Hydrogels
Friday, 17 September 2010: 1245 – 1300 hours

INCORPORATION OF TRIPOLYPHOSPHATE NANOPARTICLES INTO FIBROUS POLY(LACTIDE-CO-GLYCOLIDE) SCAFFOLDS FOR TISSUE ENGINEERING

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Aim:
Poly(lactide-co-glycolide) (PLGA) has been widely used for scaffolding materials in tissue engineering. It degrades mainly via hydrolysis of the ester bonds into lactic acid and glycolic acid leading to the decrease in pH of the surrounding microenvironment. The current study was designed to quickly neutralize the acidic degradation products of PLGA fibrous scaffolds by incorporating tripolyphosphate (TPP) nanoparticles into PLGA fibers.

Method:
A homogeneous mixture of PLGA and TPP was first obtained by water-in-oil emulsion-dispersion followed by freeze-drying. The dried blend was melt-spun to yield fibers which were processed into scaffolds and subsequently immersed into phosphate-buffered saline (PBS) to verify the degradation properties. The pH of the saline was monitored for a duration of 80 days. The amount of TPP was optimized to obtain a PLGA based scaffolds without acidic degradation problems. Cellular compatibility of the modified and pristine scaffolds was evaluated using rabbit adipose-derived stem cells (rASCs).

Results:
It was shown that TPP particles within the fibers were roughly 100 nm in diameter and mainly located inside fibers instead of on the superficial layer. The acidic degradation of PT-16 and PT-64 (PT-X is termed when the monomer molar ratio of TPP to PLGA was 1:X) was significantly improved as the pH values of their respective solutions were maintained in a well neutralized state during the degradation. PT-64 and PT-16 scaffolds could well support the attachment and proliferation of rASCs.

Conclusions:
Hence, the incorporation of TPP nanoparticles via an emulsion-dispersion method could be an effective strategy to improve/adjust the acidic degradation of PLGA and further pave the way for clinical applications of such polyesters.

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Session 8C: Synthetic Scaffolds & Hydrogels
Friday, 17 September 2010: 1300 – 1315 hours

FABRICATION OF THICK CELL SHEET VIA ELECTROCHEMICAL REACTIONS ON POROUS MEMBRANE CULTURE SUBSTRATE

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Aim:
The purpose of this study is to fabricate a transplantable thick cell sheet by employing the electrochemical detachment of cells from a porous membrane culture substrate.

Methods:
The oligopeptide, CCRRGDWLC, was designed to contain an arginine-glycine-aspartate (RGD) domain in the center and cysteine at both ends. Since cysteine contains a thiol group, the oligopeptide was chemically adsorbed onto a gold layer on a porous membrane via the formation of a gold-thiolate bond. Fibroblasts seeded on the membrane were grown to form a thick cell layer. Then, by applying a negative potential, the gold-thiolate bonds were reductively cleaved, and the cell sheet was detached along with the desorption of the peptide.

Results:
To evaluate the cell detachment quantitatively, cells were attached to the membrane substrate at a low density, and the detached cells were counted after the application of the potential. Over 90% of the cells detached within 5 min. Because of the oxygen supply through the membrane, the cells attached to the substrate grew and formed a 50-μm-thick cell sheet after 14 days of culture; this sheet is significantly thicker than the sheets formed in a conventional culture dish. The proposed approach was further employed to stack the detached cell sheets to obtain 200–300 μm thick multilayered sheets.

Conclusion:
Thick cell sheets were prepared via electrochemical cell detachment. This cell sheet engineering approach appears to be a promising tool for tissue engineering and regenerative medicine applications.

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Friday, 17 September 2010: 1315 – 1320 hours

PREPARATION OF CRYOPOLYMERS FOR BONE ENGINEERING

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[b] *Instituto de Ciencia y Tecnología de Polímeros, CSIC, Madrid, Spain; CIBER-BBN, Spain*

The aim of this work is to test the ability of the cryopolymerization technique to produce scaffolds for bone engineering. Cryopolymerization is a processing procedure that generates highly hydrophilic and elastic porous materials. This study involves the manufacture and optimization of composite scaffolds based on 2-(Dimethylamino) ethyl Methacrylate (DMAEMA), 2-hydroxyethyl methacrylate (HEMA) and a bioactive ceramic -tricalcium phosphate (TCP). DMAEMA is both a pH-responsive cationic poly-electrolyte, and a thermo-sensitive polymer. HEMA is a non-ionic polymer with a reported good biocompatibility and haemocompatibility. TCP is a highly reactive ceramic that supports adhesion, differentiation and proliferation of mesenchimal stem cells and osteoblasts.

Cryogels were prepared by free radical copolymerization in an aqueous solution containing the ceramic component, the monomers, the initiator ($Na_2S_2O_7$), the activator (N,N,N´,N´-Tetramethylethylene diamine) and the croslinkers at -20ºC. Specimens with different monomer/water ratio (5-40), ceramic content (0-20%) and crosslinker concentration (0-2%) were prepared.

25 mm long and 13 mm width porous specimens were obtained for all the compositions assayed. Total monomer amount was optimized at 10%. Greater percentages yield specimens with isolated pores. Crosslinker/monomer ratio was optimized to a 1:300 ratio. The thawing rate was a factor that affected critically the properties of the cryogels. The density of the freeze dried specimens is a 28 % lower than the density of the specimens that were thawed before extracting the solvent suggesting that polymerization continues during the heating process. SEM images also show specimens with thicker, denser struts and smaller pore sizes for the thawed specimens than for the freeze dried specimens. A pore size distribution between 50 m and 1 mm was obtained.

DMAEMA/HEMA ratios up to 25/75 were studied. Greater porosity (75%) and pore size (1 mm) was obtained for a 75/25 monomer ratio. Swelling ability is also greater for 75/25 specimens, up to 400%, than for any other composition. However elastic modulus, 1.5 kPa, is not significantly lower than the modulus of denser compositions.XRD patterns of ceramic loaded specimens shows that the reinforcing phase is TCP. No transformation into apatite could be observed. 5% ceramic loaded specimens produce an increase in the elastic modulus of the specimens from 1125 to 1161 Pa for a 75/25 specimen while not affecting significantly the porosity of the specimens

As a summary, thawing/solvent extraction steps play a crucial role in the porosity of obtained scaffolds. Scaffolds with a 75% of porosity and pore sizes up to 1mm can be obtained with the cryopolymerization technique. It is possible to incorporate highly reactive TCP into these scaffolds.

Acknowledgements:
This work was supported by grant No. MAT2007-63355, CICYT, Spain and fellowship CNPQ, Brazil.

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Friday, 17 September 2010: 1320 – 1325 hours

IN VITRO COMPARATIVE STUDY OF MSC SEEDING ON WHITE AND DARK POLY(CAPROLACTONE) TRIFUMARATE SCAFFOLD

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Aim:
Dark poly(caprolactone) trifumarate (PCLTF) is a successful candidate for bone tissue engineering scaffold. Recent development of this polymeric scaffold has introduced a whiter form which takes less synthesization time and is convenient for tissue staining work. This is an in-vitro comparative study on both the white and dark PCLTF scaffolds.

Method:
White PCLTF scaffold was synthesized using PCL triol (900 M.W.), fumaryl chloride, and potassium carbonate at a molar ratio of 0.9:1:1.5 and dissolved in methylene chloride. Dark PCLTF scaffold was synthesized as above but triethylamine was substituted with potassium carbonate and dissolved in ethyl acetate. Both white and dark PCLTF macromer were chemically crosslinked before molded in cylindrical scaffold (Ø 8 x 2 mm). Biodegradability of the two scaffolds was measured by percentage weight loss on day 3, 7, 10, 14 and 21 (n=5). Static seeding was employed using rat bone marrow mesenchymal stromal cells (MSC) in which 20µl of 2 M cells/ml was seeded directly onto scaffold surface and incubated in 5% CO_2 at 37°C. Seeded scaffolds were tested for biochemical assays on day 3, 7, 14, 21 and 28 and processed for scanning electron microscopy (SEM) on day 1, 14 and 28.

Results:
Both synthesized white and dark PCLTF macromer were characterized by the presence of -C=C-H and C=C bonds via FT-IR and NMR spectroscopy. Prior to seeding, MSC was characterized by the presence of α-smooth muscle actin (ASMA), CD105+, CD31-, CD45- via immunofluorescence and flow cytometer. Dark scaffold is significantly higher in weight loss by day 21 ($p<.01$), yielding 28.44 ± 9.50% compared with white scaffold 9.26 ± 0.42%. ECM (collagen, proteoglycan/GAG and elastin) and DNA content have increased up to day 28 with no significant difference between the white and dark PCLTF scaffolds. MTT assay exhibited false positive (purple) on both white and dark PCLTF. SEM revealed significant cellular attachment up to day 28.

Conclusions:
Preliminary result showed that there was no cell growth preference in either two PCLTF scaffolds, with white scaffold having a slower biodegradability rate. Overall, this successful in-vitro study has suggested that the improved version of the white PCLTF scaffold is suitable for tissue engineering scaffold.

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Friday, 17 September 2010: 1325 – 1330 hours

INVESTIGATION OF OXYGEN DIFFUSION THROUGH POROUS SCAFFOLD FOR SIMULATING MASS TRANSPORT PHENOMENON

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[2] *Division of Biosciences and Biotechnology, POSTECH, Korea*
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Aim:
Oxygen and nutrients dissolved in the medium are transported by diffusion from the surface of the scaffolds within the limit of 100 μm . Therefore, the primary challenge for tissue engineering of large and complex organs is to construct a vascular supply for nutrient and oxygen transport. This research addresses the measurement of the effective oxygen diffusion coefficient according to scaffold porosity and the effect of cell proliferation. The resultant findings can form the basis for designing artificial vascular networks in scaffolds.

Method:
Porous scaffolds were fabricated using a salt leaching method with poly(e-caprolactone). The scaffold was located between the donor and receiver chambers, which were filled with water having high and low oxygen concentrations, respectively, such that oxygen diffused from donor to the receiver chamber. The oxygen concentration in the donor chamber was fixed and the change of that in the receiver was measured every 5 minutes for 200 minutes. Using these data, the effective diffusion coefficient was calculated based on Fick's law. Two kinds of scaffold having different porosities (66% and 53%) were used for the experiments. To investigate the effect of cell proliferation on the effective diffusion coefficient, NIH3T3 cells were cultured on scaffolds for 5 days.

Results and Conclusions:
The scaffold having the higher porosity showed a higher effective oxygen diffusion coefficient compared to the scaffold with lower porosity. Moreover, the effective oxygen diffusion coefficient decreased with cells proliferation. Therefore, one must consider the effect of cell proliferation on the oxygen diffusion phenomenon in design of artificial vascular network.

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Session 8D: Novel Therapeutic Targets
Friday, 17 September 2010: 1130 – 1200 hours

THERAPEUTIC TARGETS FOR SPINAL CORD INJURY: NOVEL BIOLOGY GLEANED FROM MULTIMODALITY STUDIES

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[2] Department of Chemical Engineering, MIT, Cambridge, MA, USA
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Clinical therapy for traumatic spinal cord injury (SCI) still remains elusive. The biology of neural stem cells (NSCs) makes them ideally suited to reconstructing the damaged central nervous system (CNS) through cell replacement. Emerging evidence, however, increasingly suggests that NSCs may repair the CNS through multimechanistic strategies that are often concurrent. They may serve not only as tissue engineering mediators, but also as investigative reporters to search for novel therapeutic targets. We recently applied retrievable drug-releasing polymer seeded with human NSCs (hNSCs) or mesenchymal stromal stem cells (MSCs) in SCI models using rats or GFAP$^{-/-}$ Vimentin$^{-/-}$ (GV) mice. Buoyed by tangible results derived from these studies we propose to discuss that how a polymer-based retrievable implant containing stem cells may hold marked promise for providing a unique range of insight regarding essential neurological mechanisms/targets required for repairing the adult mammalian spinal cord after injury. We will present data elucidating molecular events underlying rapid loss of donor cells in acutely injured spinal cord, and counteracting strategies proved effective in SCI rats using a retrievable scaffolds seeded with hNSCs that was shielded by drug-releasing polymer. Additionally, data obtained by adopting similar approaches will be analyzed for understanding the critical role of distal spinal cord adaptation in the processes of invoking neuroplasticity and rehabilitation post SCI in GV mice. Our findings may provide a multimodality bioengineering approach for investigating new neurobiological mechanisms that could serve as therapeutic targets and help formulate treatment tactics for enhancing clinically meaningful functional restoration following traumatic SCI.

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Friday, 17 September 2010: 1200 – 1230 hours

RESORTING TO NATURE FOR INSPIRATION TO ENGINEER SCAFFOLDS FOR REPAIR AND REGENERATION OF SKELETAL TISSUE

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There is increasing demand for synthetic materials that can regenerate lost or diseased bone and cartilage. Clinically available modalities for treating large bone defects are limited in their success. Significant challenges remain in the regeneration of these biomechanically functional tissues. Implanted materials are known to affect cellular physiology and function. Using the basis of "functional tissue engineering" we have developed novel 3D scaffolds with clinically relevant attributes for skeletal tissue and vascular ingrowth. These scaffolds exhibited mechanical properties that are superior to the clinically available ones, as measured by compressive strength. Such highly porous, interconnected and mechanically strong scaffolds are suitable for treating large bone defects in load-bearing applications.

Scaffolds and biomaterials used for skeletal tissue regeneration need to be biocompatible, osteo-inductive, osteoconductive and mechanically compatible with bone/cartilage to meet the requirements for skeletal tissue engineering. The current generation of synthetic scaffolds does not combine the required porosity, mechanical properties and bioactivity.

This presentation will highlight some of our newly developed novel highly porous and mechanically strong scaffolds that promote the migration, proliferation and differentiation of bone and endothelial cells for effective skeletal tissue integration and vascularization. Innovative biodegradable and bioactive biomaterials for bone/cartilage augmentation will permit greater control over the location and quality of bone regeneration, allowing faster healing.

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Session 8D: Novel Therapeutic Targets
Friday, 17 September 2010: 1230 – 1245 hours

BRAIN RESPONSE POST IMPLANTATION OF ELECTROSPUN SCAFFOLDS FUNCTIONALISED WITH BRAIN DERIVED NEUROTROPHIC FACTOR

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Aim:
To implant functionalised nanofibrous scaffolds within the rat brain evaluating both the endogenous neurite infiltration and associated inflammatory response within the brain parenchyma. Optimised nanofibrous scaffolds will ultimately be targeted at repairing damaged neural pathways in the brain and spinal cord.

Method:
Polycaprolactone (PCL) was electrospun and functionalised with brain-derived-neurotrophic factor (BDNF). The attachment was then confirmed using ELISA before they were implanted within the caudate putamen of adult Wistar rats. At various time points (1,3,7,14,21,28 and 60 days) the brains were removed and sectioned into 40 μm slices, before being processed for immunohistrochemistry. We used antibodies for: neurones, axons, astrocytes, microglia/macrophages, cell proliferation, programmed cell death and synapse formation.

Results:
Implantation of unmodified electrospun PCL scaffolds in the brain produced a foreign body reaction that peaked after 4 days (microglia) and 7 days (astrocytes) before it began to recede to homeostatic levels. Interestingly we found little evidence of scar formation (astrocytic encapsulation) and that this was independent of the fiber alignment. Neurite penetration was influenced by the fiber alignment, with it only being observed on randomly orientated nanofibrous scaffolds. When BDNF was tethered to the surfaces of the fibers, there was less gliosis and it returned to basal levels more rapidly. However, the BDNF did encourage astrocytes to migrate into the scaffolds, which was not observed for untreated PCL. Furthermore, the BDNF turned off axonal sprouting within the brain parenchyma. This is interesting as such sprouting is well documented following CNS injury and was observed following implantation of the unmodified scaffolds.

Conclusions:
The immobilisation of BDNF to electrospun scaffolds has a profound effect on the in vivo response within the adult brain. While it can control the severity of the immune reaction, a combination of proteins is likely to be required for CNS regeneration, as it eliminates axonal sprouting following injury.

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Friday, 17 September 2010: 1245 – 1300 hours

LIVER TARGETTED SIRNA DELIVERY USING PULLULAN-PEI CONJUGATES

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Aim:
Efficient and stable siRNA delivery to the target tissue for various diseases is important in the use of the nucleic acid medicines. We previously found the very high affinity of the pullulan with liver parenchymal cells. In the present study, a novel pullulan-polyethylenimine (PEI) conjugates has been developed as liver targeting siRNA carrier molecules and their targeting efficiency has been evaluated in vitro and in vivo.

Method:
Pullulan is a water-soluble polysaccharide consisting of three α-1,4-linked glucose polymers with different α-1,6-glucosidic linkages. Sequences of anti-apoB siRNA used in this study were as follows: 5'-GUCAUCACACUGAAUACCAAUdTdT-3' (sense) and 5'-dTdTCACAGUAGUGUGACUUAUGGUUA-3' (antisense) for treating. The siRNA were labeled with fluorescent probes. PEI/siRNA or pullulan- PEI/siRNA complexes were injected into mice through the tail vein, and the their live accumulation tendency was evaluated.

Results:
The incidence of mortality was found to increase with an increase in the cationic/anionic (C/A) ratio of PEI/siRNA complexes. Introduction of pullulan moiety to PEI dramatically reduced mouse death after systemic injection. After systemic injection, the PEI/fluorescein-labeled siRNA complex increased the level of fluorescence in the lung and the PEI-pullulan/siRNA complex led to an increased fluorescence level in the liver.

Acknowledgment:
This work was supported by a research grant from the Ministry of Health, Labour and Welfare (MHLW) and by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO) Japan.

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Session 8D: Novel Therapeutic Targets
Friday, 17 September 2010: 1300 – 1315 hours

MIXED MICELLES TO DELIVER DRUGS FOR ENHANCING SKELETAL MUSCLE REGENERATION

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Aim:
Current treatments for the muscle wasting and weakness common in many diseases, such as muscular dystrophy, sarcopenia and cancer cachexia, tend to have short half-lives *in vivo* and may promote side-effects, such as cardiac hypertrophy. This study aimed to develop safer and more effective methods of treating muscle wasting and weakness, by means of controlled release of the β_2-adrenoceptor agonist, formoterol, from tailored microsphere delivery vehicles.

Method:
Formoterol was encapsulated in mixed triblock copolymer micelles, for controlled release from within gelatin microspheres, produced by a water-in-oil emulsion technique and cross-linked with glutaraldehyde. The release of formoterol from microspheres incorporating micelles of different compositions was measured *in vitro* using phosphate buffered saline containing collagenase to simulate *in vivo* degradation of gelatin.

Results:
Direct loading of formoterol into gelatin microspheres resulted in complete release of formoterol within the first hour in buffer solution. The micelles slowed release significantly, with rapid formoterol release observed over the first 12 hours and then gradual release over 7 days. Altering the composition of the mixed micelles allowed some control over release rates while maintaining the bioactivity of formoterol.

Conclusions:
The incorporation of micelles to entrap formoterol slowed the release kinetics of the drug from gelatin microspheres. Once fine-tuning of the release has been completed *in vitro*, the ability of the loaded microspheres to improve muscle function and treat muscle wasting will be tested *in vivo*.

Supported by a project grant from the National Health & Medical Research Council (454461).

ORAL PRESENTATION – ABSTRACT 142
Session 8D: Novel Therapeutic Targets
Friday, 17 September 2010: 1315 – 1330 hours

THE EFFECT OF THE AGAL EPITOPE IN THE RESPONSE TO ECM IN A NONHUMAN PRIMATE MODEL

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The Galα1,3Galβ1,4GlcNAc-R (Gal) Epitope is a major factor in the hyperacute rejection of xenograft organ transplants in humans and old world primates. Biologic scaffold materials used for tissue reconstruction and composed of xenogeneic extracellular matrix (ECM) may contain the Gal epitope. However, the effect this epitope has upon the host response is controversial. The present study investigated the effect of the Gal epitope upon the host response to a porcine-derived ECM in an African Green monkey (*Cholrocaebus aethiops*) abdominal wall resection model. Histologic methods, serology, complement-dependent cytotoxicity (CDC) and gene expression profiling were used to evaluate the host response to allogeneic and both wild-type and Gal-deficient xenogeneic scaffold materials. Although expression of the Gal epitope induced an increase in serum anti-Gal antibodies in recipients, no other differences were noted in the host response between test articles. All ECM scaffolds were well tolerated and showed constructive remodeling during the test period. Recipients of all test articles showed no histologic or humoral evidence of sensitization when a second scaffold was implanted 45 days after the original surgery. The findings of the present study show that the Gal epitope within porcine-derived ECM scaffold material causes a serum antibody response, but no adverse effect upon tissue remodeling.

KEYNOTE ORAL PRESENTATION – ABSTRACT 162
Session 9A: Craniofacial 3
Friday, 17 September 2010: 1430 – 1500 hours

PERIODONTAL REGENERATION WITH PERIODONTAL LIGAMENT-DERIVED CELL SHEETS: A TRANSLATIONAL RESEARCH FOR THE CLINICAL TRIAL

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Aim:
The final goal of periodontal therapy is regeneration of the entire periodontal tissue including alveolar bone, periodontal ligament (PDL), and cementum. We previously showed that PDL-derived cell sheets fabricated on temperature-responsive culture dishes regenerated true periodontal tissue in a canine model. We therefore intend to start a clinical application of the cell sheet tansplantation and are exploring a safe and effective protocol of application to the patients. Previous studies suggested that several cell sources have an ability to regenerate periodontal tissues. In this study, we compared the regenerative abilities of cell sheets from different cell sources in a canine model.

Methods:
Cells were harvested from bone marrow, periosteum, and PDL of four dogs, respectively. Cell sheets were created on temperature-responsive culture dishes and transplanted to the surgically-created one-wall infrabony defects (5 mm x 5 mm x 5 mm) autologously. The bone defects were filled with β-tricalcium phosphate. The regeneration of periodontal tissues was evaluated from hisological and histomorphometrical analysis.

Result:
The thickness of newly formed cementum and the ratio of fibers vertically inserted to the root surface in the PDL group were significantly higher than those in the bone marrow and the preiosteum groups. No significant differences were observed in both the ratio of bone regeneration and the apical extension of junctional epithelium within all groups.

Conclusions:
These results indicate that PDL tissue is a suitable cell source for the periodontal regeneration.

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Session 9A: Craniofacial 3
Friday, 17 September 2010: 1500 – 1515 hours

RESORBABLE COMPOSITE SCAFFOLDS FOR CRANIOFACIAL BONE TISSUE ENGINEERING

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Aim:
Bone loss associated with trauma, osteo-degenerative diseases and tumors has tremendous socioeconomic impact related to personal and occupation disability and health care costs. In the present climate of increasing life expectancy with an ensuing increase in bone-related injuries, orthopaedic surgery is undergoing a paradigm shift from bone-grafting to bone engineering, where a scaffold is implanted to provide adequate load bearing and enhance tissue regeneration. We aim to develop composite scaffolds for bone tissue engineering applications to replace the current gold standard of autografting.

Methods:
Medical grade polycaprolactone-tricalcium phosphate (mPCL/TCP) scaffolds (80/20 wt%) were custom made using fused deposition modelling to produce 1x1.5x2 cm sized implants for critical-sized pig cranial implantations, empty defects were used as a control. Autologous bone marrow stromal cells (BMSCs) were extracted and pre-cultured for 2 weeks, dispersed within fibrin glue and injected during scaffold implantation. After 2 years, microcomputed tomography and histology were used to assess bone regenerative capabilities of cell versus cell-free scaffolds.

Results:
Extensive bone regeneration was evident throughout the entire scaffold. Clear osteocytes embedded within mineralised matrix and active osteoblasts present around scaffold struts were observed. Cell groups performed better than cell-free scaffolds.

Conclusions:
Bone regeneration within defects which cannot heal unassisted can be achieved using mPCL/TCP scaffolds. This is improved by the inclusion of autogenous BMSCs. Further work will include the inclusion of growth factors including BMP-2, VEGF and PDGF to provide multifunctional scaffolds, where the three-dimensional (3D) template itself acts as a biomimetic, programmable and multi-drug delivery device.

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Session 9A: Craniofacial 3
Friday, 17 September 2010: 1515 – 1530 hours

GMP-GRADE CULTURED HUMAN ORAL MUCOSAL EPITHELIAL CELL GRAFTS FOR TREATMENT OF ESOPHAGEAL ULCERATION BY ENDOSCOPIC SUBMUCOSAL DISSECTION

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Aim:
In previous study, since cultured epithelial cell grafts were demonstrated its effectiveness in promoting re-epithelialization and suppressing inflammation causing esophageal scarring and stenosis after endoscopic submucosal dissection (ESD) in animal model, human oral mucosal epithelial cells (hOEC) were fabricated in conformity with Good Manufacturing Practice (GMP) guideline as a pre-clinical study.

Methods:
hOEC derived from seven healthy volunteers were cultured on temperature-responsive cell culture inserts in GMP-grade Cell Processing Center. Cultured hOEC were harvested as an intact contiguous cell sheet from temperature-responsive surface by reducing temperature. The hOEC graft was transplanted on canine esophageal ulcer model after ESD. Transplanted hOEC sheet and canine esophagus were subjected to histological analysis.

Results:
The reproducibility of the fabrication of hOEC sheets was confirmed in the method and the results indicated that animal-derived materials such as 3T3 feeder layer and fetal bovine serum were successfully excluded from the culture condition. Furthermore, the environment of culture room and safety cabinet was maintained to be sterility assurances during the fabrication. hOEC sheets were confirmed to graft on canine esophageal ulcer model after ESD.

Conclusions:
hOEC sheets were fabricated without anima-derived materials and suggested its efficacy as a medical device that promote the re-epithelialization of esophageal ulcer after ESD.

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Session 9A: Craniofacial 3
Friday, 17 September 2010: 1530 – 1545 hours

PIG MANDIBULAR RECONSTRUCTION BY ADIPOSE-DERIVED STEM CELLS AND FUNCTIONALIZED LASER-SINTERED POROUS PCL SCAFFOLD WITH PLATELET RICH PLASMA :IN-VITRO AND IN-VIVO STUDY

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Aim:
Polycaprolactone (PCL) is a bioresorbable polymer with potential applications for bone and cartilage repair. In this work, the three-dimentional and porous PCL scaffolds were designed and it was fabricated via selective laser sintering (SLS). However, pure PCL is not suitable for cell attachment. The aim of this study is to evaluate the osteogenic potential of adipose-derived stem cells (ASCs) in functionalized laser-sintered PCL scaffold with platelet rich plasma (PRP).

Method:
In the in-vitro study, the laser–sintered PCL scaffold was seeded with ASCs. It was divided into three groups. Group I: PCL/ASCs were cultured in osteogenic medium. Group II: PCL/ASCs were cultured in control medium. Group III: PCL/PRP/ASCs were cultured in osteogenic medium. Alkaline phosphatase activity, RT-PCR of ALP, osteocalcin, RunX II were used to assess the osteogenic ability. SEM and confocal microscope were used to observe the interaction between scaffold and cell. In In-vivo study, the 3 cm porcine mandible defect was created and it was reconstructed with either PCL only or PCL/PRP/ASCs. CT was used to evaluate the bone regeneration 3 months, 6 months after operation. The Young's modulus of both groups was measured and compared with normal bone. H&E stain and IHC stain of osteocalcin, collagen type I were done for confirmation of bone regeneration.

Results:
In in-vitro study, alkaline phosphatase activity and RT-PCR all showed the best osteogenic potential in group III(PCL/PRP/ASCs) comparing with other groups. SEM and confocal microscope showed the cells were well attached to PCL in group III. All these data confirms that the PCL combined with PRP was suitable for osteogenic differentiation and attachment of ASCs. In in-vivo study, both groups showed new bone regeneration in PCL scaffold. However, the bone density was less and loose in PCL group and the Young's modulus was only 30% of normal bone. In contrast, the continual and firm bone formation was found in PCL/PRP/ ASCs gorup and the Young's modulus was 90% of normal bone. H&E stain, IHC of osteocalcin, collagen type I all proved the new generation tissue was bone.

Conclusions:
In conclusions, modification of the laser-sintered PCL scaffold by PRP enhances the affinity and osteogenic potential of ASCs.

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Session 9A: Craniofacial 3
Friday, 17 September 2010: 1545 – 1600 hours

BONE REGENERATION BY OCTACALCIUM PHOSPHATE (OCP) COLLAGEN COMPOSITES IN CANINE ALVEOLAR CLEFT MODEL

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Aim:
Octacalcium (OCP) and porcine atelocollagen composites (OCP/Col) clearly enhanced bone regeneration in a rat cranial defect model and dog extraction socket model. In this study, we examined whether OCP/Col would enhance bone regeneration in an alveolar cleft model in an adult dog, which was assumed to reflect patients with alveolar cleft.

Method:
Disks of OCP/Col or collagen were prepared and implanted into the artificial alveolar cleft model, and bone regeneration by OCP/Col or collagen was investigated by macroscopically, radiographically, histologically, and crystallographically at four and ten months after implantation.

Results:
Macroscopically, the OCP/Col-treated alveolus was apparently augmented. And the augmented alveolus was occupied by radiopacity, also the boundary between the original bone and the OCP/Col-treated defect was indistinguishable. In histological analysis, newly formed bone was filled and bridged with the defect, and a small quantity of the remaining implanted OCP granules was detected. The X-ray diffraction patterns of OCP/Col implanted for four months were no difference with those of dog bone. In collagen-treated alveolus, the concaved alveolar ridge was still remained and it was mostly filled with fibrous connective tissue, while small amount of newly formed bone was observed at the defect margin.

Conclusions:
Bone repair had apparently originated if OCP/Col was implanted into the artificial maxillary alveolar cleft model in an adult dog, and OCP/Col would be notable bone regenerative material to substitute for autogeneous bone.

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Session 9B: Biological Scaffolds 3
Friday, 17 September 2010: 1430 – 1500 hours

ELASTIC BIOMATERIALS THAT UTILIZE HUMAN TROPOELASTIN

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Aim:
We have defined the molecular basis of tropoelastin-directed interactions and developed prototype synthetic elastin-based biomaterials that exploit the molecule's unique structural and cell signaling properties. These constructs include synthetic vascular conduits for small-diameter applications and elastic dermal substitutes.

Method:
Elastin is a vital protein component of the elastic, extracellular matrix in vasculature, skin and lungs. Elastin is derived from crosslinked tropoelastin and contacts cells responsible for elastogenic production and maintenance.

Results:
Tropoelastin enhanced endothelial cell attachment and proliferation, and was incorporated into a conduit to mimic mechanical properties of the human internal mammary artery. The synthetic graft was primarily recombinant human tropoelastin and responded elastically at low pressures, displaying a burst pressure equivalent to that of the human internal mammary artery. Tropoelastin and synthetic elastin demonstrated low thrombogenicity. Initial implantation revealed that these grafts suture well and are impermeable. In parallel studies, synthetic human elastin dermal substitutes supported primary fibroblast and keratinocyte attachment, spreading and proliferation. Open weave dermal substitutes provided for penetrating fibroblast infiltration.

Conclusions:
Tropoelastin-based constructs present biologically relevant functionality encompassing cell interactions, elasticity and versatility. Applications include suturable vascular conduits and dermal substitutes.

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Session 9B: Biological Scaffolds 3
Friday, 17 September 2010: 1500 – 1515 hours

CURRENT PROGRESS TOWARDS UNDERSTANDING AND RE-ENGINEERING RESILIN-INSPIRED PROTEINS AND BIOMATERIALS.

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Aim:
To better understand the role of gene and protein sequence, molecular weight and cross-linking chemistry in determining favorable expression and biomaterial properties in resilin-inspired proteins (RIPs), and to exploit this information for future development of tunable high-resilience and durable biomaterials.

Method:
To overcome problems of solubility and expression levels in RIPs, we are currently optimising a directed evolution approach to generate libraries of chimeric constructs (resilin and resilin-silk block copolymers), each unique in nucleotide and/or amino acid sequences that will be assessed in high-throughput expression systems.

To increase the Young's modulus of RIPs, we are comparing 3 approaches to increase modulus: modification of the crosslinking chemistry, increasing the primary molecular weight of RIPs, and introduction of structural motifs (e.g. silk domains) to create novel RIPs.

Results:
Through sequence analysis of constructs generated, together with solubility and expression analysis and biomaterials testing of candidate proteins, we hope to gain insights regarding the role of sequence composition in expression, solubility and ultimately biomaterial properties of RIPs. This will increase our capability to generate favorable constructs in the future.

Modifying the crosslinking chemistry and increasing the primary molecular weight of RIPs both yield increased modulus (up to 20-fold) for existing RIPs, with further increases anticipated through combination of the approaches. Block co-polymers are currently under investigation.

Conclusions:
RIPs with optimal purification characteristics will allow us to engineer resilient, durable and strong biomaterials that are tuneable for targeted biomedical applications. Data being obtained at present will be critical in designing these proteins and biomaterials.

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Session 9B: Biological Scaffolds 3
Friday, 17 September 2010: 1515 – 1530 hours

DESIGN OF NOVEL COLLAGEN-LIKE PROTEIN SCAFFOLDS FROM STREPTOCOCCUS PYOGENES

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Aim:
To develop recombinant collagen-like proteins from bacteria for design of novel, functional scaffolds for tissue engineering and regenerative medicine.

Method:
The recombinant collagen-like domain of S. pyogenes Scl2.28 was cloned in pCold III and expressed in E. coli. Expressed protein was purified using Ni-NTA-sepharose and other chromatography procedures. The collagen (CL) domain was made by tryptic digestion and purified by chromatography. Various constructs were designed to incorporate specific biological motifs (heparin binding motif, MMP sites, integrin site) into the backbone collagen sequence, and between repeating CL domains.

Results:
Cell compatibility of CL protein constructs were examined using 3 cell lines and showed that the CL protein was not cytotoxic [1]. For immunogenicity evaluation, 2 strains of mice were immunised with and without adjuvant (IF). Neither strain showed any significant antibody response even when adjuvant was used. The injections did not lead to adverse reactions, indicating that acute and sub-chronic toxicity were absent. Freeze dried CL protein was stabilised by glutaraldehyde giving a material that was stable at >37°C and which supported cell attachment while not causing loss of cell viability. Depending on the design of the construct, cell binding and other functionalities were observed, consistent with the design features.

Conclusions:
These data suggest that bacterial collagen-like proteins could be useful for medical and tissue engineering applications, and that constructs could be designed to specific desired cell binding, degradation and other biological functions.

[1] Peng et al. (2010) Biomaterials, 31: 2755-2761.

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Session 9B: Biological Scaffolds 3
Friday, 17 September 2010: 1530 – 1545 hours

IN VIVO TISSUE RESPONSE AND *IN VITRO* DEGRADATION OF CHITOSAN-PBS BASED SCAFFOLDS

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[3] *Institute for Health and Life Sciences - ICVS, University of Minho, Campus de Gualtar, Braga, Portugal*

Aim:
Previous studies showed that chitosan-poly(butylene succinate) (Ch-PBS) fiber mesh scaffolds evidenced remarkable biological performance with mouse and human MSCs[1,2]. Thus, it is required to study the kinetics of biodegradation of the biomaterials *in vivo,* since these scaffolds are intended to provide a temporary support for the cells *in vivo*.

This work consisted on the study of the biodegradation process *in vitro,* using enzymes in concentrations similar to those found in the human body, and in *vivo,* using the rat subcutaneous model.

Method:
In vitro degradation studies were carried out using lipase and/or lysozyme at concentrations similar to the human blood serum, in static and dynamic conditions for 1, 3, 6 and 12 weeks. Water uptake and weight loss measurements, micro-Computed Tomography (μ-CT) analysis (specifically porosity, interconnectivity, mean pore size and fiber thickness) were performed.

Scaffolds were subcutaneously implanted in Wistar rats for the same time periods and used for histology and immunohistochemistry analyses.

Results:
Weight loss results showed that lysozyme and/or lipase have a strong effect on the scaffolds degradation in vitro.

Implanted scaffolds showed normal and mild inflammatory response, with the formation of new blood vessels inside and around the implant. It was also observed a pronounced degradation of the scaffolds after 12 weeks.

Conclusions:
The degradation rate of the scaffolds must be tuned appropriately with the growth rate of the new tissue. It was clear that physiological levels of lysozyme have a positive effect on the scaffolds degradation. *In vivo* degradation of scaffolds was visible by micro-CT analysis, and images of H&E stained sections show the pores completely filled and fibers replaced with cells. In vivo implantation led to a normal tissue response and to some degradation after a period of 3 months.

[1] Costa-Pinto AR, Salgado AJ, Correlo VM, Sol PC, Bhattacharya M, Charbord P, Delorme B, Reis RL, Neves NM. Adhesion, proliferation, and osteogenic differentiation of a mouse mesenchymal stem cell line (bmc9) seeded on novel melt-based chitosan/polyester 3d porous scaffolds. Tissue Engineering 2008;14(6):1049-52.
[2] Costa-Pinto AR, Correlo VM, Sol PC, Bhattacharya M, Charbord P, Delorme B, Reis RL, Neves NM. Osteogenic differentiation of human bone marrow mesenchymal stem cells seeded on melt based chitosan scaffolds for bone tissue engineering applications. Biomacromolecules 2009; 10, 2067–2073

ORAL PRESENTATION – ABSTRACT 297
Session 9B: Biological Scaffolds 3
Friday, 17 September 2010: 1545 – 1600 hours

DERMAL REPAIR: ELECTROSPUN ELASTIN-BASED DERMAL SUBSTITUTES FOR THE TREATMENT OF SEVERE BURNS

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We present an elastic, fibrous human protein-based and cell-interactive dermal substitute. This novel scaffold is directed towards facilitating wound closure with minimal contracture in order to contribute to improved prognosis in severe second and third degree burns. The dermal replacement scaffold is based on synthetic human elastin.

Elastin imparts elasticity and resilience to skin through an abundant elastic fibre network that spans the skin dermis. Evidence suggests that elastin suppresses the differentiation of fibroblasts with a proliferating phenotype into contractile myofibroblasts which are responsible for excessive wound contraction and scarring following injury. The elastin precursor tropoelastin and elastin fragments are identified chemo-attractants for dermal fibroblast migration.

The production of recombinant human tropoelastin (rhTE) is tightly controlled and highly reproducible. Gram quantities of high purity protein are readily available in the laboratory and implantation studies reveal subcutaneous tolerance. rhTE is chemically cross-linked to form synthetic human elastin (SHE).

rhTE was electrospun into open-weave, fibrous scaffolds that closely mimic the fibrous nature of the skin dermis. 3D electrospun SHE scaffolds are robust and consist of flat, ribbon-like fibres with widths that are similar to those of native dermal elastic fibers. The scaffolds display elasticity close to that of natural elastin.

Fibroblasts attached, spread and proliferated on SHE fibers and infiltrated into open-weave SHE scaffolds. The cells persisted on SHE scaffolds for at least 14 days post-seeding and expressed extracellular matrix proteins fibronectin and type I collagen.

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Session 9C: Stem Cells 4
Friday, 17 September 2010: 1430 – 1445 hours

MESENCHYMAL STEM CELL DIFFERENTIATION ON LATERALLY GRADED POROUS SILICON WITH SUBMICRON PORE SIZES

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Aim:
It is known that surface topography influences cell proliferation and differentiation. Here, we aimed to investigate if changes in the diameter of submicron pore with uniform orientation will lead to changes in mesenchymal stem cell behaviour. Porous silicon, pSi, was chosen for this study because of its good biocompatibility and biodegradability. Using mesenchymal stem cells, MSCs, a specific aim of this study was to identify the optimal pore size for osteogenesis on the one hand and for adipogenesis on the other.

Method:
pSi samples were prepared from p-type silicon with a resistivity of 0.0005 Ω·cm. Silicon was etched in a 25% HF/ethanol solution with current densities of 60 mA/cm^2 for 90 s by placing a Pt electrode perpendicular to the silicon surface on one side of the silicon. After etching, the freshly etched pSi surfaces were thermally oxidised, ozone oxidised, and silanised with (3-aminopropyl)-triethoxysilane (APTES). Rat MSCs were harvested and seeded on the pSi for 6 days and then cultured in either osteogenic or adipogenic medium for 3 weeks. Osteogenesis and adipogenesis of rMSCs were examined by calcein blue, and nile red staining, respectively.

Results:
Laterally graded pSi with pore sizes ranging from several nanometers to 1 micron were fabricated. Cell attachment was dependent was sensitive to pore size. Furthermore, we identified regions on the gradient where osteogenesis and adipogenesis of MSCs occurred preferentially.

Conclusions:
The results suggest that the submicron porous structure modulates MSCs attachment and differentiation. Laterally graded pSi appears to be useful tool to screen the suitable pore size for contact guidance and stem cell behaviour.

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Friday, 17 September 2010: 1445 – 1500 hours

COMBINED ANALYSES OF MICRORNAS AND MRNA TRANSCRIPTOMES IN MESENCHYMAL STEM CELLS DERIVED FROM UMBILICAL CORDS, MILK TEETH, AND ADULT TEETH

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Aim:
Mesenchymal stem cells (MSCs) from the Wharton jelly of umbilical cords, milk teeth, and adult teeth are among various types of tissue MSCs that can be collected from such disposable tissues for future clinical use. To understand biology of these MSCs at transcriptomic levels, we have integrally analyzed these cells on both microRNAs (miRs) and messenger RNA (mRNA).

Method:
Total RNA were isolated from five MSCs for each type of origins. MicroRNAs were analyzed with quantitative RT-PCR and mRNAs with Affymetrix human U133A chips. TargetScan database 5.0 was used for prediction of the targeted genes for each miR. MetaCore database was used for pathway enrichment analysis. Statistical analyses used for miR and mRNA expression data were Mann-Whitney test, principle component analysis (PCA), Pearson's correlation analysis, and hierarchical clustering.

Results:
All MSCs derived from the Wharton jelly of umbilical cords, milk teeth and adult teeth expressed cell surface markers that are characteristic for mesenchymal stem cells, and they could be induced to differentiate into adipose tissues, bone, and cartilage. PCA and hierarchical analysis revealed that miR profiles of both teeth MSCs clustered together whereas that of cord MSCs was far apart. According to both miR and mRNA analyses, significantly highly expressed genes in teeth MSCs than in cord MSCs included *SATB2, TNFRSF11B, NQO1, PMAIP1, ADAMTS5, FGF5, ANKRD28, METTL7A, STARD13, NEDD4,* and *RASA1*. These genes are involved in ossification, bone development, regulation of apoptosis, and anatomical structure development, being compatible with the functional differences between teeth MSCs and cord MSCs.

Conclusions:
Analyses of transcriptomes usually result in differentially expressed genes at the number more than we could easily comprehend. Integral analyses from both miRs and mRNA perspectives are instrumental in converging abundant data into the core biological information.

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Session 9C: Stem Cells 4
Friday, 17 September 2010: 1500 – 1515 hours

STUDIES ON LIVER PROGENITOR CELL SPHEROIDS *IN VITRO,* AND IN A MURINE *IN VIVO* VASCULARIZED CHAMBER MODEL

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Aims:
To assess:
(**1**) ***in vitro,*** the growth/maturation of adult (BMOL) and embryonic (BMEL) murine liver progenitor cell spheroids, and
(**2**) ***in vivo***, BMOL and BMEL **spheroid** versus single cell suspension survival/development when implanted in murine tissue engineering chambers.

Methods:
In vitro: 20,000 BMOLs or BMELs were placed in 15µL of 20% methylcellulose in growth media. A single spheroid formed/well within 24 hours. Dissociation experiments determined spheroid cell number and 2 - 10 day spheroids were morphologically prepared for PAS, caspase 3, Ki 67,or broad spectrum cytokeratin labelling.

In vivo: Vascularized chambers were created on both epigastric pedicles in SCID mice.

One chamber received a single cell suspension in Matrigel™ (200,000 BMOLs or 100,000 BMELs) whilst the opposite chamber received either 9 BMOL, or 3 BMEL spheroids. Chambers are harvested at 2 and 6 weeks and morphologically evaluated.

Results:
In vitro: Two day spheroids were optimal for *in vivo* implantation - BMOL spheroids contained 22,000 cells, BMELs - 35,000 cells. Both spheroid types included central apoptotic cells (caspase 3 labelling), and proliferating cells (Ki67 positive) peripherally, with little cytokeratin/PAS labeling.

In vivo: Two week *in vivo* BMOL spheroid chambers contained liver cells, and were larger, and more vascular than BMOL single cell chambers which contained few liver cells. BMEL spheroid **and** single cell containing chambers were large, vascular and most included masses of organized mature liver cells. Six week chambers have not been harvested.

Conclusions: BMOL spheroids enhance liver cell survival and growth *in vivo*. BMELs may not require a spheroid configuration for adequate growth *in vivo.*

ORAL PRESENTATION – ABSTRACT 341
Session 9C: Stem Cells 4
Friday, 17 September 2010: 1515 – 1530 hours

BONE MARROW-DERIVED MONONUCLEAR STEM CELL IMPLANTATION IN PATIENTS WITH BUERGER'S DISEASE

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Tissue Engineering and Nanomedicine Research Center, Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Patients suffering from Thromboangiitis obliterans (TAO) have endothelial cell dysfunction and the severity of the disease lies in the need for amputation in more than a quarter of all sufferers.

We report the safety and feasibility of autologous implantation of circulating mononuclear cells for patients suffering from Buerger's disease following bone-marrow mobilization with granulocyte colony stimulating factor (5 mcg/kg/day for 5-7 days). Five patients participated in this study. Mononuclear cells were separated by Cobas Spectra cell separator. MNCs, CD34+ and CD133+ cells were enumerated prior to intramuscular injection into the affected foot/limb muscles at multiple sites on the collection day. Stem cell injection prevented disease progression in all five patients. In this small cohort of patients with critical limb ischemia, quality of life improved significantly over a two year period. Also, pain-free walking distance in all patients showed significant improvement.

Autologous bone-marrow derived mononuclear cell therapy collected from peripheral blood following G-CSF mobilization is effective, safe and results in sustained clinical results for patients with severe peripheral occlusive arterial disease.

ORAL PRESENTATION – ABSTRACT 20
Session 9C: Stem Cells 4
Friday, 17 September 2010: 1530 – 1545 hours

CELL SHEET THERAPY FOR MYOCARDIAL INFARCTION IN A PIG MODEL

Dehua Chang, Tatsuya Shimizu, Yuji Haraguchi, Masayuki Yamato & Teruo Okano
Tokyo Women's Medical University, TWIns, Tokyo, Japan

Aim:
Recently, mesenchymal stem cells (MSCs) have been reported to repair chronically infracted myocardium with direct injection to the defect area. However, it is very difficult to localize the injected cells onto the ischemia area to regenerate sufficient cardiac mass. To overcome the problem, we have utilized our cell sheet technology based on temperature-responsive culture dishes.

Method:
Bone marrow stem cells (BMSC) were harvested from the pig. Once the cells reach confluence for 7 days after being cultured, a monolayer BMSC detached itself from temperature-responsive culture dish when the temperature was reduced from 37 °C to 20 °C. A multilayer was prepared by stacking three-monolayer of cells. Myocardial infarction was created by the ligation of the left anterior descending branch of the left coronary artery. A multilayered cell sheet was then transplanted onto the ischemia area. The echocardiography was performed on the myocardial infarction model.

Results:
Multilayer cell sheet were produced that retained all cell-cell contacts. The cells in the multilayer were positive for CD29, CD90, CD146 and CD73 indicating the undifferentiated MSCs. EF and FS were changed and cardiac function was improved after cell sheet transplantation.

Conclusions:
The cell sheet improved the cardiac function in pigs with myocardial infarction. The present study represents progress toward the clinical application of cell sheet of MSCs as a powerful treatment vehicle for human heart disease.

ORAL PRESENTATION – ABSTRACT 119
Session 9C: Stem Cells 4
Friday, 17 September 2010: 1545 – 1550 hours

DIFFERENTIATION OF SIDE POPULATION CELLS ISOLATED FROM HUMAN AMNIOTIC MESENCHYMAL CELL INTO VASCULAR ENDOTHELIAL CELLS UNDER HYPOXIC CONDITION

Naoko Maruyama[1], Yuji Nishikubo[1], Kenichi Kokubo[1,2], Toshihiro Shinbo[1], Minoru Hirose[1], Mamoru Kobayashi[3], Norio Sakuragawa[3] & Hirosuke Kobayashi[1,2]
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[2] *Regenerative Medicine and Cell Design Research Facility, Kitasato University School of Allied Health Sciences, Sagamihara, Japan*
[3] *Bioresource Application Institute Co., Ltd, Gamagori, Aichi*

Aim:
Human amniotic mesenchymal side polulation (SP) cells have pluripotency and weak immunogenicity. These cells have promising features in regenerative medicine. The aim of the present study was to determine whether hypoxic condition is appropriate for the differentiation of the SP cells into vascular endothelial lineage.

Method:
The human amniotic mesenchymal cells were isolated from the enzyme-treated amniotic membrane and were stained with Hoechst33342. SP cells were negatively selected by sorting the stained human aminiotic mesenchymal cells out using FACS. The SP cells were cultivated in induction medium (DMEM/F12 + FBS + VEGF) in normoxia (20%) or hypoxia (1%) for 1 or 2 weeks. The expressions of endothelial markers as vWF, VE-cadherin, VCAM, FLk-1/KDR and Flt-1 were evaluated by real-time PCR and fluorescent immunostaining. The protein expression of Oct3/4, a stem cell marker, was also evaluated by fluorescent immunostaining.

Results:
Gene expressions of Flt-1, VE-cadherin and vWF were most pronounced after 2 weeks cultivation in hypoxia. Protein expressions of KDR and VE-cadherin were observed only after 2 weeks cultivation in hypoxia. The KDR was expressed irrespective of the conditions investigated. Expression of Oct3/4 disappeared after 2 weeks cultivation.

Conclusions:
Hypoxia enhanced the induction of amniotic mesenchymal SP cells treated with VEGF into vascular endothelial lineage.

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Session 9C: Stem Cells 4
Friday, 17 September 2010: 1550 – 1555 hours

CAN VISIBLE LIGHT ENHANCE PLURIPOTENT GENE EXPRESSION AND IMPROVE THE DIFFERENTIATION ABILITY OF AMNIOTIC FLUID STEM CELLS?

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[2] *Department of Reproduction, National Research Institute for Child Health and Development, Okura, Setagaya-ku, Tokyo, Japan*

Aim:
Stem cells derived from human second-trimester amniotic fluid (AF) have high capability to differentiate into several types of cells originated from mesenchymal lineages, and are known to have higher differentiation ability into some other types of cells originated from endoderm and ectoderm than mesenchymal stem cells derived from bone marrow. In our previous study, we demonstrated the visible light can regulate the gene expression of neural model cells. Therefore, we investigated the effect of visible light on the expression of pluripotent genes of AF stem cells. We further investigated the effect of visible light on the differentiation ability of the AF stem cells.

Method:
The AF was cultured on tissue culture flask in the medium containing 20% fetal bovine serum. The cells were exposed to blue light (470nm, 1mW/cm^2) in a CO2 incubator at 37^{o}C. RNA was extracted and changed into cDNA followed by PCR. The pluripotent genes (Oct4 and Sox2) of AF stem cells with and without irradiation of visible light were investigated. The differentiation ability of AF stem cells into adipocytes, neurons and osteoblasts was investigated and analyzed by staining methods.

Results:
The pluripotent gene expression of Oct4 and Sox2 on AF stem cells increased by the irradiation of blue light. AF stem cells also showed higher differentiation ability (osteogenic and neural differentiation) under irradiation of blue light.

Conclusions:
Visible light treatment on AF stem cells is effective to maintain their pluripotency in several passages, and leads to high ability to differentiate into osteogenic and neural cells.

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Session 9C: Stem Cells 4
Friday, 17 September 2010: 1555 – 1600 hours

CULTURE AND IDENTIFICATION OF BONE MARROW MESENCHYMAL STEM CELLS ON POLY (N-ISOPROPYLACRYLAMIDE) COPOLYMER FILM

Lei Yang[1], Tianqing Liu[1], Jianren Lu[2], Kedong Song[1], Lili Jiang[1] & Shuang Wu[1]
[1] *Dalian R&D Center for Stem Cell and Tissue Engineering, Dalian University of Technology, Dalian, China*
[2] *Biological Physics Group, School of Physics and Astronomy, The University of Manchester, Schuster Building, Manchester, UK*

Aim:
The poly (N-isopropylacrylamide) (PNIPAAm) and its derivatives can be used for cell recovery based on their unique thermo-responsive property. However little is known about whether the cell properties will change after the cells are cultured on or harvested from the PNIPAAm-containing surfaces by temperature shift. This research investigated the growth activities and biological functions of the bone marrow mesenchymal stem cells (BMMSCs) recovered by temperature drop using PNIPAAm copolymer films and compared to those harvested by trypsinization.

Method:
The purified BMMSCs from SD-Rats were characterized by immunophenotype analysis (CD29, CD44, CD45 and CD90), differentiation inspection (osteogenesis, adipogenesis and chondrogenesis), extracellular matrix (fibronectin, laminin and collagen) and transmembrane (integrin) protein detection. The identified BMMSCs were cultured on 24-well culture plates with or without PNIPAAm copolymer films respectively. When reaching confluence, the cells were recovered by temperature drop and trypsinization respectively and then replanted on other plates with or without films. After the BMMSCs were passaged using two recovery methods for three times, the cell morphology observation, immunophenotype analysis, differentiation inspection, protein detection were performed.

Results:
The experimental results indicated that the BMMSCs harvested by decreasing temperature showed no significantly different from the control groups in cell morphology, immunophenotype and differentiation, whereas more proteins were remained around or within the cell membranes after utilizing the method of low-temperature-induced cell detachment.

Conclusions: It is concluded that the harvested BMMSCs can not only preserve self-properties, but also retain adhesion-related proteins compared to those passaged by trypsinization. The recovery method of low-temperature-induced cell detachment using PNIPAAm-containing substrates may partial replace traditional enzymatic digestion especially for enzyme-sensitive cells.

ORAL PRESENTATION – ABSTRACT 67
Session 9D: Embronic Stem Cells in Tissue Engineering
Friday, 17 September 2010: 1430 – 1445 hours

MULTIPOTENT MESENCHYMAL-LIKE STEM CELLS DERIVED FROM HUMAN PARTHENOGENETIC EMBRYONIC STEM CELLS

Yao Chen, Ai Ai, Zheng Ya Tang, Guang Dong Zhou, Wei Liu, Wen Jie Zhang, Yilin Cao
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Aim:
Human parthenogenetic embryonic stem (hpES) cells are pluripotent that could differentiate into cells of all three germ layers. The aim of this study is to detect whether hpES could be differentiated into mesenchymal stem cells (MSC)-like cells with multipotent differentiation capacity, and compare these cells with fertilized egg derived embryonic stem (hES) cells.

Method:
hpES cells and hES cells were differentiated into embryonic bodies (EBs) first. Day 10 EBs were then cultured in tissue culture plate. After 7 days, cells migrated from EBs were collected and expanded. The cell surface marker expression profile and differentiation potential of expanded cells were compared between those cells established from hpES and hES cells.

Results:
Mesenchymal-like cells were achieved from both hpES cells and regular hES cells. Cells from those two types of cells exhibited similar cell surface marker expression profile. However, hpES derived MSC showed stronger osteogenic differentiation capacity but weaker adipogenic differentiation capacity compared with hES derived MSC.

Conclusion:
hpES cells could be differentiated into MSC-like cells with multipotent differentiation capacity. But the differentiation potential of hPES derived MSC were not as same as cells differentiated from regular hES.

ORAL PRESENTATION – ABSTRACT 121
Session 9D: Embronic Stem Cells in Tissue Engineering
Friday, 17 September 2010: 1445 – 1500 hours

CELL SUBSTRATE INFLUENCES THE MOTILITY AND MODE OF COLONY FORMATION OF HUMAN EMBRYONIC STEM CELLS

Jennifer Turner, Drew Titmarsh, Ernst Wolvetang & Justin Cooper-White
The University of Queensland, Australian Institute for Bioengineering and Nanotechnology, St Lucia, QLD, Australia

Aim:
Recently there has been a shift in human embryonic stem cell (hESC) culture methods from the utilisation of feeder cells to more defined systems with commercially available substrates and media. This work aims to investigate how the surface, both traditional and defined, impacts on cell motility and mode of colony formation.

Method:
Time-lapse microscopy was used to observe cell motility and colony formation of hESCs adapted to passaging as single-cells in various cell culture medium and substrates including mouse embryonic fibroblasts and matrigel. Vector maps and velocity measurements were generated using particle tracking software. Finally clonal growth was confirmed by mixing an m-Cherry expressing cell line with a normal cell line and imaging the resulting colonies.

Results:
The hESCs were observed to form colonies by one of two methods; either cell division resulting in colony formation from a single cell, or colony formation by cell migration and aggregation. Low motility was found to foster clonal growth whereas high motility cells seek others and aggregate together prior to colony formation, resulting in a colony formed from multiple cells. Cell motility and clonal growth was independent of the medium used.

Conclusions:
We conclude that the mode of colony formation of single cell adapted hESC is linked to cell substrate mediated motility of the cells. Clearly the choice of cell substrate is an important consideration in the production of clonal reporter lines and induced pluripotent stem cells as cell motility and the method of colony formation directly impact on cell clonability.

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Session 9D: Embronic Stem Cells in Tissue Engineering
Friday, 17 September 2010: 1500 – 1515 hours

DEFINING A THRESHOLD SURFACE DENSITY OF VITRONECTIN FOR THE STABLE EXPANSION OF HUMAN EMBRYONIC STEM CELLS

Lynn YW Yap[1,2], Jian Li[3], In Yee Phang[3], Lay Ting Ong[3], Jo'an Zhu-En Ow[4], James CH Goh[5], Jonathan Hobley[3], Andre BH Choo[4], Steve KW Oh[4], William R Birch[3] & Simon M Cool[1,6]

[1] *Stem Cells and Tissue Repair Group, Institute of Medical Biology, Agency for Science, Technology and Research (A*STAR), Singapore*
[2] *National University of Singapore, Graduate School for Integrative Sciences and Engineering, Singapore*
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[5] *Department of Orthopedic Surgery, Tissue Engineering Program, National University of Singapore, Singapore*
[6] *Department of Orthopaedic Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore*

Aim:
Expansion of human embryonic stem cells (hESCs) in a defined system that is free of animal components is required to realize the clinical efficacy of hESCs and their derivatives. Current method for pluripotent human embryonic stem cells (hESCs) expansion relies on Matrigel™ which is a basement membrane substrates isolated from murine sarcoma cells which precludes the use of these cells in regenerative medicine. Our aim is to determine a threshold density of a defined extracellular matrix protein, vitronectin (VN) that can support long-term hESCs culture.

Method and Results:
This study reports the successful propagation of hESCs (HES-3 and H1) for more than 20 passages on culture plates, coated from 5 μg/ml of human plasma-purified VN solution. Cells maintain the expression of pluripotent markers Tra1-60 and OCT-4 (>90%) and are karyotypically normal after 20 continuous passages. *In-vitro* and *in-vivo* differentiation of hESC by embryoid body formation and teratoma yielded cells from the ecto- , endo- and mesoderm lineages. Definitive germ layers were stained with GFAP, Desmin and Cytokeratin which confirms the pluripotentiality of these cells. Immobilized VN was characterized using a combination of X-ray photoemission spectroscopy (XPS), atomic force microscopy (AFM), and quantification of the VN surface density with Bradford protein assay. Surface density of VN was tuned, *via* the concentration of depositing solution, revealed a threshold surface density of 125 ng/cm^2, which is required for hESCs attachment and proliferation but not enough for teratoma formation.

Conclusions:
Cell attachment and proliferation assays on VN surface densities above 250ng/cm^2 show the substrate properties to be equally viable in maintaining pluripotent hESCs.

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Session 9D: Embronic Stem Cells in Tissue Engineering
Friday, 17 September 2010: 1515 – 1530 hours

SUBSTRATE STIFFNESS AFFECTS THE CHONDROGENIC DIFFERENTIATION IN EMBRYONIC STEM CELLS

Zheng Yang[1,4,5], Vinitha Denslin[2], Shy Chyi Wuang[3], Chwee Teck Lim[3,5], Eng Hin Lee[2,5]

[1] *Tissue Engineering Program, Life Sciences Institute, National University of Singapore, Singapore*
[2] *Department of Orthopaedic Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore*
[3] *Division of Bioengineering and Department of Mechanical Engineering, National University of Singapore, Singapore*
[4] *Research Centre of Excellence in Mechanobiology, National University of Singapore, Singapore*
[5] *Department of Oral Maxillo-Facial Surgery, Faculty of Dentistry, National University of Singapore, Singapore*

Embryonic stem cells (ESCs) represent a potential cell source for regenerative therapy and tissue engineering due to their indefinite self-renewal ability and the capability to differentiate into various cell types. One of the challenges in utilizing ESCs in regenerative therapy is to control their differentiation process. Stem cells differentiation is affected by biochemical cues in the form of soluble growth factors and insoluble components of extracellular matrix, and physical cues in the form of matrix mechanical properties and architecture. In this study, we examine the influence of matrix stiffness on ESC differentiation. We employed polydimethylsiloxane (PDMS) of varying stiffness with Young's modulus of 8, 16, 20, 30, 50 kPa, as determined by atomic force microscopy indentation. Human ESC cultured on the matrigel-coated PDMS was able to adhere without apparent difference in attachment and expansion in mTerS-1medium. However, by day 7, mRNA analysis by real-time PCR revealed a biasing towards early mesendoderm lineages on matrix stiffness of 20-40 kPa. Interestingly, an up-regulation of chondrogenic master gene, Sox 9, was detected with ESC grown on matrix of around 20 kPa, without apparent influence on other mesodermal lineages such as osteogenic and cardiovascular lineages. When subjected to a spontaneous differentiation culture, chondrogenic differentiation of ESCs was enhanced on substrate of 20-30 kPa, as determined by RNA analysis by real time PCR, type II collagen immunostaining and ELISA quantification. Our results suggests that the mechanical environment influences both early and terminal ESC differentiation and that mechanical conditions should be taken into consideration when directing ESC differentiation or when designing scaffolds for stem cell-based tissue engineering.

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Session 9D: Embronic Stem Cells in Tissue Engineering
Friday, 17 September 2010: 1530 – 1545 hours

GENERATION OF MATURE BETA CELLS FROM EMBRYONIC STEM CELLS

Weiwei Wang, Sha Jin & Kaiming Ye
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Introduction:
The treatment for type 1 diabetic patients relies upon insulin injection. While insulin treatment is lifesaving, it is not a cure[1]. The restoration of near-physiological insulin secretion capacity in type 1 patients can be achieved through cell transplantation[2]. This treatment has not yet become available for the vast majority of diabetic patients due to the scarcity of human donors and immune rejections in recipients. Thus, new sources of transplantable cells need to be identified. In this work, we presented a new approach to produce more mature biologically functional -cells from embryonic stem (ES) cells. We demonstrated here that more mature cells can be generated by differentiating ES cells within three-dimensional scaffolds. The study was first performed using mouse ES cell[4]. Human ES cells were tested upon the success in directing mouse ES cell differentiation into mature cells in 3D microenvironment.

Materials and Methods:
The mES-D_3 cell line (ATCC CRL-1934) was used. The cells were cultured on a layer of mitotically inactivated mouse embryonic fibroblast cells and undifferentiated ES cells were maintained in DMEM supplemented with 15% FBS, 0.1 mM β-mercaptoethanol, 1% nonessential amino acids, 2 mM L-glutamine, 1% penicillin/streptomycin, and 10^3 U/ml leukemia inhibitor factor. ES cells were pre-differentiated by passaging free of feeder cells two or three times on gelatin-coated Petri dishes in the presence of LIF. LIF was withdrawn from the medium in order to initiate ES cell differentiation and EB formation. To prepare 3-D cultures, pre-differentiated ES cells were mixed with neutralized type I rat tail collagen solution, and the cell-collagen mixture was transferred into a 24-well plate and incubated at 37°C, 5% CO_2 for 2 h to achieve gelation. Before initiating the inducement, growth medium was used to allow cells proliferation in collagen gel for 2 days. A modified five-step protocol was used to differentiate ES cells within three-dimensional scaffolds.

For the differentiation of human ES cells within 3D scaffolds, H9 ES cell line from WiCell Inc. was used. The differentiation procedures and the fabrication of 3D scaffolds are the same as those described above.

Results:
In the first trial of using mouse ES cells, we demonstrated that islet-like structure can be formed from the 3D differentiated ES cells. About 50% of cells formed in these cell clusters expressed both insulin and C-peptide, two characteristic markers for cells. These cells also expressed high level of glucose transport-2 and pdx-1 biomarker, two hallmarks of mature cells. The glucose challenging experiments indicated that the cells are capable of secreting both insulin and C-peptide when grown in high glucose containing medium, suggesting the biological function of these insulin-secreting cells formed from ES cells within 3-D scaffolds.

We also found that the pancreatic differentiation of human ES cell was greatly affected by the biomaterials used for fabricating of 3D scaffolds. We will report these at the conference.

References:
1. Madsen, O. D. 2005. Stem cells and diabetes treatment. Apmis 113:858-75.
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3. Wang, X and K. Ye (2009) Three-dimensional differentiation of embryonic stem cells into islet-like insulin-producing clusters. Tissue Engineering 15, 1941-1952

Acknowledgments:
This work is partly supported by NSF (CBET-0756455) and JDRF 5-2009-381.

ORAL PRESENTATION – ABSTRACT 232
Session 9D: Embronic Stem Cells in Tissue Engineering
Friday, 17 September 2010: 1545 – 1600 hours

HIGH HEPATIC POTENTIALS IN LIVER TISSUE MICRO-SIZED FLOW-CULTURE SYSTEM USING MURINE OR HUMAN ES/IPS CELL

Yoh-Ichi Tagawa[1,2]
[1] *Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Japan*
[2] *PRESTO, Japan Science & Technology Agency, Japan*

Aim:
The liver is a vital organ playing major roles such as metabolism, detoxification, and protein synthesis. In the process of drug discovery and development, compounds should receive to be investigated the pharmacokinetics and metabolism in vitro liver model, however it is still difficult to culture hepatocytes keeping multiple hepatic functions. It is also clear to be difficult to maintain the multiple hepatic functions in the single culture of ES or iPS cell-derived hepatocytes. Liver tissue reconstruction including parenchymal cells, as hepatocytes, and non-parenchymal cells, as endothelial cells, should be necessary for maintaining hepatic functions *in vitro*. Our purpose is to establish liver tissue micro-sized flow-culture system being close to in vivo liver using murine or human ES/iPS cells.

Method:
Murine ES/iPS cells were differentiated into hepatic tissue consisting of hepatocytes and endothelial cells. On the other hand, human ES/iPS cells were differentiated to hepatic lineage cells and then co-cultured with activated endothelial cells to form hepatic sinusoid-like structure. The media was supplied to the PDMS-micro-culture system by a syringe pump.

Results:
Both murine and human ES/iPS cell-derived hepatic tissues strongly expressed liver specific genes, and had high ammonia metabolism potentials and cytochrome P450 activities in the micro-sized flow-culture system.

Conclusions:
We succeeded to reconstruct liver/sinusoid-like tissue using murine or human ES/iPS cells and to culture it in the micro-sized flow-culture system. These hepatic tissues could have higher hepatic potentials in culture for a long period. In the future, we expect that our hepatic tissue culture system could be useful as an application of evaluating drug metabolism and hepatotoxicity in the development of new drugs and chemicals.

POSTER ABSTRACTS

(in number/day order)

Poster 1
Abstract 14
Displayed on Wednesday all day & Thursday morning

ALTERNATIVE THERAPEUTIC STRATEGY FOR AORTIC ANEURYSM USING DOXYCYCLINE CONTROLLED RELEASING BIODEGRADABLE FIBER

Aika Ogata[1,2], Ryotaro Hashizume[1], Makoto Satake[3], Hiroaki Kaneko[3], Yuichi Ueda[1], Toshiyuki Moritan[4] & Yuji Narita[1]
[1] *Department of Cardiac Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[2] *Department of Medical Imaging, Graduate School of Health Science, Suzuka University of Medical Science, Suzuka, Japan*
[3] *Teijin Technology Innovation Center, New Business Development Group, TEIJIN LIMITED, Tokyo, Japan*
[4] *Department of Clinical Engineering, Faculty of Medical Engineering, Suzuka University of Medical Science, Suzuka, Japan*

Aim:
Aortic aneurysm (AA) is a life-threatening disease without surgical intervention. However, the surgery for AA has high mortality and morbidity rate. Therefore, drastic alternative treatment is required. The pathogenesis of AA is degradation of extracellular matrix with increased matrix metalloproteinases (MMP) and loss of structural of the aortic wall. In addition, AA involves tissue inflammation including IL-1β, -6, TNF-α. Meanwhile, it is known that doxycycline (Doxy) (is tetracycline derivative antibiotic agent) inhibits MMPs. In this study, we demonstrated the effects of Doxy controlled releasing biodegradable fiber (DCRBF) for AA in mice.

Method:
DCRBF containing 5wt% Doxy with PLA was made by electrospinning methods. AA was induced for apolipoproteinE deficient mice with continuous administration of angiotensin-II. The AA mice were treated with 25mg DCRBF on the abdominal aorta (group DCRBF). After 4wks, whole aortic tissue were harvested and compared with group DCEBF and control group (group C).

Results:
Elastin content of the aorta was preserved (group DCRBF vs group C = 25.6 vs 35.8 μg/mg, $p<0.05$), and MMP-2, 9 were decreased in the presence of DCRBF. In addition, expression of IGF-1 and TIMP-1 were increased (group DCRBF vs group C = 5.7 vs 11.6 ng/ml, $p<0.01$, and 1.5 vs 2.2 pg/ml, $p<0.05$), and expression of IL-6 and TNF-α were decreased in the presence of DCRBF (group DCRBF vs group C = 3.6 vs 2.1 pg/ml, $p<0.01$, and 53.8 vs 41.3 pg/ml, $p<0.05$).

Conclusions:
These results suggested that local administration of DCRBF might become alternative therapeutic strategy for AA.

Poster 2
Abstract 16
Displayed on Wednesday all day & Thursday morning

EFFECT OF LEUCINE COATING FOR BONE FORMATION IN POROUS HYDROXY APATITE SCAFFOLD

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[1] *Department of Endodontics, Osaka Dental University, Japan*
[2] *Tissue Engineering Research Group, Health Research Institute, National Institute of Advanced, Industrial Science and Technology, Japan*

Aim:
Effect of leucine for osteogenesis in a scaffold in vivo was estimated. The dental pulp is surrounded by dentine. So, cylindrical scaffolds with a hollow center were used.

Method:
Cylindrical porous hydroxyapatite scaffolds (total porosity: 55%, diameter: 8 mm, height: 10 mm) with a hollow center (diameter: 4 mm) were designed and manufactured. Bone marrow cells (BMCs) were obtained from bone shafts of femora of 6-week-old male Fischer 344 rats. Hydroxyapatite scaffolds were soaked in 100mM leucine solution followed by seeding of BMCs at 1×10^6 cells/ml of suspension. The scaffolds without soaking in leucine solution were received BMCs by suspension at 1×10^6 or 1×10^7 cells/ml. They were implanted in dorsal subcutis of 7-week-old syngeneic rats and removed 4 weeks postoperatively.

Results:
Quantity of osteocalcin in the scaffolds was measured by immunochemical method. Serial paraffin sections cut into 6 μm were obtained to calculate percentage of pores with bone to total ones. Results were analyzed by Welch's test ($p<0.01$). By seeding of BMCs at 1×10^6 cells/ml concentration, 6.49 ng/scaffold osteocalcin was detected in leucine prepared scaffolds and 3.96 ng/scaffold without leucine. Leucine induced bone in 53.9% of pores by BMCs suspension at 1×10^6 cells/ml. In the scaffold without leucine, bone was contained in 54.2% of pores by BMCs suspension at 1×10^7 cells/ml and 28.5% at 1×10^6 cells/ml.

Conclusions:
BMCs suspension at 1×10^7 cells/ml or more was essential for bone formation in pores of hydroxyapatite scaffold. However, leucine induced conspicuous bone formation in the pores with a smaller number of BMCs.

Poster 3
Abstract 20
Displayed on Wednesday all day & Thursday morning

CELL SHEET THERAPY FOR MYOCARDIAL INFARCTION IN A PIG MODEL

Dehua Chang, Tatsuya Shimizu, Yuji Haraguchi, Masayuki Yamato & Teruo Okano
Tokyo Women's Medical University, TWIns, Tokyo, Japan

Aim:
Recently, mesenchymal stem cells (MSCs) have been reported to repair chronically infracted myocardium with direct injection to the defect area. However, it is very difficult to localize the injected cells onto the ischemia area to regenerate sufficient cardiac mass. To overcome the problem, we have utilized our cell sheet technology based on temperature-responsive culture dishes.

Method:
Bone marrow stem cells (BMSC) were harvested from the pig. Once the cells reach confluence for 7 days after being cultured, a monolayer BMSC detached itself from temperature-responsive culture dish when the temperature was reduced from 37 °C to 20 °C. A multilayer was prepared by stacking three-monolayer of cells. Myocardial infarction was created by the ligation of the left anterior descending branch of the left coronary artery. A multilayered cell sheet was then transplanted onto the ischemia area. The echocardiography was performed on the myocardial infarction model.

Results:
Multilayer cell sheet were produced that retained all cell-cell contacts. The cells in the multilayer were positive for CD29, CD90, CD146 and CD73 indicating the undifferentiated MSCs. EF and FS were changed and cardiac function was improved after cell sheet transplantation.

Conclusions:
The cell sheet improved the cardiac function in pigs with myocardial infarction. The present study represents progress toward the clinical application of cell sheet of MSCs as a powerful treatment vehicle for human heart disease.

Poster 4
Abstract 22
Displayed on Wednesday all day & Thursday morning

EFFECT OF TRANSFORMING GROWTH FACTOR BETA3 ON RAT VOCAL FOLD FIBROBLASTS

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Aim:
Vocal fold scarring occurs after inflammation, injury, phonosurgery and radiotherapy. It causes hoarseness and decreases the quality of life. Histologically, thick disorganized collagen bundles and decreased hyaluronic acid (HA) have been seen in the lamina propria. Hence, the viscoelastic property of the lamina propria is aggravated, and phonation disorders are observed. There is no satisfactory treatment for vocal fold scarring.

Skin wounds made during the first one-third to one-half of gestation heal perfectly with no scars. Fetal wounds express very high levels of Transforming growth factor beta3 (TGFb3) compared to adult wounds. Some literatures have reported the efficacy of TGFb3 for the regeneration of the skin after wounding. Fibroblasts organizes various extracellular matrix (ECM) components after wounding. Hence, it is of interest how TGFb3 affects gene expression of ECM components in the vocal fold fibroblasts, such as HA that is essential for pliable mucosa. In this study, we investigated the effect of TGFb3 on vocal fold fibroblasts.

Method:
Fibroblasts were harvested from vocal folds of Sprague-Dawley rats and incubated. After the cells grew to about 70% confluency, they were cultured with TGFb3 at concentrations of 1, 10, 100 ng/mL. The cells were collected on days 1, 3, 6 and the expressions of mRNAs which relate ECM components were examined by real-time reverse transcription-polymerase chain reaction.

Results:
The expression of HA synthase mRNAs increased significantly when TGFb3 was administered.

Conclusions:
These results suggest that TGFb3 may have potential of regenerating pliable vocal folds by inducing the expression of HA.

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MALFUNCTION OF MESENCHYMAL STEM CELLS IN HUTCHINSON-GILFORD PROGERIA SYNDROME

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Aim:
Hutchinson-Gilford progeria syndrome (HGPS) is a rare, fatal genetic disorder that is characterized by accelerated aging in children. The disease is caused by autosomal dominant mutation in the lamin A/C gene (C-T mutation at position 1824). The HGPS patients appear normal at birth, but develop symptoms resemble normal aging within the first year of their life, including severe growth retardation, alopecia, loss of subcutaneous fat, osteoporosis, and progressive arteriosclerosis, which seems to affect mainly mesenchymal lineages. Here we aim to study the function of mesenchymal stem cells (MSC) with lamin A/C mutation by using induced pluripotent stem cells (iPSC) as a model system.

Method:
HGPS Patient and control fibroblasts were reprogrammed into iPSC by the over-expression of transcription factors Oct4, Sox2, C-myc and Klf4. Fully reprogrammed iPSC lines are then differentiated in vitro into mesenchymal lineage of cells.

Results:
Both patient and control iPSC derived MSCs can be further differentiated towards the bone, cartilage and fat lineages. We observe that patient iPSC-derived MSCs, but not controls, are very sensitive to hypoxia and substrate deprivation stress in vitro and fail to mediate in the restoration of circulation in a murine hind limb recovery model.

Conclusions:
Our results suggest that HGPS pathology is a pre-mature depletion of MSCs which are needed for tissue renewal. The stem cell depletion may occur due to the susceptibility of the stem cells to stress conditions.

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LITHIUM CHLORIDE (LICL) IMPROVES ADIPOSE-DERIVED STEM CELLS (ASC) PROLIFERATION AND AFFECTS ITS ADIPOGENIC/OSTEOGENIC DIFFERENTIATION

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Aim:
To determine the effects and mechanisms of LiCl on proliferation and trans-differentiation of ASCs.

Methods:
To measure LiCl's effect on ASC proliferation, culture dishes were seeded with ASC supplemented with 0, 1, 4, 8, or 12mM LiCl. After 3 days of growth the cells were trypsinized and quantified. To measure LiCl's effect on cell differentiation, ASCs were induced either adipogenic or osteogenic media both of which were supplemented with 0, 1, 4, 8, or 12mM LiCl. After an appropriate induction time, cells were harvested and checked for key adipogenic/osteogenic marker gene expression.

Results:
At low levels (4mM), LiCl decreased ASC doubling time by 19.14%, an indication of improvement of proliferation. However, at LiCl concentrations of 8mM and 12mM, it quickly increased doubling time by 217% and 268%, respectively, inhibiting proliferation. In addition, it was found that adipogenic differentiation was linearly and drastically decreased with the addition of lithium to the culture media. Oil-Red O analysis showed a 50% decrease in adipogenic differentiation with 12mM LiCl. However, expression of adipogenic-specific gene (Peroxisome proliferator-activated receptor, PPAR-) was not affected. This suggests that LiCl likely affected adipogenic differentiation downstream of PPAR-. Finally, we found that LiCl at lower levels (1mM) inhibited osteogenic differentiation while at higher levels (4-8mM) increased osteogenic differentiation. This was observed in both alkaline phosphatase assay and osteogenic gene expression.

Conclusion:
This study suggested that LiCl affect ASC proliferation and differentiation and may be exploited as therapeutic intervention to enhance the proliferation and osteogenic differentiation of ASCs.

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THE BIOLOGICAL FUNCTION OF ADIPOSE DERIVED STEM CELLS FOLLOWING LABELING WITH ^{18}F-FDG FOR IN VIVO TRACKING WITH MICROPET IMAGING

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Aim:
Our objective is to characterize the effects of 18F-FDG on rat Adipose-derived stem cells (ASCs) viability, proliferation, trans-differentiation and cell function in vitro.

Methods:
Samples of 104 ASCs were labeled with 1.85, 3.70, and 7.40 MBq FDG for 90 minutes. Cell proliferation and viability were measured using Trypan blue exclusion assay. Cell differentiation and cell function were examined in cells labeled with 3.70 MBq FDG. Expressions of several specific genes were determined through RT-PCR measurements in cells subjected to adipogenic and osteogenic inductions. Cell function was examined through the expression of several growth factors (VEGF, HFG, and IGF-I). In all experiments non-labeled rASC samples were used as control groups.

Results:
Our results showed that the cell viability did not change significantly (90-100%), over 3 days post labeling for the amount of FDG used, but cell size increased as FDG activity increased. Labeled rASCs adipogenic differentiation was 25% lower than that in non-labeled cells. Growth factors indicating the cell functions were expressed in both groups, but with slightly lower intensities in the FDG-labeled group. Over all, FDG did not impose severe cytotoxicity in rat ASCs.

Conclusion:
For in vivo cell tracking studies the parameter of interest is the amount of radiotracer that is present in the cells being labeled and consequent biological effects. Our data show that labeling of ASCs with FDG was biologically safe at the levels of radioactivity used in our experiments. Cell viability, transdifferentiation and cell functions were not severely affected by labeling with FDG.

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ISOLATION OF MYOGENIC PROGENITORS FROM HUMAN AMNIOTIC FLUID

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Aim:
Stem cells isolated from amniotic fluid are known to be able to differentiate into different cell types, being thus considered as a potential tool for cellular therapy of different human diseases. We investigated whether these human stem cells could differentiate into myogenic lineage in vitro under influence of a conditioned medium.

Method:
Amniotic fluid stem cells were isolated from amniocenteses fluids. Myogenic lineage differentiation was induced by culture on plastic plates precoated with matrigel and 3 μM 5-aza-2'-deoxycytidine.

Results:
Optical microscopy examination of amniotic fluid stem cells showed the cells to be fibroblast-like. A typical doubling time of the amniotic fluid stem cells was about 36 h. Reverse transcription-polymerase chain reaction analysis evidenced the presence of genes expressed in amniotic fluid mesenchymal stem cells, namely FGF-5, Oct-4, GATA-4, vimentin and CK-18 genes. These cells consistently expressed embryonic stem cells markers (SSEA-4, Oct-4) and mesenchymal stem cells markers (CD 90, CD 105 and CD 73) in flow cytometry analysis. After 7 days in culture in the specific medium, amniotic fluid stem cells expressed the myogenic lineage-specific markers, MyoD and desmin in Western blot analysis. The stem cells were also found to express MyoD and desmin in immunocytochemistry.

Conclusions:
These data suggest that amniotic fluid stem cells were able to differentiate into myogenic cells in vitro.

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A POROUS POLY(L-LACTIC ACID) MEMBRANE DESIGNED FOR CULTURING HUMAN PERIOSTEAL SHEETS AS AN OSTEOGENIC GRAFTING MATERIAL

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Aim:
We have reported a series of clinical and basic studies indicating human cultured periosteal sheets constitute a promising osteogenic graft material for periodontal regenerative therapy. Although multi-layered cell sheets can be easily prepared with a minimum of physical manipulation, poor initial adhesion of excised periosteum tissue segments (PTSs) to culture dishes critically limits successful preparations. To stabilize adhesion and facilitate implantation, we have developed a novel, biodegradable, porous poly-(L-lactic acid) membrane (pPLLAM) and tested its function in static expansion cultivation of periosteal sheets.

Method:
The microstructure of pPLLAMs were examined by SEM. PTSs excised from the alveolar bone of healthy volunteers were placed on pPLLAMs (or empty dishes) and cultured in Medium199+10%FBS. Initial adhesion was evaluated by simple mechanical agitation. Cell growth and mineralization was evaluated by the cyto-histological method. Differential gene expression (pPLLAM vs. dish alone) was examined by DNA microarray.

Results: pPLLAMs possessed many micropores (□20-30 □m) and micropore-like structures on the upper surface suitable to the retention of PTSs on the membrane. Global gene expression analysis demonstrated that periosteal sheets cultured on pPLLAMs upregulated many cell adhesion molecules. Under osteogenic induction, cells expanded from PTSs penetrated into deep pore regions and increasingly produced proteoglycans in parallel with in vitro mineralization.

Conclusions:
Our pPLLAM could reduce the risk of unsuccessful preparations and increase the efficiency of tissue-processing and implantation. As a functional scaffold, its use should facilitate periodontal regenerative therapy using cultured periosteal sheets.

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THE EFFECTS OF □ AND □ POLY (VINYLIDENE FLUORIDE) PHASE CONFORMATIONS ON CELL ATTACHMENT AND PROLIFERATION

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Background:
Membranes of poly (vinylidene fluoride) (PVDF) have potential for use in biomedical applications due to their stability and biocompatibility. Interestingly, PVDF possesses variable piezoelectric properties, depending on the polymorph conformations present. Although many reports describe PVDF as a suitable biomaterial, few considered the specific effects that different polymorph conformations exert on cellular behavior. We hypothesized that different polymorphs, specifically □- and □-phase PVDF, will exert direct but different influences on cell attachment and proliferation.

Aim:
To study the effect of □- and □-phase PVDF on cell attachment and proliferation.

Method:
PVDF films were fabricated using N, N-Dimethylformamide (DMF) and hexamethylphosphoramide (HMPA) by solvent casting. Samples were characterized by differential scanning calorimetry (DSC), fourier transformed infrared spectroscopy (FTIR) and x-ray diffraction (XRD).

Results:
Films containing 85% □-phase PVDF and predominant □-phase PVDF were produced and used to evaluate in-vitro attachment and proliferation of L929 cells. Cell metabolic activity on both PVDF conformations increased about 3 folds over the 1-week culture period, with higher cell metabolic activity observed on □-phase dominant PVDF on day 5 of culture, compared to β-phase dominant PVDF. Cells grown on □-phase dominant PVDF were spindle-shaped, well-spread and expressed spotted paxillin in focal adhesion points mainly localized to perinuclear regions of the cells, while a high proportion of cells on □-phase dominant PVDF were round, less spread and expressed relatively fewer paxillin spots.

Conclusions:
Our results suggested that □- and □-phase PVDF conformations can evoke different cellular behavior, which can potentially be useful for different biomedical applications.

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IN VITRO CYTOTOXICITY AND GENOTOXICITY OF SILVER-NANOPARTICLES CONTAINING HYDROGEL IN HELA CELLS

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Aim:
Silver-nanoparticles containing hydrogel （Ag-NPs-Gel）was used in care of cervicitis due to its antimicrobic activity. However, the potential toxicity of Ag-NPs-Gel is unclear. The aim of this study is to evaluate potential cytotoxicity and genotoxicity of Ag-NPs-Gel on cells, and analyze the mechanisms of cell and Ag-NPs-Gel interactions.

Method:
MTT assay, in vitro micronucleus test and DNA microarray were used in this study, and HeLa cells were selected as target cells.

Results:
After HeLa cells were exposed in different-dose of Ag-NPs-Gel for 48h, a significant proliferation inhibition and cell apoptosis in a dose-dependent manner were observed. Ag-NPs-Gel exposure increased the frequency of micronucleus formation. Furthermore, DNA microarray analysis demonstrated that about 208 genes were up-regulated in the Ag-NPs-Gel treated cells at 24h, and 160 genes were continued up-regulated upto 48h. To verify that the gene up-regulations are resulted from the effects of silver-NPs contained in Ag-NPs-Gel, commercial Ag-NPs were tested in the same experimental system, and the results showed a very similar gene up-regulation pattern in two groups; furthermore the special metal-binding protein family were up-regulated in both of Ag-NPs-Gel and Ag-NPs, but not in the hydrogel (without silver-NPs) group, indicating that the gene up-regulations were resulted from the toxicity of silver-NPs. Gene ontology analysis showed that the HeLa cells responded to Ag-NPs-Gel covered a broad rang of functional gene groups changes.

Conclusions:
This study investigated cytotoxic and genotoxic effects of Ag-NPs-Gel to HeLa cells, and demonstrated that Ag-NPs-Gel induced cell proliferation inhibition, apoptosis etc., covered a broad rang of functional gene group up-regulation.

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POROUS BIOACTIVE GLASS CERAMICS FOR BONE TISSUE REGENERATION

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Bioactive glasses (BGs) can bond chemically to and integrate with the living bone in the body through the formation of a biologically active apatite layer at the implant tissue interface without the formation of fibrous tissue. Both the chemical and structural conditions of BGs, including their composition, porosity, specific surface area, and particle size, appear to have important effects on bone-forming bioactivity.

Hierarchically 3D porous BG balls with well interconnected meso- and macro-pore structures were fabricated. The pore structure of the balls can be controlled by changing the reaction time in chloroform. Both pore size and pore structure were increased and varied with increasing reaction time in chloroform. That is, BG balls contain only nano-sized pore (mesopore) before reaction in chloroform. The nano-sized pore changes to macro-sized pore by time and the ratio of macro/nano-sized pore increases with increasing reaction time. The balls have oblong pore structure with a size of several hundred micrometer and these macropore are comprised of several tens of micrometer pores, several of micrometer pores, and several tens of nanometer pores after 24h reaction. The balls obtained show superior bone-forming activity. The pore structure characteristics of the BG balls, such as therir specific surface area and pore volume, substantially affect their biodegradability and accordingly influence their biocompatibility as well as immunotoxicity both in vitro and in vivo. More detailed results on this topic will be reported in the near future.

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IN VITRO AND IN VIVO PROLIFERATION AND DIFFERENTIATION OF HUMAN ADIPOSE-DERIVED STEM CELLS IN A NOVEL INJECTABLE TEMPERATURE-SENSITIVE HYDROGEL

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Aim:
The in vitro and in vivo proliferation and differentiation of human adipose-drived stem cells (hADSCs) in a novel hydrogel.were investigated.

Method:
To our knowledge, a novel injectable temperature-sensitive chitosan/glycerophosphate/ collagen (C/GP/Co) hydrogel was firstly prepared. The hADSCs were harvested and seeded into above hydrogel by mixing at low temperature, then were cultured in 96-well plates in vitro. The cell morphology and viability were both detected, and the expansion fold of hADSCs was also assayed. Following to this, the biocompatibility of the hydrogel was investigated using subcutaneous injection into 8-week-old SD rats. For in vivo study, adipose-induced hADSCs was mixed with C/GP/Co hydrogel and subcutaneously injected into 4-week-old nude mouse and the adipogenic differentiation in vivo was further observed.

Results:
The results showed that within 5 days of in vitro culture, the hADSCs were well adhering to the hydrogel with good morphology and the cell viability was high. Moreover, cells number increased by 30% after 7 days of culture. Immunostaining analysis showed that the C/GP/Co hydrogel had good biocompatibility within 4 weeks. Staining results of cell-hydrogel complex implants after 4 weeks of implantation in vivo indicated that a great number of adipocytes were generated with vascularization while the results of complex implants with non-induced hADSCs were negative.

Conclusions:
The hADSCs have good capability of proliferation and differentiation in this novel biocompatible C/GP/Co hydrogel.

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REMARKABLE OSTEOGENIC DIFFERENTIATION OF HUMAN MENSTRUAL BLOOD DERIVED STEM CELLS IN PRESENCE OF HUMAN PLATELET RELEASATE

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Aim:
Menstrual blood has been identified as an easily accessible, non-controversial and renewable stem cell source. So, the challenge remains to develop robust protocols to generate different lineages such as osteoblasts from menstrual blood derived stem cells (MenSCs). The aim of this study was to find out substitution effect of fetal bovine serum (FBS) with human platelet releasate (HPR) as a major growth factor source during osteogenic differentiation of MenSCs.

Methods:
Human MenSCs were isolated of menstrual blood by discontinuous density gradient centrifugation and plastic adherence. After characterization of isolated cells, osteogenic differentiation of MenSCs was performed in a medium including ß-glycerophosphate, ascorbate and dexamethasone. Parallel histochemical and molecular experiments were carried out using either culture media containing FBS or HPR- supplemented media.

Results:
Flow cytometric analysis illustrated that MenSCs were positive for stem cells markers such as CD9 (95%), CD29 (99%), CD44 (99%) and CD73 (98%) while negative for CD34(0.6%), CD45 (0.22%) and CD38(0.06%) and mildly positive for stro1 (2%) and CD133(7%). Unlike MenSCs differentiated in medium containing FBS, nodule-like structures were observed in induced cells in presence of HPR. Mineralization as judged by Alizarin red staining was strongly more positive in cells differentiated in presence of HPR than that fortified with FBS (60±10% in HPR group against 5±4% in FBS group). The supporting role of HPR was further confirmed by the mRNA expression of osteobalst specific markers including alkaline phosphatase and osteocalcin.

Conclusion:
HPR is an efficient and potent substitute for FBS during osteogenic differentiation of MenSCs.

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PREPARATION OF POLYMER-BASED MULTIMODAL IMAGING AGENT TO VISUALIZE THE PROCESS OF BONE REGENERATION

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Aim:
With the recent development of molecular or cell biology and tissue engineering technology, the regeneration and repairing of various tissues and organs have been experimentally and clinically demonstrated. In this circumstance, it is practically indispensable to develop the technology of accurate and non-invasive imaging for the process of regeneration and repairing. Although various imaging techniques have been investigated, a single technique does not always meet to all the requirements of diagnosis. One trial to tackle the issue is design a multimodal imaging system, which can compensate the deficiency of single imaging modality. The objective of this study is to prepare a polymer-based multimodal imaging agent co-containing fluorescence and magnetic resonance (MR) imaging probes to visualize the process of bone regeneration and repairing.

Method:
Pamidronate (PA), a bisphosphonate with a high affinity for the hydroxyapatite of bone tissues, was chemically introduced to pullulan with different molecular weights, followed by the conjugation of PA-pullulan with two imaging probes (PA-pullulan-F/M).

Results:
Irrespective of the pullulan molecular weight, the PA-pullulan-F/M had an affinity for the hydroxyapatite and an MR imaging ability. When intravenously injected to a mouse model of bone formation, the accumulation of PA-pullulan-F/M to the bone tissue newly formed was confirmed by the in vivo fluorescence imaging technique. The time profile of signal intensity corresponded well with that of bone formation which was evaluated by measuring the calcium deposition.

Conclusions:
These findings clearly indicate that the PA-pullulan-F/M is a feasible polymer-based multimodal agent to image the process of bone formation.

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MOLECULAR DESIGN OF ULTRASOUND-RESPONSIVE DELIVERY SYSTEM FOR THROMBOLYTIC THERAPY

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Aim:
Tissue-type plasminogen activator (t-PA) is clinically used to treat thrombosis. However, the therapeutic applications are often limited due to adverse side effects. This study is undertaken to prepare a novel t-PA delivery system where the t-PA activity is suppressed in the blood circulation, but is recovered upon ultrasound irradiation.

Method:
Gelatin with different molecular weight was mixed with t-PA in aqueous solution, followed by agitation at 37°C for 30 min. Then, zinc acetate, calcium acetate or magnesium acetate was added and agitated at 37°C for 30 min to form gelatin–t-PA complexes. The t-PA activity was evaluated by the fibrin plate method. The cytotoxicity of complexes was assayed in the culture of L929 cells. Thrombolytic effect of complexes with or without ultrasound was examined in a rabbit thrombosis model.

Results:
When zinc ions were added to with gelatin–t-PA complexes, the t-PA activity was suppressed most strongly to 57% of original activity among calcium and magnesium ions. Upon ultrasound exposure in vitro, the t-PA activity was recovered to the original level. No cytotoxicity of complexes was observed at the concentration used for the in vivo experiment. The life-period of t-PA in the blood circulation was prolonged by the complexation with gelatin and zinc ions. Combination of complex with ultrasound irradiation resulted in complete recanalization in the thrombosis model.

Conclusions:
It is concluded that the zinc-stabilized t-PA–gelatin complex is a promising t-PA delivery system which can control the thrombolytic activity by the local ultrasound irradiation.

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COMB-TYPE GRAFTED POLY(N-ISOPROPYLACRYLAMIDE) GEL MODIFIED SURFACES FOR RAPID DETACHMENT OF CELL SHEET

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Aim:
A novel approach of tissue engineering, cell sheet engineering, was proposed by our laboratory. An intact cell sheet could be formed on the normal-type Poly(N-isopropylacrylamide) (PIPAAm) gel modified tissue culture polystyrene (TCPS) (NG-TCPS), which could provide potential applications as an engineered tissue. However, the detachment of cell sheet is a slow process. For the manipulation and transplantation of cell sheet in clinical application, temperature-responsive cell culture surface for allowing cell sheet to detach rapidly itself becomes an important tool.

Method:
PIPAAm macromonomer was prepared by the etherification reaction of the hydroxyl terminal moieties of PIPAAm with acryloyl chloride. Solution containing (N-isopropylacrylamide) monomer and PIPAAm macromonomer was spread on the surface of TCPS, and then the surface was subjected to electron beam irradiation, resulting in comb-type grafted PIPAAm gel modified TCPS (GG-TCPS).

Results:
At 37 ^{o}C, bovine aortic endothelial cells (BAECs) were well adhered and spread on GG-TCPS as well as NG-TCPS regardless of the macromonomer concentration. By lowering temperature to 20 ^{o}C, BAECs detached themselves more rapidly from GG-TCPS compared with NG-TCPS. Upon lowering temperature, the grafted polymer was speculated to accelerate the hydration of modified PIPAAm gel, resulting in a rapid cell sheet detachment.

Conclusions:
This novel temperature responsive cell culture surface is thought to be useful for cell sheet tissue engineering.

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EVALUATION OF POLYMERIC DERMAL WOUND DRESSING CONTAINED ALLICIN

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A novel composite material with allicin was developed for the application of wounds dressing. The poly(hydroxyl ethyl methacrylate) was polymerized as the polymeric matrix. allicin was encapsulated into the porous polymeric matrix through the coupling agent. The combination between allicin and poly(HEMA) were achievement by UV radiation. The morphology of polymeric matrix was observed by scanning electronic microscopy. The binding and structure of the composites were analyzed by using UV-Vis spectrophotometer and FTIR spectrum. The hydrophilic property, water loss rate and permeability of materials are measured in this study. The relationship between the antibiotics of wound dressing and the content of allicin was investigated. The result shows that these performances are more excellent than commercial artificial skins.

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THE COMBINATION OF STROMAL CELL-DERIVED FACTOR-1 AND BONE MORPHOGENETIC PROTEIN-2 FOR BONE TISSUE ENGINEERING

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Aim:
The aim of this study is to investigate the osteogenic properties of combined stromal cell-derived factor-1 (SDF-1) and bone morphogenetic protein-2 (BMP-2) for bone tissue engineering.

Method:
Expression of CXCR4 (a cell-surface receptor of SDF-1) and runx2 (early marker of osteogenesis) genes in rat MSC treated with SDF-1, BMP-2, and mixed SDF-1/BMP-2 was evaluated by real time RT-PCR. Then, SDF-1, BMP-2, and mixed SDF-1/BMP-2 were incorporated into gelatin hydrogel as the release carrier, and cultured with MSC *in vitro* in an osteogenic medium to assess the CXCR4 and runx2 expression. The gelatin hydrogel incorporating SDF-1, BMP-2, and mixed SDF-1/BMP-2 was further studied *in vivo* in a critical ulna defect of Wistar rat to observe bone regeneration.

Results:
MSC treated with BMP-2 and mixed SDF-1/BMP-2 showed up-regulated CXCR4 expression while those treated with SDF-1 expressed CXCR4 gene as equally as non-treated cells. This indicates that BMP-2 and SDF-1/BMP-2 can activate cell recruitment via a receptor of SDF-1. Up-regulation of runx2 gene was also found in MSC treated with BMP-2 and SDF-1/BMP-2. The similar result of MSC cultured in the gelatin hydrogel incorporating SDF-1, BMP-2, and mixed SDF-1/BMP-2 was also observed. In addition, the gelatin hydrogel incorporating mixed SDF-1/BMP-2 showed a significantly higher runx2 expression of MSC and bone regeneration 4 weeks post-operatively, compared with that incorporating either SDF-1 or BMP-2.

Conclusions:
The combination of SDF-1/BMP-2 had a novel osteoinductive activity for bone tissue engineering.

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LOCALISATION OF HUMAN BONE MARROW-DERIVED OSTEOBLASTS IN BOTH ECTOPIC AND ORTHOTOPIC OSTEOGENIC PROCESS

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Aim:
Cell therapy is one of the potential strategies to enhance bone defect healing as it allows the direct introduction of osteogenic/mesenchymal stem cells. Cell-cell interaction between host and donor cells plays a very important role in the regulation of bone healing process after in vivo cell transplantation. However, it is still unclear how transplanted donor cells behave in vivo and interact with host cells during the bone healing process. The purpose of this study was to investigate the fate of the in vivo transplanted human osteoblasts (hOBs) derived from bone marrow and their interaction with the host cells in the new bone formation sites using in situ hybridization technique that allows differentiation between human and mice cells.

Methods:
The human bone marrow stromal cells (hBMSCs) were isolated and cultured on collagen I scaffolds in osteogenic differentiation media for two weeks. Then these cell-carrying scaffolds were implanted into subcutaneous area of four SCID mice and skull bone defects of twelve SCID mice. The scaffolds seeded with undifferentiated hBMSCs or scaffolds alone were used as controls. All these mice were sacrificed four weeks after the implantation. The tissue samples were then harvested, fixed, embedded in paraffin and sliced. New bone formation was detected by micro-CT, histology and immunohistochemistry. In situ hybridization against human repetitive *alu* sequences was used to distinguish transplanted human cells from mice cells in the sites of transplantation.

Results:
Micro-CT, H&E and immunohistochemical staining against bone markers revealed remarkable new bone formation within the skull defects after the transplantation of hOBs and hBMSCs while only mineral precipitation along collagen fibres was observed in the subcutaneously implanted scaffolds. No new bone formation was detected in the treatment with scaffold alone. In situ hybridization against human *alu* sequences revealed that donor cells were detected in newly formed osteocytes, osteoblasts and cells supporting the new bone formation in the orthotopic sites. Interestingly, it was also noted that human cells were involved in the reconstitution of bone marrow component, mainly the new formed bone marrow stromal tissues. No hematopoietic cell in the newly formed bone marrow was positive for the *alu* probe. No *alu* positive cell was found in the treatment with scaffold only. In the ectopic sites it was found that in the scaffolds with hOBs or hBMSCs, the mice cell migration into the scaffolds was significantly enhanced, which was demonstrated by the increased cell numbers inside these scaffolds compared with the scaffolds without human cells.

Conclusions:
The transplanted cells involved in the formation of new bone and bone marrow tissues in the orthotopic sites directly. The donor cells can recruit host cells into the transplantation sites in tissue formation and orthotopic osteogenic process.

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THE EFFECT OF THE ELECTROSPUN SILK FIBROIN NERVE GUIDANCE CONDUIT ON NERVE REGENERATION OF RAT'S DEFECTED SCIATIC NERVE

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Nerve conduit was made by electrospun silk fibroin (SF). For testing compatibility this biomaterial with cells, Schwann cell (RT4-D6P2T) was cultured on 2D SF sheet in vitro. And Sprague-Dawley rats were used for in vivo study. The sciatic nerve of the rat was cut 8 mm and 10 mm of SF nerve conduit (SFNC) was transplanted. Only defects and autograft groups were used by control. The paw pressure (PP) and angle stance ankle (ASA) tests were used for the motor or sensory functional recovery every week for 10 weeks after surgery, respectively. SFNC group were showed higher recovery both motor and sensory function than only defects group. Especially, autotomy index result is very interesting. SFNC group does not appear grade 3 of autotomy index while other groups show up higher percent (only defect group: 40%, autograft group: 25%). Also, histological figures using myelin stain was present newly formed nerve fiber in SFNC transplanted group at 10 weeks after operation. These results suggested that SFNC may be a good guidance role in nerve fiber migration from distal to proximal and thus it will help nerve regeneration effectively.

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ANALYSES OF THE CELL VIABILITY AND METABOLISM OF MULTI-LAYERED CELL SHEETS AND A POSSIBLE SOLUTION FOR OVERCOMING THE DIFFUSION LIMIT

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Aim:
The tissue's ischemic environments inducing cell death make the production of thicker three-dimensional (3D) tissues difficult. For evaluating thickness limitation of tissue, cell viability and metabolism of 3D tissue model, which is composed of multi-layered cell sheets, were investigated. In addition, we explored a method to create thicker cell-dense tissues.

Method:
Human endometrium-derived mesenchymal stem cells (EMSCs) were cultured on temperature-responsive culture dishes. Confluently cultured EMSCs were harvested intact a contiguous cell sheet only by lowering temperature. The obtained cell sheets were successfully layered into 3D cell-dense tissues. Engineered 3D tissues were assessed by histological analyses and biochemical assays after 1, 3, and 7 days cultivation. To overcome the diffusion limit, a cultivation system using semipermeable membranes was examined.

Results:
Glucose consumption and lactate production in the culture media increased in accordance with the number of cell sheets (single to triple). Histological analyses and cell viability assays showed that viable tissues were found in single- to triple-layered cell sheets (~40 μm) and damaged tissues in over quadruple layers. When multi-layered cell sheets were cultured on semipermeable membranes, cell viabilities were improved, resulting in fabricating thicker tissues (~100 μm) than on normal culture dishes.

Conclusions:
While the thickness of viable engineered 3D tissues is limited in normal culture condition, thicker cell-dense tissues were successfully created using bidirectional media exposure.

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DIFFERENTIATION INTO NEURAL CELLS FROM HUMAN AMNIOTIC MESENCHYMAL CELL IN SERUM FREE MEDIUM

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Aim:
Human amniotic mesenchymal cells (hAMCs) are considered to be a promising cell source for regenerative medicine because they have multipotency and weak immunogeniscity. The aim of the present study was to clarify the appropriate condition for effective differentiation of the hAMCs into neural cells in serum free medium.

Method:
The hAMCs were isolated from the enzyme-treated amniotic membrane. The cells were cultivated in various serum free induction medium: 1) DMEM/F12 + KSR on collagen-coated dish, 2) DMEM/F12 + KSR + Dkk-1 + Lefty-A on collagen-coated dish, 3) DMEM/F12 + KSR on the dish coated with the human solubilized amnion products. 4) DMEM/F12 + KSR + Dkk-1 + Lefty-A on the dish coated with the human solubilized amnion products. The gene expressions of neural markers as Tuj1, NSE and NF-M were evaluated by real-time PCR. The protein expressions of Oct3/4, a stem cell marker, and Tuj1, the neuron specific marker, were also evaluated by fluorescent immunostaining.

Results:
The gene expression of Tuj1, NSE, and NF-M were increased in hAMCs after cultivation irrespective of the culture conditions investigated. The expression of Oct3/4 disappeared after 2 week, while that of Tuj1 increased after 2 weeks cultivation.

Conclusions:
Serum free media investigated were sufficient enough for the induction of the hAMCs into neural cells.

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BORON DOPED NANOCRYSTALLINE DIAMOND FILMS AS POTENTIAL SUBSTRATE FOR BONE TISSUE REGENERATION.

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Aim:
The aim of this study was to investigate the adhesion, growth viability and osteogenic differentiation of human osteoblast-like MG 63 cells in cultures on nanocrystalline diamond films with different boron concentration.

Method:
NCD films were deposited on silicon substrates in a microwave PECVD reactor and doped with 133, 1000 and 6700 ppm of boron, which is known to increase the electrical conductivity of NCD. The cell number and viability was evaluated on microphotographs after staining cells with a LIVE/DEAD kit on days 1, 3, and 5 after seeding. The concentration of osteocalcin, a marker of osteogenic cell differentiation, was measured semiquantitatively by an enzyme-linked immunosorbent assay (ELISA).

Results:
All substrates significantly supported the adhesion, growth, viability and osteogenic differentiation of MG 63 cells. On day 6, the cell number on NCD films with 1000 ppm of boron (200,306 ± 4,323 cells/cm^2) and 6700 ppm of boron (211,834 ± 7,205 cells/cm^2) highly exceeded the cell numbers on the nanodiamond films without boron (154,192 ± 18,734 cells/cm^2) and control polystyrene dishes (93,668 ± 12,964 cells/cm^2). The cell viability was almost 100% on all samples in all culture intervals.

The concentration of osteocalcin in cells cultured on all types of NCD films was significantly higher (by 24 to 33 %) than the values obtained from cells grown on polystyrene dishes.

Conclusions:
Boron-doped nanocrystalline diamond films have a great potential for its use in bone regeneration.

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DEVELOPMENT AND EVALUATION OF A NOVEL AUTOMATIC CELL CULTURE SYSTEM OF PERFUSION FLOW USING BONE MARROW STEM CELLS

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Aim:
The purpose of this study was to develop the novel perfusion bioreactor system for human alveolar bone tissue engineering using alveolar bone marrow stem cells.

Method:
We have developed and evaluated a novel automatic cell culture system of perfusion flow using alveolar bone marrow stem cells. The present study in this paper was to improve the automatic perfusion flow bioreactor system related to a novel cell culture chamber system with sequence control function.

Results:
Cell viability showed that cell growth rate cultured (without medium perfusion) using the novel flow perfusion chamber system revealed a similar trend ($p>0.05$) compared to that of static culture. Also, the cell viability under the static culture (without perfusion) and flow perfusion culture (flow rate: 0.03 ml/min) with the developed perfusion bioreactor system was compared. The cell growth rate of the flow perfusion culture was significantly higher ($p<0.05$) than that of static culture for 4 days. The characteristics of the bioreactor developed in this study was fast to culture stem cells with mechanical stimulation and to monitor live cell imaging while promoting healthy cellular activity outside of an incubator environment.

Conclusions:
Through experimental results, the developed perfusion bioreactor could increase cell growth with proper flow based on mechanical stimuli.

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PREPARATION, FABRICATION AND BIOCOMPATIBILITY OF A NOVEL INJECTABLE TEMPERATURE-SENSITIVE CHITOSAN-BASED HYDROGEL

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Aim:
This paper introduces a novel type of injectable temperature-sensitive chitosan/ glycerophosphate/collagen (C/GP/Co) hydrogel that possesses great biocompatibility for the culture of adipose tissue-derived stem cells.

Method:
The C/GP/Co hydrogel is prepared by mixing 2.2% (v/v) chitosan with 50% (w/w) β-glycerophosphate at different proportions and afterwards adding 2 mg/mL of collagen. The gelation time of the prepared solution at 37°C was found to be of around 12 minutes. The inner structure of the hydrogel presented a porous spongy structure, as observed by scanning electron microscopy. Moreover, the osmolality of the medium in contact with the hydrogel was in the range of 310-330 $mmol.kg^{-1}$.

Results:
These analyses have shown that the C/GP/Co hydrogels are structurally feasible for cell culture, while their biocompatibility was further examined. Human adipose tissue-derived stem cells (ADSCs) were seeded into the developed C/GP/Co hydrogels, and the cellular growth was periodically observed under an inverted microscope. The proliferation of ADSCs was detected using cck-8 kits, while cell apoptosis was determined by a Live/Dead Viability/Cytotoxicity kit. After 7 days of culture, cells within the C/GP/Co hydrogels displayed a typical adherent cell morphology and good proliferation with very high cellular viability.

Conclusions:
It was thus demonstrated that the novel C/GP/Co hydrogel herein described possess excellent cellular compatibility, representing a new alternative as a scaffold for tissue engineering, with the added advantage of being a gel at the body's temperature that turns liquid at room temperature.

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NANOFIBROUS PLGA SCAFFOLDS LOADED WITH NANODIAMOND AS SUBSTRATES FOR ADHESION AND GROWTH OF HUMAN BONE-DERIVED CELLS

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Aim:
Electrospinning is a promising technique for creating advanced nanostructured biomaterials for tissue engineering. In this study, composite nanofibrous membranes containing a copolymer of L-lactide and glycolide (PLGA) and diamond nanoparticles were fabricated and used as substrates for the adhesion and growth of human osteoblast-like MG 63 cells.

Method:
For electrospinning, PLGA was dissolved in a mixture of methylene chloride and dimethyl formamide (2:3) at a concentration of 2.3 wt.%, and the nanodiamond powder was added at a concentration of 0.7 wt.% (after evaporation of the solvents, this concentration in pure PLGA reached about 23 wt.%). The composite nanofibrous membranes were then prepared using Nanospider™ equipment (Elmarco Ltd., Czech Republic), seeded with MG 63 cells and incubated in a medium DMEM with 10% of fetal bovine serum.

Results:
Both PLGA and PLGA-nanodiamond scaffolds enabled attachment, spreading and subsequent proliferation of MG 63 cells, although the cell numbers on nanodiamond-containing meshes, obtained on days 1, 3 and 7 after seeding (from 11,300±1,600 to 200,300±10,300 cells/cm^2) were significantly lower than the values on cells on pure PLGA meshes (from 48,500±4,000 to 268,600±12,200 cells/cm^2). This cell behaviour might be attributed to the presence of bigger and more numerous material clusters in the composite PLGA-nanodiamond meshes. Nevertheless, the cell spreading area on PLGA-ND membranes (428 ± 19 μm^2) was similar to that on pure PLGA meshes (475 ± 14 μm^2).

Conclusions:
Composite PLGA-ND meshes gave relatively good support for the adhesion and growth of human bone-derived cells. Thus, after some improvements, this material has potential for the use in bone tissue engineering.
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VASCULAR ENDOTHELIAL AND SMOOTH MUSCLE CELLS IN STATIC AND DYNAMIC CULTURES AND COCULTURES ON ELECTROSPUN NANOFIBROUS SCAFFOLDS

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Aim:
Nanofibrous scaffolds are promising materials for applications in tissue engineering, including the construction of bioartificial vascular wall. Therefore, nanofibrous membranes, made of an aromatic polyamide (Aramid), were colonized with endothelial cells (EC) and vascular smooth muscle (VSMC). Aramid was chosen for its good mechanical properties, and was also used for creating a bilayer of EC and VSMC in a pulse perfusion bioreactor.

Method:
The Aramid meshes were prepared by electrospinning in a Nanospider equipment (Elmarco Ltd., Liberec, Czech Republic) and seeded with bovine pulmonary artery EC or rat aortic VSMC (~17,000 cells/cm^2). For construction of bilayer of VSMC and EC, the Aramid membranes were first seeded with VSMC, and after 4 days in the static culture system, the VSMC layer was seeded with EC and cultured for additional 4 days. The sample was then placed into a perfusion bioreactor (ProVitro GmBH, Germany) with a pulse flow of the culture medium for 17 days.

Results:
On Aramid, both EC and VSMC adhered in numbers very close to the seeding densities (16,500 ± 1,500 and 15,300 ± 1100 cells/cm^2, respectively). On day 7, the EC and VSMC on Aramid reached densities 33,600 ± 3,000 and 106,800 ± 9900 cells/cm^2, respectively, which was comparable to the values on control glass coverslips (31,500 ± 2,300 and 106,500 ± 8,800 cells/cm^2). In the perfusion bioreactor, a continuous bilayer of VSMC and EC was formed, and these cells were well-stained for their differentiation markers, i.e. alpha-actin and von Willebrand factor, respectively. In the control static culture system, only islets of both cell types were observed.

Conclusions:
Aramid nanofibrous fabrics gave good support for vascular tissue reconstruction *in vitro*. Further studies will be directed to degradable nanofibrous scaffolds, providing a temporary support for vascular tissue regeneration.

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DEVELOPMENT OF FAST-HARDENING CALCIUM PHOSPHATE BONE CEMENT WITH SINTERED ANIMAL BONES

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Aim:
The objective of this study was to develop fast-hardening calcium phosphate cement (CPC) for bone regeneration with sintered animal bones.

Method:
CPC consists generally of a solid phase (powder) and an aqueous phase (solution). For the preparation of a powder of CPC, the bones from horse and pig were soaked in oxygenated water for 48h to eliminate soft tissues and other organic components. Then, the bones were annealed in an electric furnace at 1200°C for 2h. The 2.0, 3.0, 3.5% chitosan solutions (CS) in 2% acetic acid were used as an aqueous phase of CPC. Chitosan is a biocompatible and biodegradable natural polymer which has the ability to shorten a hardening time of CPCs.

Result:
The peak patterns of X-ray diffraction (XRD) and Energy dispersive X-ray spectroscopy (EDX) show a hydroxyapatite (HA) pattern which means that the pig and horse bone powders are calcium phosphate composite and can be bioceramic materials for bone regeneration. A vicat needle was used to measure the hardening time of CPC, a hardening time of pig bone powder with 3.5% CS was much faster (11min) than distilled water (>60min). The average compressive modulus of CPC with horse bone powder and CS ranged from 23~42 MPa was significantly higher than CPC with distilled water (16~18 MPa). In vitro Cytotoxicity (MTT) assay with MG-63 (osteoblast-like cell) was no significant difference between cultured cell with CPC and cell only.

Conclusions:
Through experimental results, the CPC with CS may be useful for bone regeneration in orthopedic application.

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INFLUENCE OF DEACETYLATION IN CHITOSAN-BASED, LASER-ACTIVATED SURGICAL ADHESIVE ON CELL GROWTH AND ATTACHMENT

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Biomaterials, like chitosan, are ideal as artificial extracellular matrices; providing a three-dimensional space for cells to form into new tissue. They can also support the delivery of cells and bioactive factors. SurgiLux® is a novel chitosan-based, laser-activated bioadhesive film designed for sutureless surgery and to promote tissue regeneration. The degree of deacetylation (DDA) in chitosan has been shown to influence the physiochemical and material properties of its various devices. By extrapolation we hypothesise that DDA will influence the physiochemical, material and biological properties of SurgiLux®.

The DDA in SurgiLux® did not appear to have any effect on crystallinity of the thin films but decreased their tensile strength from ~36kpa to ~46kpa. Despite this, the repair strengths for sutureless anastomosis of sheep intestine pieces were similar , ~9kpa. Increasing the DDA increased the average surface roughness (Ra) of the SurgiLux® films, as determined using confocal laser scanning microscopy 0.62µm to 0.78µm. This increase in coarseness of the surface topography as measured by Ra supported a relative increase in the attachment of C2C12 myoblastic cells.

In conclusion, varying the DDA of SurgiLux® did not unduly affect its material or bioadhesive properties but influenced cell attachment and growth. This suggests that SurgiLux® can be readily manipulated to promote cellular responses while retaining its inherent adhesive function, such a device has potential in tissue engineering.

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NANOSCALE PRESENTATION OF CELL ADHESION MOTIFS VIA BLOCK-COPOLYMER SELF ASSEMBLY: EFFECT OF LIGAND SPACING ON MESENCHYMAL STEM CELL BEHAVIOUR

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Aim:
Classically soluble factors have been investigated for their effects on mesenchymal stem cell (MSC) growth and differentiation, however, more recent work has shown that the mechanical environment, including substrate modulus and ligand type and availability can also influence MSC properties. Here we aim to determine how the spatial arrangement of cell adhesion ligands affects MSC behaviour.

Method:
We use self-assembled polystyrene-block-poly(ethylene oxide)-copolymers (BCP) to present RGD-peptide to MSCs with lateral spacings, ranging from 34 to 62 nm. These surfaces are used to investigate how lateral spacing of adhesion ligand affects MSC adhesion, morphology and differentiation.

Results:
We demonstrate that adhesion of MSCs to the BCP surfaces is through specific attachment to the presented RGD-motif and that this is mediated by □5, □V, □1 and □3 integrins. As the lateral spacing of the peptides is increased, the ability of the MSCs to spread is diminished and that the morphology changes from a classical fibroblastic morphology and defined stress-fibres, to less-spread cells with numerous cell protrusions and few stress fibres. In addition, the ability of MSCs to form mature focal adhesions is significantly reduced on substrates with increased lateral spacing. Finally, we investigate differentiation and using qRT-PCR determination of gene expression levels and a quantative alkaline phosphatase assay, show that MSC osteogenesis is reduced on surfaces with increased lateral spacing, whilst adipogenic differentiation is increased.

Conclusions:
We show that lateral spacing of adhesion ligand affects MSC size, shape, cytoskeleton, ability to form focal adhesions and differentiation along the osteo and adipogenic lineages.

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EFFECT OF LITHIUM CHLORIDE ON ß-CATENIN EXPRESSION, PROLIFERATION AND OSTEOGENIC DIFFERENTIATION OF HUMAN MENSTRUAL BLOOD DERIVED STEM CELLS

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Aim:
Menstrual blood derived stem cells (MenSCs) appears unique stem cells that have been identified recently. The special traits of MenSCs can be related to cell signaling pathways. Lithium chloride (LiCL) has been shown to implicate the effects of Wnt signaling on cell proliferation and differentiation. In this study, we investigated the influence of lithium chloride on ß-catenin expression, proliferation and osteogenic differentiation of MenSCs.

Methods:
MenSCs were isolated from healthy women using combining gradient density centrifugation with plastic adherence. After characterization of isolated cells, cell proliferation of MenSCs in presence of 10-15 mM LiCl was evaluated by MTT assay. ß-catenin expression of treated cells was examined using immufluoroscent technique. Thereafter, osteogenic differentiation ability of MenSCs osteocytes in media containing 10-15mM liCl was traced by Alizarin red staining.

Results:
Flow cytometric analysis revealed that both mesenchymal stem cell markers including CD9, CD29, CD44, CD105, CD73 and embryonic stem cells markers such as Oct-4 are expressed on menstrual blood stem cells. MTT value was decrease depending on LiCl concentration. The proliferation of MenSCs cultivated in culture media containing 15mM LiCl was approximately twofold less than that grown without LiCl ($P<0.01$) as to nuclear accumulation of ß-catenin protein in cells treated by LiCl was greater than cells without LiCl. However, calcium assembly in differentiated cells into osteoblast judged by Alizarin red staining were not gross divergent between groups.

Conlusion:
LiCl probably suppress MenSCs proliferation via mediating nuclear accumulation of ß-catenin in a dose dependent manner but no affect on osteogenic differentiation.

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TEMPORAL RESPONSE OF ZONAL HUMAN OSTEOARTHRITIC CHONDROCYTES TO COMPRESSIVE STIMULATION

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Aim:
Articular cartilage has a depth-dependent (zonal) structure that serves to distribute loads in diarthrodial joints. We hypothesise that chondrocytes from distinct zones respond differently to compressive loading, and that the cellular temporal response will aid in the rational development of loading protocols for enhancing engineered cartilage. Therefore, we aimed to determine the time-dependent changes in mRNA and protein synthesis of human OA chondrocytes from the superficial (S) and middle/deep (M/D) zones following compressive loading.

Method:
Human S and M/D chondrocytes from osteoarthritic joints (P2) were encapsulated in 2% alginate and compressed (1Hz, 15% strain, 1hr) after 2weeks of pre-culture. The changes in cartilage-related mRNA (PRG4, collagen II, aggrecan, collagen I) were determined at multiple time-points before and after compression using qRT-PCR. Collagen production was estimated by ^{3}H-proline incorporation.

Results:
S and M/D chondrocytes produced different gene expression profiles after compression. For S chondrocytes, collagen II and aggrecan mRNAs were up-regulated (~2-fold) 4 hrs after compression, compared to free-swelling controls. Collagen I was up-regulated more than 3-fold immediately after compression, but PRG4 expression was not affected by compression. In contrast, M/D chondrocytes showed no clear changes in any of the genes within the first 8 hrs after the compression. ^{3}H-proline data remain to be analysed.

Conclusions:
Differences in response of zonal chondrocytes to compression indicate different loading regimes may be needed for optimal tissue formation in such constructs. Future experiments will determine whether stimulatory effects can be enhanced by repeated loading following rest periods indicated by the single loading study.

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DIFFERENTIATION OF SIDE POPULATION CELLS ISOLATED FROM HUMAN AMNIOTIC MESENCHYMAL CELL INTO VASCULAR ENDOTHELIAL CELLS UNDER HYPOXIC CONDITION

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Aim:
Human amniotic mesenchymal side polulation (SP) cells have pluripotency and weak immunogenicity. These cells have promising features in regenerative medicine. The aim of the present study was to determine whether hypoxic condition is appropriate for the differentiation of the SP cells into vascular endothelial lineage.

Method:
The human amniotic mesenchymal cells were isolated from the enzyme-treated amniotic membrane and were stained with Hoechst33342. SP cells were negatively selected by sorting the stained human aminiotic mesenchymal cells out using FACS. The SP cells were cultivated in induction medium (DMEM/F12 + FBS + VEGF) in normoxia (20%) or hypoxia (1%) for 1 or 2 weeks. The expressions of endothelial markers as vWF, VE-cadherin, VCAM, FLk-1/KDR and Flt-1 were evaluated by real-time PCR and fluorescent immunostaining. The protein expression of Oct3/4, a stem cell marker, was also evaluated by fluorescent immunostaining.

Results:
Gene expressions of Flt-1, VE-cadherin and vWF were most pronounced after 2 weeks cultivation in hypoxia. Protein expressions of KDR and VE-cadherin were observed only after 2 weeks cultivation in hypoxia. The KDR was expressed irrespective of the conditions investigated. Expression of Oct3/4 disappeared after 2 weeks cultivation.

Conclusions:
Hypoxia enhanced the induction of amniotic mesenchymal SP cells treated with VEGF into vascular endothelial lineage.

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THERMO-SENSITIVE HYDROGEL FOR DRUG AND CELL DELIVERY

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Aim:
Poloxamer is amphiphilic non-ionic block polymers. It exhibits reverse thermal gelation and has a non-toxic nature. In this study, we investigated whether poloxamer could improve the wound healing by delivering drugs or cells.

Method:
To evaluate Substance P (SP) as a drug of wound, fibroblasts were cultured medium containing SP. A model of cutaneous wound healing was utilized to determine wound closure rates in BALB/c nude mice. An 8-mm diameter full-thickness circular skin flap was excised form the midline just below the scapulae using a skin biopsy punch. To evaluate the poloxamer as drug delivery vehicle, animals were assigned to 3 groups; control, hydrogel (SP-incorporated poloxamer), and Intrasite (Smith & Nephew Inc.). To evaluate the poloxamer as cell delivery vehicle, the wound was treated by poloxamer containing mesenchymal stem cells (MSCs). The wound were evaluated 7 days after the treatment.

Results:
Fibroblasts cultured with SP showed higher proliferation rate than did control. Mice treated with SP-incorporated poloxamer showed considerable dermal regeneration. However, Intrasite still remained in the treated sites. Also, Intrasite treated mice failed to form epidermis as well as dermis. The wounds of poloxamer containing MSC treated mice regenerated epidermis and dermis.

Conclusions:
As a carrier and scaffold for cells and drugs, poloxamer can be used for other tissue regeneration as well as wound healing.

Acknowledgements
This study was supported by a grant of the Korea Healthcare technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A091141).

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CELL SUBSTRATE INFLUENCES THE MOTILITY AND MODE OF COLONY FORMATION OF HUMAN EMBRYONIC STEM CELLS

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Aim:
Recently there has been a shift in human embryonic stem cell (hESC) culture methods from the utilisation of feeder cells to more defined systems with commercially available substrates and media. This work aims to investigate how the surface, both traditional and defined, impacts on cell motility and mode of colony formation.

Method:
Time-lapse microscopy was used to observe cell motility and colony formation of hESCs adapted to passaging as single-cells in various cell culture medium and substrates including mouse embryonic fibroblasts and matrigel. Vector maps and velocity measurements were generated using particle tracking software. Finally clonal growth was confirmed by mixing an m-Cherry expressing cell line with a normal cell line and imaging the resulting colonies.

Results:
The hESCs were observed to form colonies by one of two methods; either cell division resulting in colony formation from a single cell, or colony formation by cell migration and aggregation. Low motility was found to foster clonal growth whereas high motility cells seek others and aggregate together prior to colony formation, resulting in a colony formed from multiple cells. Cell motility and clonal growth was independent of the medium used.

Conclusions:
We conclude that the mode of colony formation of single cell adapted hESC is linked to cell substrate mediated motility of the cells. Clearly the choice of cell substrate is an important consideration in the production of clonal reporter lines and induced pluripotent stem cells as cell motility and the method of colony formation directly impact on cell clonability.

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MULTILAYER CELL SHEET THERAPY FOR A MYOCARDIAL INFARCTION MODEL

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Aim:
It is critical for a multilayer cell sheet to attach on infracted myocardial tissue within a limited time to maximize the cell sheet based therapy. Our previous study showed that it takes 30 min for bone marrow stem cells (BMSC) to attach firmly onto the normal heart tissue of pig. The goal of this study is to optimize suitable timing for the attachment of multilayer BMSC sheet on the infracted myocardial tissue.

Method:
BMSC were harvested from the bone marrow of pig. Once the cells reach confluence for 7 days after being cultured in mesenchymal stem cell (MSCs) basal medium, a monolayer BMSC detached itself spontaneously from temperature-responsive culture dish when the temperature was reduced from 37 °C to 20 °C. Myocardial infarction was created by the ligation of the left anterior descending branch of the left coronary artery in pig. A multilayered cell sheet was then transplanted onto the ischemia area on left ventricle.

Results:
A multilayer of BMSC was prepared by stacking three-monolayer of cells. Histological analyses and scanning electron microscopy demonstrated that a firm adhesion was formed between MSCs sheets and the ischemia heart tissue within 30 minutes, which is the same as normal heart tissue.

Conclusions:
The timing of cell sheets adhesion provides an important information for the clinical application of BMSC sheet-based therapy for heart infraction tissue repairing.

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POROUS POLY(L-LACTIDE) SCAFFOLDS WITH BMP-2 RELEASING SURFACE-IMMOBILIZED *NANO*-HYDROXYAPATITE

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Aim:
Nano-hydroxyapatite (n-HAp) has been used as a main component for bone-regenerative scaffolds due to its cell-friendly surfaces and osteoconductivity. As another bioactive component, BMP-2 has been widely adopted to offer the scaffolds with enhanced osteoinductive property. BMP-2 plays a significant role for stem cell differentiation to osteoblasts, which cannot be accomplished by the use of n-HAp alone. In particular, the sustained release of BMP-2 at its free form is known to be effective in promoting new bone formation. Herein, we report on porous poly(L-lactide) (PLLA) scaffolds with surface-immobilized n-HAp that can release BMP-2 in a controlled manner. We aim to demonstrate the synergistic effect of released BMP-2 with surface-immobilized n-HAp on *in vivo* osteogenic potentials.

Method:
PLLA pellets were dissolved in with a mixture of 1,4-dioxane and water (87/13, v/v) at 75 °C to produce the transparent solution (7%, w/v). The solution was then cast to 10 mm in diameter with 5 mm in thickness Teflon mold. The samples were frozen in a deep-freezer at -20 °C, followed by lyophilization. The pore surface of PLLA scaffolds was then functionalized with primary amine groups. The surface immobilization of n-HAp was performed by the EDC-mediated reaction of phosphate groups of surface-functionalized n-HAp with surface amines of PLGA scaffolds. BMP-2 was incorporated onto the anionic brushes on immobilized n-HAp surfaces by incubation in PBS.

Results:
Porous PLLA scaffolds with a regular and interconnected pore structure were fabricated using a thermally induced phase separation (TIPS) process. The pore size of the PLLA scaffolds was in the range from 100 to 200 μm. Themogravimetric analysis (TGA) showed that 11 wt% of N-HAp was immobilized on the scaffold pore surface. X-ray photoelectron spectroscopy (XPS) analysis showed that n-HAp was stably immobilized on the pore surfaces of PLLA scaffolds. FITC-labeled BMP-2 was utilized to visualize the BMP-2 loading on the pore surface of PLLA scaffolds. BMP-2 could be efficiently bound on the phosphate anionic brushes of n-HAp surfaces. The loading efficiency of BMP-2 on the surface was estimated 97%. BMP-2 could be released continuously for more than one month.

Conclusions:
We developed a novel approach for biodegradable porous scaffolds that have pore surfaces featuring osteoconductivity and osteoinductivity. The scaffold described in this work may display the synergistic effects due to each inherent activity of n-HAp and BMP-2 for effective bone formation. The detailed discussions on *in vivo* bone-regeneration of this novel scaffold will be presented.

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FABRICATION OF CHITOSAN-ALGINATE-HYALURONATE-HEPARAN SCAFFOLDS FOR NEURAL STEM/PROGENITOR CELL CULTURE

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Neural stem/progenitor cells (NS/PCs) therapy is a promising strategy for brain disorders, traumatic brain injury and neurodegenerative disorders. However, a major problem is to build a compatible culture model for NS/PCs proliferation and differentiation. In this study, a new porous three-dimensional natural polymer scaffold comprised of chitosan, alginate, hyaluronate and heparan sulfate (C-A-Ha-He) was fabricated, and several structural determinants of its biological activity including the mean pore size, the porosity and degradation rate were identified. By evaluating the cellular proliferation, adhesion rate and alkaline phosphatase activity, we assessed the status of NS/PCs in the C-A-Ha-He scaffolds. Compared with chitosan-alginate scaffolds, C-A-Ha-He scaffolds provided a more suitable environment for NS/PCs proliferation and differentiation after 7 days. Moreover, immunostaining and observation by electron microscope indicated that NS/PCs in the C-A-Ha-He scaffolds might form an artificial neural network where neurons and glia establish connections and exhibit synaptic activities. These findings provide a biological basis for future application in screening medicine or transplantation of this artificial construct in neural repair.

Acknowledgements:
Financial supported by the National Science Foundation of China (30800288) and Doctoral Program Foundation of Institutions of Higher Education of China (20070141050) is greatly acknowledged.

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IN VITRO THREE DIMENSIONAL CHONDROGENESIS OF MURINE BMSCS PURIFIED BY HYPOXIA CULTURING AND MAGNETIC-ACTIVATED CELL SORTING WITH LINEAGE

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[2] *National Tissue Engineering Center of China, Shanghai, China*

Aim:
Mouse is an ideal model to study cell biology and differentiation mechanisim of stem cells in tissue engineering. However, due to lack of appropriate methods to proliferate and purify murine bone marrow stromal cells (mBMSCs), three dimensional (3D) cartilage construction based on mBMSCs has never been accomplished. This study explored the feasibility of *in vitro* 3D chondrogenesis of mBMSCs purified by hypoxia culturing and magnetic-activated cell sorting (MACS) with lineage.

Methods & Results:
The current results demonstrated that mBMSCs cultured under hypoxia (4%) had stronger capacity of proliferation compared to normal oxygen (20%) group. Furthermore, hypoxia cultured BMSCs included a lower level of hematopoetic lineage, suggesting that hypoxia culture can purify at a certain extent mBMSCs. In vitro differentiation assays further demonstrated that hypoxia cultured BMSCs had stronger adipogenic, osteogenic, and chondrogenic potentials. In order to obtain a higher percentage of BMSCs, we further enriched mBMSCs by MACS with hematopoetic lineage markers. These enriched mBMSCs expressed higher levels of Sca-1, CD90, c-kit, and CD105 but negative for CD31, CD34, and CD45. After seeded onto PLGA scaffolds and *in vitro* chondrogenically induced for 10 weeks under hypoxia, mBMSCs formed typical cartilage-like tissue with positive expression of collagen II and chondromodulin 1. Moreover, the engineered cartilage could retain its cartilage phenotype even after 10 weeks of subcutaneous implantation.

Conclusions:
These results indicated it is feasible to engineer a 3D cartilage with purified mBMSCs by hypoxia culturing and MACS with lineage. This study provides an ideal animal model for investigating differentiation mechanism of BMSCs in the future.

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CURRENT TOPICS ON THE JAPANESE REGULATION FOR THE MARKETING APPROVAL OF HUMAN CELL/TISSUE-BASED MEDICAL PRODUCTS: PMDA'S PERSPECTIVE AND ACTION ON THEIR EVALUATION AND DEVELOPMENT

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PMDA is a Japanese regulatory agency which contributes to improve the public health and safety by reviewing license application and conducting post-marketing safety measures of pharmaceuticals and medical devices, and also responsible for providing relief compensation for sufferers from adverse drug reaction and infections by pharmaceuticals or biological products. We, office of biologics II, review the marketing license application of cells/tissue-based medical products.

Our mission is to ensure quality, safety and efficacy of medical products as well as to promote the efficient development of these products through scientific review and consultation. The recent rise of social expectation on the realization of regenerative medicine, in cooperation with progression on the rerated cellular science, encourages us to extend our supportive action on this field to build common base of knowledge for the evaluation of human cell/tissue-based medical products with introduction of our point of view for public. In this presentation, we will introduce the current Japanese regulation on the human cell/tissue-based medical products, and our recent regulatory actions including contribution to preparation of new guidelines for them, including comparison to those in EU and US.

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RHEOLOGICAL AND STRESS RELAXATION PROPERTIES OF PHEMA AND PHEMA-TiO_2 COMPOSITE HYDROGELS

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Aim:
The study aims to characterize the viscoelastic properties of poly (2-hydroxyethyl methacrylate) (PHEMA) and PHEMA-TiO_2 hydrogels. The effect of shear stresses on the stress relaxation behavior of PHEMA and PHEMA-TiO_2 hydrogels was also investigated.

Method:
The viscoelastic and stress relaxation properties were examined using a HAAKE MARS II Rheometer (HAAkE, Germany). The geometry is demonstrated in Figure 1.

Results:
The complex modulus, $|G^*|$ is dependent on the HEMA contents in the hydrogels (Fig.2). The influence of TiO_2 nanoparticles on viscoelastic properties of the hydrogels was less significant in comparison to that of HEMA contents. The difference of $|G^*|$ was due to the changes of morphological structure of the hydrogels.

A rapid recovery was found in the hydrogels after the stress was released (Fig.3). The hydrogels containing a high HEMA content presented higher shear stress resistance, attributed to their less porous structure. 40HEMA and 40HEMA-7.5TiO_2 hydrogels can completely recover at a stress of 250Pa, while the permanent deformation was noticed for both 30HEMA (2%), and 30HEMA-7.5TiO_2 (3%) hydrogels.

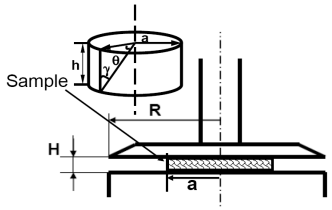

Figure 1 Experimental set-up for rheological test

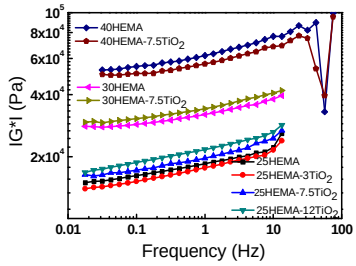

Figure 2 The complex modulus $|G^*|$ of the hydrogels

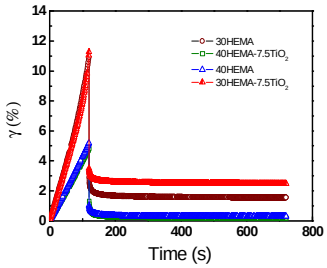

Figure3 Creep-recovery of hydrogels at τ=250Pa

Conclusions:
The HEMA contents have shown a great impact on $|G^*|$ values and the stress resistance. TiO_2 nanoparticles addition had little effect on the $|G^*|$, but it caused more permanent deformation for the hydrogel at a same stress. Our findings suggest that the dynamic mechanical responses of PHEMA hydrogels can be optimized by altering the composition of the materials.

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POLYHYDROXYBUTYRATE AND ETHYL CELLULOSE COMPOSITES AS BIOMATERIALS

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[2] *Australian Nuclear Science & Technology Organisation, Lucas Heights, NSW, Australia*

Polyhydroxyalkanoates (PHAs) are hydrophobic biopolyesters produced by a range of microorganisms. The most studied member of the PHA family is Polyhydroxybutyrate (PHB), which is widely used as a biomaterial in medical implantation devices. This biocompatible and biodegradable biomaterial is an FDA approved product. However, due to its brittle nature, slow degradation in vivo and relative high manufacturing costs, applications of PHB are restricted. One strategy to overcome PHBs impediments is by blending with other biopolymers such as ethyl cellulose (EtC) to improve material properties, promote degradation under physiological conditions and reduce cost.

This study characterised the properties of various loading capacities of EtC blended with PHB and their influence on physiological degradation and cellular biocompatibility. PHB/EtC films demonstrated that average surface roughness (R_a) increased as EtC loading increased. The mechanical strength (6.83±1.05 to 7.12±1.29 MPa) was maintained in PHB/EtC film with blend ratios of 80/20 and 60/40 respectively. Furthermore, crystallinity of the films was reduced and degradation rates were enhanced with increases in EtC loadings. In addition, results revealed a hydrogen-bonding interaction between the two polymers in the blends. Cellular biocompatibility was analysed using neural associated olfactory ensheathing cells (OECs) which demonstrated that PHB/EtC blends maintained OEC growth and cellular morphology when compared to PHB films.

Our results show that blending PHB with EtC improved the material properties and degradation of the resultant composite films while maintaining cellular compatibility. Our results suggest that these composite films have potential for neural tissue engineering strategies in regenerative medicine applications.

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COMPARISON OF DIFFERENT BIOREACTOR TO CULTURE FETAL MESENCHYMAL STEM CELLS FOR BONE TISSUE ENGINEERING

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Bioreactors provide dynamic culture conditions for efficient exchange of nutrients and the mechanical stimulus necessary for generation of effective tissue engineered bone grafts (TEBG). We have shown that biaxial rotating (BXR) bioreactor-matured human fetal mesenchymal stem cell (hfMSC) mediated-TEBG can heal rat critical sized femoral defects. However, it is not known whether optimal bioreactors exist for bone TE (BTE) applications. We systematically compared this BXR bioreactor with three most commonly used systems: Spinner Flask (SF), Perfusion and Rotating Wall Vessel (RWV) bioreactors, for their application in BTE applications.

The BXR bioreactor achieved higher levels of cellularity and confluence (1.4-2.5x, $p<0.05$) in large 785mm^3 macroporous scaffolds not achieved in the other bioreactors operating in optimal settings. BXR bioreactor treated-scaffolds experienced earlier and more robust osteogenic differentiation on von Kossa staining, ALP induction (1.2-1.6x, $p<0.01$) and calcium deposition (1.3-2.3x, $p<0.01$). We developed a MicroCT quantification method which demonstrated homogenous growth of hfMSC in BXR bioreactor-treated grafts, but not with the other three.

BXR bioreactor enabled superior cellular proliferation, spatial-distribution and osteogenic induction of hfMSC over other commonly used bioreactors. In addition, we developed and validated a non-invasive quantitative microCT-based technique for analyzing neo-tissue formation and its spatial distribution within scaffolds.

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HEPARIN-CONJUGATED FIBRIN AS INJECTABLE SYSTEM FOR SUSTAINED DELIVERY OF BONE MORPHOGENIC PROTEIN-2

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Aim:
We developed an injectable system for long-term delivery of bone morphogenetic protein-2 (BMP-2) by covalently conjugating heparin to fibrinogen to promote bone regeneration. We hypothesized that long-term BMP-2 release from heparin-conjugated fibrin (HCF) gel would promote bone formation as compared to short-term BMP-2 release.

Method:
Heparin-conjugated fibrinogen formed an injectable, HCF gel when mixed with thrombin. 1 μg BMP-2 was mixed with 50 μl HCF or normal fibrin gel and implanted into rat hindlimb muscle. 8 weeks after implantation soft x-ray, micro CT, histological analysis, western blot analysis and calcium quantification assays were performed.

Results:
In contrast to 83.7±7.6% of BMP-2 released from normal fibrin gel for the initial 3 days, 89.4±3.8% of BMP-2 was released from HCF for 13 days. BMP-2 released from HCF significantly increased alkaline phosphatase activity of cultured osteoblasts as compared to that of BMP-2 released from normal fibrin gel. BMP-2 released from HCF maintained BMP-2 bioactivity during the released period and was advantageous for bone regeneration as compared to short term BMP-2 delivery from normal fibrin gel. X-ray radiography, micro CT, histomorphometry, calcium assay, and western blot analysis showed that BMP-2-loaded HCF can induce extensive bone formation as compared to short term BMP-2 delivery.

Conclusions:
Since clinically benign and injectable HCF can deliver BMP-2 over a long term, HCF system would have advantages for clinical applications for bone regeneration.

Acknowledgement:
This study was supported by a grant (SC3220) from 21st Centry Frontier Program, ministry of Education, Science, and Engineering, Republic of Korea.

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SPINNER FLASK CULTURE INDUCES REDIFFERENTIATION OF DEDIFFERENTIATED CHONDROCYTES

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Aim:
Condrocytes dedifferentiate during culture with several passages, and cartilage regenerated by implantation of dedifferentiated chondrocytes may be suboptimal. We hypothesized that a stirred suspension bioreactor (i.e. spinner flask) culture system would induce formation of chondrocyte aggregates and redifferentiation of dedifferentiated chondrocytes.

Method:
Condrocytes were isolated from rabbit articular cartilage and cultured in monolayer or spinner flasks for 5days. On 3 and 5 days, we collected cells and carried out scanning electron microscopy, histological analysis, live and dead assay and RT-PCR.

Results:
P1 chondrocytes cultured in monolayer for 5 days showed a polygonal morphology, which is a characteristic of differentiated chondrocytes. In contrast, P4 chondrocytes showed a large, spread cellular appearance, which is a characteristic of dedifferentiated chondrocytes. Culture in 3D stirred suspension bioreactors (spinner flasks) induced the aggregate formation of both P1 chondrocytes and P4 chondrocytes. And chondrocytes cultured in spinner flasks significantly increased mRNA expression of differentiation markers at day 3. Importantly, spinner cultures induced redifferentiation of dedifferentiated chondrocytes, as type I collagen expression was significantly lower and type II collagen expression was significantly higher in chondrocytes cultured in spinner flasks than in chondrocytes cultured in monolayer.

Conclusions:
Following a 3D stirred suspension bioreactor (spinner flasks) culture system can induce formation of aggregates and redifferentiation of dedifferentiated chondrocytes. This system is easily scalable and could be useful for large-scale culture of chondrocytes for clinical applications.

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FABRICATION OF POLYCAPROLACTONE/ELASTIN COMPOSITE SCAFFOLDS FOR TISSUE ENGINEERING

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The aim of this study was to fabricate three-dimensional (3D) porous polycaprolactone (PCL)/elastin composites. Porous structures of PCL with average pore size greater than 300 μm were formed using gas foaming process. The PCL foams were then soaked in a solution of elastin and a cross-linking agent to prepare composite scaffolds. The PCL/elastin composites were prepared under a range of conditions: ambient pressure, vacuum, and high pressure CO_2. The presence of elastin within the 3D structures of the PCL foam imparted hydrophilicity and improved the swelling behavior of fabricated composites. The results of scanning electron microscopy and Fourier transform infrared analysis showed that immersing the PCL foam in an elastin solution under atmospheric conditions results in a skin-like formation of crosslinked elastin on the top surface of the PCL with no penetration into the 3D structures. Impregnation of the PCL foam under vacuum for 24h slightly improved the penetration of elastin into the PCL foam but the elastin fibers were not homogenously distributed within the PCL structure. However, elastin fibers penetrated and were uniformly distributed within the 3D structure of PCL, when the crosslinking reaction was performed using high pressure CO_2. The presence of open pores on the top surfaces of composite hydrogels fabricated under high pressure CO_2 can promote cellular growth throughout the matrices for tissue engineering applications.

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PREPARATION SELF-EMULSIFYING DRUG DELIVERY SYSTEM FOR IMPROVEMENT STABILITY &SOLUBILITY OF DOCETAXEL

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Aim:
The main goal of this experiment was a preparation of a self-microemulsifying emulsion using PLGA, Tetraglycol, Labrasol, and Cremophor ELP to improve stability of docetaxel (Dtx) drug.

Method:
PLGA, Dtx, Tetraglycol, Labrasol, and Cremophor ELP was added into the vial, followed by heating with stirring and vortex mixing until Dtx had perfectly dissolved. The particle size, size distribution, and surface charge of resultant Dtx-loaded SMES were determined by dynamic light scattering (DLS). The shape and surface morphology were investigated by atomic force microscopy (AFM). B16F10 cells were incubated without and with Dtx-loaded SMES, and Taxol for 7 days and cell viability was determined by MTT assay.

Results:
The average size of Dtx-loaded SMES was in the range of 80–90 nm. We found that the formulation of the Dtx-loaded SMES prepared in DW and BSA showed a little change in the particle size for 7 days. The zeta-potential of the Dtx-loaded SMES indicates the negative surface charges. The cytotoxicity for Dtx-loaded SMES and Taxol for comparison was evaluated using B16F10 melanoma cell line. Dtx-loaded SMES was somewhat more effective in inhibition of B16F10 cell than Taxol. Thus, it appeared that the slightly improved inhibition of B16F10 cell may be due to the Dtx-loaded SMES's stability.

Conclusions:
We prepared the Dtx-loaded SMES to improve their stability. We confirmed that Dtx-loaded SMES showed an inhibitory effect for proliferation of B16F10 melanoma cells.

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PREPARATION AND CHARACTERIZATION OF POLYMERIC MICELLES CONSISTING OF POLY(PROPYLENE GLYCOL) AND POLYESTERS

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[1] *Department of Molecular Science and Technology, Ajou University, Korea*
[2] *Department of Polymer engineering, Pukyong National University, Korea*

Aim:
We confirmed that the PPG-polyesters triblock copolymers formed micelles and hence may act as potential hydrophobic drug carriers.

Method:
PPG and toluene were introduced into a flask. Toluene was then distilled off completely. To PPG was added the CH_2Cl_2, followed by the individual addition of CL, TMC, or VL. The polymerization was initiated by the addition of HCl at 25 °C. After 24 h, the reaction mixture was poured into n-hexane to precipitate a polymer. The obtained polymer was redissolved in CH_2Cl_2 and then filtered. The polymer solution was concentrated by rotary evaporator and dried in vacuum to give a colorless polymer of quantitative yield.

Results:
PPG-polyester triblock copolymers were successfully prepared by the individual polymerization of CL, TMC, or VL using terminal alcohol of PPG as an initiator via an activated monomer mechanism. This method offered new biomedical materials with well-defined molecular architecture. The micelle formation of PPG–polyester copolymers in an aqueous phase was confirmed by NMR, DLS, AFM and PL. CMCs and partition equilibrium constant (K_v) of the triblock copolymers prepared in this work depended on the polyester segments. The diameters of micelles, measured by DLS and AFM, were 400-600 nm.

Conclusions:
We prepared PCL–PPG–PCL, PVL-PPG-PVL, PTMC-PPG-PTMC triblock copolymers via activated monomer mechanism and confirmed their possibility as a potential hydrophobic drug delivery vehicle.

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LOCAL DELIVERY OF ANTIBIOTICS FROM A LASER ACTIVATED SURGICAL ADHESIVE

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SurgiLux® is a chitosan based, laser activated surgical adhesive. Its morphology as a flexible, thin film makes it ideal for sutureless surgery avoiding suture trauma and supporting wound healing. An advancement of this biomaterial would be the incorporation of local drug delivery technology. In particular, delivery of antibiotics at the possible site of infection would provide SurgiLux® with an unprecedented advantage in the wound healing market, while providing the ideal conditions for contamination free cellular growth.

This study incorporated gram positive, gram negative and broad spectrum antibiotics (Vancomycin, Ofloxacin and Tobramycin) into SurgiLux® films at various concentrations. The activity of these films, post-lasering, was investigated against bacterial strains responsible for severe ocular inflammatory diseases, including *Pseudomonas aerigenosa, Staphylococcus aureus, S. epidermidis* and *Escherichia coli*.

Zone of clearance studies confirmed that antibiotics were successfully incorporated into the SurgiLux® films and gradually released with effectiveness against the selected strains. Bacteria kill-curves of CFU/mL (colony forming units) plotted against 'release incubation time' showed rapid CFU/mL reduction of 10^5 to 0 CFU/ mL from 0-3 hours indicating release concentrations greater than the MBC (minimum bacteriocidal concentration) were maintained over a 28 day release and quantified using LC-MS. In addition, the influence of these composite films on the growth and attachment of human stromal fibroblasts was investigated using a cell-cycle approach.

This study demonstrates that SurgiLux® can be utilised for local antibiotic delivery in wound tissue engineering applications while maintaining its adhesive properties.

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PROTEOMIC ANALYSIS OF RAT PODOCYTES UNDER HIGH CONCENTRATIONS OF GLUCOSE

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Aim:
We aimed to look for comprehensive proteome changes in primary the cultured glomerular epithelial cells (GECs, podocytes) under hyperglycemic conditions and to identify additional potential mechanisms related with early diabetic nephropathy (DN).

Method:
We performed proteome analysis of podocytes by 2-DE and identified alterations in protein expression under high glucose concentrations (30 mM glucose) 3 days after treatment. Differentially expressed GECs proteins between normal and high glucose concentrations were identified using ESI-Q-TOF MS/MS and were confirmed by Western blotting.

Results:
High glucose exposed GECs showed increased ROS and more cellular hypertrophy. Protein A was up-regulated and proteins B, C and D were down-regulated in high glucose treated GECs. The expression of protein B decreased significantly under high glucose conditions compared with normal conditions ($p<0.05$) by Western blotting. The expression of vimentin (not significantly changed) and CLP36 (down-regulated), expected to be involved podocytes pathology in DN, was also observed by Western blotting.

Conclusions:
These results will provide better understanding of the development of early DN via glucose metabolism of GECs under hyperglycemic conditions.

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INFLUENCE OF BME ON THE NEUROGENESIS OF BMSCS WITH PLGA/BME FILM

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Aim:
In this study, we figure out the effect of BME release to cells and carry out to find the proper BME content film for proliferation and differentiation of BMSCs.

Method:
We fabricated BME loaded Poly(L-lactide-co-glycolide)(PLGA) film (PLGA/BME) for neurogenesis of BMSCs and to observe the release behavior of BME. The experiments were carried out to evaluate the effect of BME on the biocompatibility and viability of neurogenesis of BMSCs from *in vitro*. We prepared PLGA/BME films using the concentrations of 200, 400, 600μM, and 1 μM of BME by the solvent evaporation method and we isolated and cultured BMSCs into the PLGA films respectively. Morphology of celluar adhesion confirmed by scanning electron microscope (SEM). Cellular viability and proliferation were assayed by WST test The effect of PLGA/BME film on the neural differentiation of BMSCs was assessed by RT/PCR.

Results:
We could observed that cells were well attached and we confirmed the cultured BMSCs were good viability increasing BME content. In addition, the neural differentiation of BMSCs were specifically presented by RT/PCR result.

Conclusions:
we expected to confirm the result of differentiation of BMSCs on PLGA/BME film has a good application for tissue regeneration. This research was supported by WCU (R31-20029) and Musculoskeletal Bioorgan Center (0405-BO01-0204-0006).

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Abstract 194
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AUTOLOGOUS AND FUNCTIONAL BLADDER TISSUE ENGINEERED IN A BIOREACTOR IMITATING THE PHYSIOLOGICAL PRESSURE ENVIRONMENT

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Aim:
Significant complications are associated to the conventional bladder augmentation, which affect its compliance and watertight function. To replace safety diseased tissues, we elaborate an autologous vesical equivalent (VE) devoid of exogenous biomaterials, and developed in a bioreactor that mimics the dynamic of bladder filling and emptying to acquire physiological properties.

Method:
Porcine fibroblasts, urothelial and endothelial cells evolve in a 3D and dynamical culture to obtain a VE easy to handle. This construction includes cyclic pressure progressively increasing up to 15 cmH2O every four hours, followed by a rapid decrease. Histology, immunohistochemistry and electron microscopy was performed. Mechanical resistance is measured by uniaxial tensile tests, and 14C-urea solution was used to quantify the degree of impermeability of our VE.

Results:
We obtain an autologous and thick matrix containing collagen I and III uniformly distributed. A differentiated urothelium, with positive marker for cytokeratin 20, is established on a well-defined basal membrane of laminin and collagen IV. Permeability evaluations displayed the same profile as the native bladder, coinciding with uroplakins organization into apical plaque observed by electron microscopy. Finally, an appropriate resistance for suturing was demonstrated, and this, even when a network formed by capillary-like structures was shown throughout the matrix with Pecam-1 staining.

Conclusions:
Our VE offers promising avenue for regenerative medicine and *in vitro* studies for therapeutic drugs. This new alternative distinguishes itself all over its autologous character, its efficiency as a barrier to urea and its endothelialization, which would significantly reduce necrosis, inflammation, contraction, and, therefore, possible rejection.

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EFFECT OF SILK CONTENT ON THE PROLIFERATION AND PHENOTYPE OF NUCLEUS PULPOSUS CELLS ON SILK/PLGA SCAFFOLDS

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Aim:
Intervertebral disc (IVD) degeneration is one of the principal causes of low back pain involving high expense within the health care system. The long-term goal is the development of a medical treatment modality focused on a more biological regeneration of the inner nucleus pulposus (NP). Silk is being rediscovered and reconsidered as potentially useful biomaterials in a range of applications. So, In this present study, we prepared the silk/PLGA scaffolds and studied to determine their suitable scaffolds for disc cell cultures.

Method:
In this study, We fabricated silk/PLGA scaffolds using 0, 10, 20, 40 and 80wt% of Silk by the solvent casting/salt leacing and solvent evaporating method. NP cells were seeded into silk/PLGA scaffolds. After 1, 3 and 7days of cell seeding, cell proliferation and viability were measured via MTT assay. In addition, morphology of cellular adhesion were observed by scanning electron microscope(SEM). RT-PCR was conducted to confirm mRNA expression of Col Ⅰ,Col Ⅱ for NP marker. And Histological appearance was evaluated by Safranin-o, H&E and MTS.

Results:
We confirmed that cell viability on silk/PLGA scaffolds of 80wt% was higher than other scaffolds. Also, RT-PCR and Histology staining results, especially silk/PLGA scaffolds of 80wt% showed that the gene characteristic of NP cells were improved.

Conclusions:
This result indicates that Silk/PLGA scaffold is useful for intervertebral disc regeneration.

This research was supported by WCU(R31-20029) and Musculoskeletal Bioorgan Center (0405-B001-0204-0006).

Poster 55
Abstract 199
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PRODUCTION OF AN AUTOLOGOUS PORCINE FIBROBLASTS SHEET IN TISSUE ENGININEERING

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Aim:
Non-urologic tissues are used to correct urethral disorders but leads to complications. Other substitutes, like acellular tissues or synthetic matrices are studied on small animal model, like rabbit, without great success. In our laboratory, the self-assembly approach allowed us to develop a graft substitute from a pig species. This model is interesting because of its physiological and functional similarities with the human urinary system.

Method:
We optimized cell culture conditions, temperature and culture medium, to produce a cellular sheet composed of porcine fibroblasts with their own matrix. We also compared the impact of the harvesting site in order to obtain an optimal extracellular matrix (ECM) synthesis *in vitro*. A characterization of collagen I, the most commonly type of collagen found in ECM, and fibronectin, an important protein for cell migration, has been performed. Also thickness measurements were taken to confirm the impact of culture temperature in ECM production.

Results:
Porcine fibroblasts obtained from oral mucosa and cultured at 39□C have faster proliferations compared to 37□C, and skin fibroblasts cultured at 39□C. Moreover, fibroblasts obtained from oral mucosa and cultured at 39□C synthesize more ECM and deliver a thicker tissue, easy to handle. Macroscopically, the formed sheets appeared uniform, and the presence of ECM proteins was confirmed with specific immunofluorescence staining.

Conclusion:
The production of our porcine fibroblasts sheet offers an interesting option in the field of urological tissue engineering. Autologous tests on a bigger animal model are now accessible to acquire *in vivo* results comparable to human profiles.

Poster 1
Abstract 205
Displayed on Thursday afternoon & Friday all day

THE EFFECT OF PROPORTION OF ACELLULAR DERMAL MATRIX AND DEMINERALIZED BONE MATRIX ON THE BONE REGENERATION IN THE RAT CALVARIAL DEFECT MODEL

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Aim:
Bone regeneration with biomaterial implants has been widely used to fill bone defects in human medicine. The purpose of this study was to investigate bone regenerative effects of several proportion of the acellular dermal matrix (AM) and demineralized bone matrix (DBM) on rat calvarial model.

Method:
Six groups were formed on the basis of the ratio of DBM to AM: negative control (left untreated); DBM alone (0.1 Mℓ), DBM group; DBM (0.07 Mℓ) and AM (0.03 Mℓ), DBMAM73 group; DBM (0.05 Mℓ) and AM (0.05 Mℓ), DBMAM55 group; DBM (0.03 Mℓ) and AM (0.07 Mℓ), DBMAM37 group; AM alone (0.1 Mℓ), AM group. After 4 and 8 weeks, the skulls were harvested.

Results:
Three-dimensional CT scan showed that hyperattenuated signs displaying newly formed bone in defect and new bone area reached more than 95% in the DBM, DBMAM73 and DBMAM55 groups comparing with the DBMAM37 (75.91%), AM (67.87%) groups and negative control (35.16%). Histologic examination revealed that the defect was filled with newly formed bone on DBM particles with neovascularization and collagen fiber around AM implant. The DBMAM73 group showed evidences of complete bone trabeculae were observed, including blood vessel in central canal and osteonal lamella. In histomorphometrical evaluation, the DBM group showed highest total healing score (12.33±2.06), and the DBMAM73 and DBMAM55 groups revealed 10.33±1.63, 10.16±2.4, higher comparing to the DBMAM37 (8.1±1.83) and AM (7.33±0.81) groups.

Conclusions:
Based on findings obtained, DBM with AM in the ratio of 7:3 or 5:5 could be useful in bone defects showing similar bone regeneration effect like DBM alone implantation.

Poster 2
Abstract 210
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NOVEL VITRIFICATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS

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Induced pluripotent stem (iPS) cells are promising as a regenerative therapy tool for bioengineering and transplantation of tissues. Cryopreservation of these cells could be an indispensable fundamental technology. Dimethyl supfoxide (DMSO) is the most effective cryoprotecitive agent (CPA) but shows high cytotoxicity. Furthermore, the extremely low viability of human embryonic stem cells and iPS cells has been obtained by ordinary slow cooling method using 10%DMSO solution. Therefore, it is necessary to use CPA that is harmless and maintain stemness and differentiation. Currently, human iPS cells have been preserved by vitrification method using special solution called DAP213, which contains 2M DMSO, 1M acetoamide and 3M propylene glycol. However, the solution shows high cytotoxicity due to its high osmotic pressure. The aim of this study was to develop the vitrification solution using novel developed polyampholytic cryoprotectant such as carboxylatedε-poly-L-lysine (PLL) for vitrification of human iPS cells. Culture medium solutions with PLL whose amino groups were converted into carboxyl groups more than 50 mol % by succinic anhydride showed excellent cryoprotective properties of various cells and antifreeze protein properties, such as ice recrystallization inhibition. We developed the vitrification solution containing ethylene glycol, sucrose and carboxylated PLL with highly stable glass state leveraging the ice recrystallization inhibition activity of carboxylated PLL, Vitrified human iPS cells with the vitrification solution showed higher recovery rate than that with DAP213. These results suggest that polyampholytes allows cryopreservation of iPS cells to be available for many kinds of applications.

Poster 3
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VARIATIONS IN THE RATIOS OF CO-CULTURED BONE MARROW STEM CELLS AND CHONDROCYTES IN PURIFIED ALGINATE MICROCAPSULES FOR TISSUE ENGINEERING

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Aim:
Adult articular cartilage tissue has poor capability of self-repair. Therefore, a variety of tissue engineering approaches are motivated by the clinical need for articular repair. Alginate has been used as a biomaterial for cartilage regeneration. However, the main drawback is the immune rejection *in vivo*. To overcome this problem, we have developed the biocompability of alginate using modifed *korbutt's* method.

Method:
Our experiments were performed that purified alginate microcapsules were fabricated by seeded various ratios of co-cultured bone marrow stem cells and cartilage cells into microcapsules. After 1, 3, 7 and 14 day of cell seeding, cell proliferation activity was measured via MTT assay. In addition, safranin-O, alcian blue, and von Kossa stain were carried out for confirmation of chondrogenesis. Moreover, differentiation at 1, 2, and 3 weeks, gene expression was examined by RT-PCR for collagen type Ⅰ, Ⅱ and aggrecan.

Results:
MTT results, cell proliferation was good in co-cultured cells. In Alcian blue, Von Kossa stain results were well shown to present the cartilaginous phenotype preculiarity. The RT-PCR results show gene encodings of collagen type Ⅰ, Ⅱ and aggrecan in experimental group.

Conclusions:
This study suggests that purified alginate microcapsules may serve as potential bone marrow stem cells differentiation and structural basis for tissue engineered articular cartilage. This research was supported by WCU (R31-20029) and Musculoskeletal Bioorgan Center (0405-BO01-0204-0006).

Poster 4
Abstract 214
Displayed on Thursday afternoon & Friday all day

EFFECTS OF SIS/PLGA FILM ON PROLIFERATION AND PHENOTYPE OF RETINAL PIGMENT EPITHELIAL CELLS

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Aim:
Transplantation of normal RPE has been proposed as a potential therapeutic modality in the surgical management of subretinal neovascularization and It is also in age related macular degeneration. In this study, we evaluate the property of SIS/PLGA film on adhesion, proliferation and phenotype of retinal pigment epithelial cells(RPEs).

Method:
Porcine small intestinal submucosa(SIS) derived from the submucosal layer of porcine intestine, both generate a minimal inflammatory response following implantation and have been approved by the FDA for many types of biomedical applications. We fabricated natural/synthetic biomaterial hybrid films using SIS into PLGA. Then RPECs were seeded on SIS/PLGA film. We confirmed morphology of cellular adhesion by scanning electron microscope(SEM). Cell proliferation activity was measured by MTT assay. RT-PCR was conducted to confirm mRNA expression of RPEs markers.

Results:
In the result of MTT assay, cell viability on SIS/PLGA films were were higher than bFGF/PLGA films and PLGA films. RPEs specific mRNA expression and protein could not be well observed on SIS/PLGA film.

Conclusions:
SIS provides suitable surface to RPEs and SIS of suitable content affect to improve cell adhesion and phenotype maintance. The SIS/PLGA film can act based on this experiment to be very useful to the tissue regeneration for the retinal function reproduction or the wound healing is provided. This research was supported by WCU (R31-20029) and Musculoskeletal Bioorgan Center (0405-BO01-0204-0006).

Poster 5
Abstract 215
Displayed on Thursday afternoon & Friday all day

FABRICATING MICROFIBERS BASED ON THE BIOMIMETIC HYDROGELS WITH 3-DIMENSIONAL BIOPRINTING SYSTEM

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Aim:
The development of vascularized tissue is one of the critical issues in tissue engineering and regenerative medicine. Conventional scaffold fabrication technologies based on bulk 3D methods can't achieve the complex tissue structures such as vascularized tissues. Novel biomimetic hydrogels were printed using 3-dimensional bioprinting system to construct 3-D vascularized tissue.

Method:
Hyaluronic acid (HA) based hydrogel was synthesized using acrylated hyaluronan and thiol containing cross-linkers such as PEG thiol or Cys containing peptides. The hydrogels were patterned into micro-sized fibers with various diameters. We analyzed the cell viability using live/dead assay. To fabricate bio-mimetic vascularized tissue, endothelial cells were cultured in the micro fiber HA hydrogels containing YIGSR and VEGF. Cellular adhesion and behavior in the fiber structure was also evaluated.

Results:
Hydrogels based micro-fibers were fabricated by 3D bioprinting systems. The diameter of the fiber was from 180 μm to 600 μm by changing printing speed. Cells in the printed hydrogels were alive and showed the viability up to 90%. Fabrication of micro fibers by changing hydrogel composition with YIGSR peptides and VEGF was also evaluated. Endothelial cells in the different composition of hydrogel showed the differential cellular behaviors.

Conclusions:
Patterning of biomimetic hydrogels into micro sized fibers was successfully achieved using 3D bioprinting systems. This method showed the possibility that it can be applied to the tissue specific vascularized scaffold by the component of hydrogels such as peptides or growth factors. The micro sized structures can be used for the regeneration of complex tissues such as heart and kidney.

Poster 6
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REGENERATION OF INTERNERTEBRAL DISC USING NUCLEUS PULPOSUS CELL ON PURIFIED ALGINATE MICROCAPSLUES

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Aim:
The application of nucleus pulposus(NP) cells is promising therapeutic approach for internertebral disc regeneration. . Alginate is a natural polymer is frequently used in biomedical applications. In this study, alginate were specially used for NP regeneration However, the main drawback is the immune rejection *in vivo.* To overcome this problem, we have developed using modifed *Korbutt's* method for alginate purification.

Method:
NP cell were seeded in purified and non-purified alginate microcapsules, and then cell viability and proliferation analyzed by MTT assay after 1, 3, 7 and 14days. RT-PCR was assessed to measure mRNA expression for specific gene of nucleus pulposus cells in purified and non-purified alginate microcapsules. NP cell-loaded purified and non-purified alginate microcapsules were implanted into the dorsal region of BALB/c-nu nude mouse, and the implanted alginate microcapsules were extracted after 2, 4 and 6 weeks. The alginate microcapsule evaluated by histology through H&E, Safranin-O, MTS and Type Ⅱ collagen staing.

Results:
The MTT result of purified alginate microcapsules showed that the cellular viability was excellent. From the RT-PCR and histology staing results, purified alginate microcapsules showed that purified alginate microcapsules improved gene characteristic of NP cells.

Conclusions:
This result indicates that purified alginate microcapsule might be ellective for intervertebral disc regeneration.

This research was supported by WCU (R31-20029) and Musculoskeletal Bioorgan Center(0405-B001-0204-0006).

Poster 7
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COMPRESSIVE STRENGTH OF POLY(L-LACTIDE-CO-GLYCOLIDE) SCAFFOLDS SEEDED NUCLEUS PULPOSUS CELLS DEPENDING ON PORE SIZE : *IN VIVO EXPERIMENT*

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Aim:
This study investigates the influence of compressive strength fabricated by PLGA scaffold for tissue-engineered bio-disc with pore size of sodium chloride particles and selecting the best suitable pore size for cell to grow in scaffold.

Method:
PLGA scaffolds were fabricated by solvent casting/salt-leaching with pore size 90~180, 180~250, 250~355 and 355~425 , 425~600 μm. Nude mice were sacrificed at 1, 2 and 4 weeks and each samples were harvested at each time point after retrival PLGA scaffolds seeding nucleus pulposus (NP) cells, which were implanted into the subcutaneous space in nude mice. To characterize the morphology, mechanical strength, NP cells compatibility, Safranin-O, Immunohistochemistry (Collagen Type II) were evaluated.

Results:
Nucleus pulposus cells were seeded in to an PLGA scaffold with various pore size. At all retrieval times, samples maintained shape. But shape of some samples were destroyed , increasing the time. As the pores become smaller, the rate of cell growth and the compressive strength of the scaffold was increased . The group of scaffolds with pore size between 90 and 250 μ m is better compressive strength ,cell proliferation and ECM production .

Conclusions:
These results demonstrated that the compressive strength of the scaffold was improved while scaffold has the pore sizes ranging from 90 to 250 μm and the good cell interconnectivity. The pore size of scaffold for cell proliferation is a key factor for cell metabolism. This research was supported by WCU (R31-20029) and Musculoskeletal Bioorgan Center (0405-BO01-0204-0006).

Poster 8
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DETERMINATION OF EFFECTIVE ACTION DISTANCE IN VITRO BETWEEN UMBILICAL CORD BLOOD-DERIVED HEMATOPOIETIC STEM/PROGENITOR CELLS AND HUMAN ADIPOSE DERIVED STEM CELLS

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Aim:
In order to investigate the optimal action distance in the coculture process *in vitro* between umbilical cord blood-derived hematopoietic stem/progenitor cells (HS/PCs) and human adipose derived stem cells (ADSCs), we thus designed a novel transwell co-culture protocol that the distance between the two culture chambers could be adjusted.

Method:
Sand papers with different specifications were utilized to adjust the cellular action distance that ranged from 10 to 450 μm, measuring by high-precision vernier caliper between two kinds of cells mentioned above. Following to this, the HS/PCs were cultured in a modified transwell at a density of 1×10^6 cells/mL supported by around 2×10^5 cells/mL of ADSCs in a 6-wellplate for 7 days. The total cell number was counted using a hemacytometer and the cell morphology was observed under a inverted microscope everyday. After 7 days of co-culture, the expansion fold, surface antigen $CD34^+$ and CFU-GM of the hematopoietic mononuclear cells (MNCs) were analyzed. Meanwhile, the surface markers (CD13, CD29, CD34, CD44, CD45, CD73, CD105, CD166 and HLA-DR) and the multi-differentiation potential (adipogenic, osteogenic and chondrogenic) of ADSCs were also assayed to identify the stemness of expanded stromal cells.

Results:
The results showed that there was an optimal communication distance, around 350μm, between these two stem cells during their co-culture. At this distance the expansion fold of the UCB-MNCs and $CD34^+$ cells achieved 15.1±0.2and 5.0±0.1 fold, respectively. The expanded ADSCs, still showed positive expression of CD29, CD44, CD166 and negative expression of CD34, CD45, and could also differentiate into osteoblasts, adipocytes and chondrocytes after respective induction.

Conclusions:
It was concluded that 350μm should be an optimal action distance between two stem cells during their 7 days of co-culture.

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SUITABILITY OF STORAGE CONTAINERS FOR CELL-THERAPY PRODUCT

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Aim:
In order to assess the suitability of several storage containers for cell-therapy product.

Methods:
Human keratinocytes and mesenchymal stem cells (MSCs) (1×10^7 cells/ml of each) were injected to glass vial, pre-filled syringe, or bag, and stored at 4°C up to 96 hrs. At 0, 12, 24, 48, 72, and 96 hrs, cells were harvested and *in-vitro* parameters for cell-therapy product, identification, viability, and cell activity, were analyzed.

Results:
Both cells expressed their own specific marker, pan-cytokeratin and CD29, irrespective of storage container. In both cells, viability was gradually decreased with the passage of time, but the decreasing rate was lowest in pre-filled syringe (keratinocytes) and glass vial (MSCs), respectively. Cell activity of keratinocytes was more highly conserved in pre-filled syringe-stored group.

Conclusions:
In-vitro parameters for cell therapy, such as viability and activity, are affected by the kind of storage container. Therefore, it may be required the selective choosing of suitable storage container according to a cell characteristics.

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APPLICATION OF HUMAN TOOTH DERIVED MATERIALS FOR TOOTH AND BONE REGENERATION

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Aim:
For dental and craniofacial regeneration, exofoliated human teeth used as bioactive material.

Method:
Teeth were decalcified with EDTA solution. The soluble proteins were analyzed by LC-MS/MS. Human mesenchymal stem cells were cultured and odonto/osteogenic differentiation assays were carried. The effect of soluble proteins, demineralized dentin and natural hydroxyapatite on dentinogenesis and craniofacial regeneration were analyzed.

Results:
We analyzed tooth soluble protein composition and found 147 different proteins. The soluble proteins stimulated odontoblast differentiation of dental pulp stem cells in 2- and 3-dimensional conditions *in vitro*. It stimulated dentin and tooth formation *in vivo* using xenogenic combination and embryonic mouse tooth bud models. The soluble proteins coated biphasic calcium phosphate scaffold significantly enhanced regeneration of calvarial defect.

We also characterized demineralized dentin, and found that it had increased nitrogen elements per unit weight. Demineralized dentin could support growth of stem cells, and induce mineralization. For improving property, we prepared composite scaffold with PLGA containing 1, 3, 5 and 10 wt% dentin. Analysis showed that scaffold (3% of dentin) induced osteo/odontogenic differentiation of DPSCs.

Dental hydroxyapatite (dHA) contained endogenous amine groups. The surface was immobilized with matrix extracellular phosphoglycoprotein (MEPE) peptide. The MEPE modified dHA showed increased osteogenesis and mineral formation *in vitro*. In calvarial and dentin defect models, it also showed significant bone and dentin regeneration.

Conclusions:
We demonstrated that soluble tooth protein, demineralize dentin and dental hydroxyapatite enhance osteo/odontogenic differentiation and bone/tooth regeneration. These results suggest that tooth derived materials are useful bioactive materials for dental and craniofacial regeneration.

Poster 11
Abstract 229
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IN VITRO CHARACTERISATION OF HUMAN ADIPOSE STEM CELLS IN FETAL BOVINE SERUM AND ALLOGENEIC HUMAN SERUM

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Aim:
Human adipose stem cells (ASCs) may have therapeutic potential, but standard expansion methods for clinical cell therapy remain to be established. ASCs are typically cultured in medium containing fetal bovine serum (FBS). Animal-derived sera, however, stage safety issues in clinical therapy, such as infections and severe immune reactions. By expanding ASCs in medium containing human serum, the problem can be avoided. To define how allogeneic human serum (alloHS) performs in ASC expansion compared to FBS, a comparative in vitro study in both serum supplements was performed.

Method:
ASCs were isolated and cultured in medium supplemented with either FBS or alloHS. The cell proliferation was assessed using PreMix WST-1 Cell Proliferation Assay. The surface marker expression of ASCs and cell cycle distribution were analyzed by flow cytometry (FACSAria®). DNA microarray was done using GeneChip® Human Genome U133 Plus 2.0 Array. Osteogenic, chondrogenic and adipogenic differentiation potential of ASCs were also evaluated.

Results:
To reach cell proliferation rate comparable with 10% FBS, 15% alloHS was required. Genes of the BMP receptor mediated signaling on the TGF-β signaling pathway, regulating e.g. osteoblast differentiation, were overexpressed in FBS. The result was further supported by differentiation analysis, where osteogenic differentiation was significantly enhanced in FBS. No major differences between culture conditions were observed in chondrogenic and adipogenic differentiation and in cell surface markers.

Conclusions:
The choice of serum had a significant effect on ASCs proliferation, gene expression and differentiation. Our data emphasize the importance of thorough investigation of ASCs for utilization in cell therapies.

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COMPARING THE PROTEIN EXPRESSION LEVELS OF TUMORIGENIC AND NON-TUMORIGENIC HEPATOCELLULARCARCINOMA CELLS

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Aim:
To elucidate the specific signaling pathway and mechanism underlying the action of cancer stem cells, we analyzed the differentially expressed proteins between tumorigenic and non-tumorigenic hepatocellularcarcinoma (HCC) cells.

Method:
In this study, CD133+ cells were purified from a HCC cell line, Huh-7 via MACS, whose purity was confirmed using FACS. The ability for self-renewal of the CD133+ cells was examined by colony-forming assay, and stemness was evaluated by spheres culture. The differences between CD133+ cells and CD133- cells were also analyzed via 2-DE. The differentially expressed proteins were analyzed and then identified by ESI-Q-TOF MS/MS. Among the identified proteins, A and F proteins were confirmed with RT-PCR and Western blotting.

Results:
A protein, lowly expressed in CD133+ cells compared to CD133- cells, was known one of the Rb regulators. However, F protein related to cell migration was highly expressed in CD133+ cells. We observed CD133+ cells have more metastatic phenotype than CD133- cells using wound healing assay and invasion assay. Moreover, in tumors derived from Huh7-induced xenografts, F protein was also co-expressed with CXCR4, responsible for tumor invasion. More studies are needed to reveal the role of the identified proteins in the undifferentiated tumerigenic cells.

Conclusions:
Although a large-scale clinical study is necessary to confirm the significance and effectiveness, the development of F-targeted therapies might have potential as a rational therapeutic strategy against tumor formation and metastasis in the progress of cancer.

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EXOGENOUS RHTRX REDUCES LIPID ACCUMULATION UNDER INFLAMMATORY CONDITIONS

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Object:
Thioredoxin (TRX), a redox-regulating protein, has been reported to have protective effects against various inflammatory diseases. The aim of this study was to investigate the action mechanism of TRX on inflammatory skin diseases.

Method:
To approach this, we performed proteomic analysis of A375 melanoma cells exposed to lipopolysaccharide (LPS) followed by treated with or without recombinant human TRX (rhTRX). Among the differentially expressed proteins, a significant decrease in expression of a 47 kDa protein (TIP47), ATP synthase, and TRX, which participate in lipid metabolism, was observed in LPS-stimulated cells. However, rhTRX attenuated the LPS-induced down-regulation of TIP47, ATP synthase, and TRX. Moreover, rhTRX reduced LPS-induced lipid accumulation in the skin of C57BL/6 mice. The protective effects of rhTRX on LPS-induced skin cell damage also involved a decrease in the release of inflammatory cytokines tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and interleukin-6 (IL-6), which contribute to lipid accumulation under inflammatory conditions.

Result and Conclusion:
Taken together, our results indicate that, as a pivotal antioxidant, TRX might play a critical role in the modulation of skin redox environments during inflammation, most likely by preventing alterations in lipid metabolism through the up-regulation of TIP47 and ATP syntase and down-regulation of inflammatory cytokines.

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ANNEXIN A2 IS A NEW REGULATOR OF H2O2-INDUCED PREMATURE SENESCENCE IN HUMAN MESENCHYMAL STEM CELLS

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Aim:
The aim of this study is to find the new regulator of cellular senescence by proteomic analysis.

Method:
For the induction of stress-induced premature senescence (SIPS), MSCs at about 75% confluence were briefly exposed to 150 μM H_2O_2 for 2 h. We showed that sublethal concentration of H_2O_2 senesce hMSCs as well as other types of cells by senescence associated β-galactosidase staining, brdU incorporation, measurement of Intracellular ROS, caspase-3/7 activity assay and cell cycle analysis. And we found differentially expressed proteins in SIPSed hMSCs compared to the control hMSCs via gel-based proteomic assay. Among the identified proteins, we evaluated the ANXA2 whether this protein is associated in cellular senescence by cloning or gene silencing.

Results:
We confirmed the senescence of hMSC during SIPS by various analyses. And we found six differentially expressed proteins in 2-DE map and identified them by ESI-Q-TOF MS/MS. Among the identified proteins, annexin A2 (ANXA2) was up-regulated on the 2-DE map and we confirmed by Western blot analysis. And up-regulation of ANXA2 induced increased expression of p53 proteins and senescent phenotypes of hMSCs.

Conclusions:
These results will provide that ANXA2 plays an important role as new regulator in cellular senescence.

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EX VIVO INDUCTION OF VASCULAR NETWORK WITHIN THE ENGINEERED 3D MYOCARDIAL TISSUES

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Aim:
In tissue engineering, current methods are limited by the restrictions of passive diffusion, preventing the creation of thick and viable tissues. Therefore, new technologies for making functional tissues with a well-organized vasculature are required. In this study, we hypothesized that it may be possible to overcome these diffusion limits by employing ex vivo microvascular bed that allowed for sufficient vascular network within tissue-engineered myocardial tissues created by our cell sheet-based tissue engineering approach.

Method:
Neonatal rat cardiomyocytes with endothelial cells were harvested as cell sheets from temperature-responsive culture dishes. Three cell sheets were layered and transplanted over the microvascular bed with an artery and a vein ex vivo. Layered cardiac cell sheets were maintained in a tissue bioreactor with culture medium. Three days after the transplantation, the perfused engineered tissues with the blood cells were histologically analyzed.

Results:
Three days after transplantation, layered cardiac cell sheets had survived and could maintain their pulsation. Immunostaining of the blood cell perfused layered cardiomyocyte sheets demonstrated that the entire CD31 positive microvasculature within the layered cardiomyocyte sheets were stained around the blood cells, indicating that vascularization was induced from the microvascular bed and that the tissues were supplied with culture medium from the underlying branch of the artery.

Conclusions:
Ex vivo vascularized myocardial tissues were successfully fabricated by transplanting the constructs over the microvascular bed with the tissue bioreactor. The present methods demonstrate the possibility for further development of engineered thick myocardial tissues with the ability for well-organized microvessels in vitro.

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DESIGN AND FABRICATION OF A COMPUTER-CONTROLLED BIOREACTOR AND BIOMECHANICAL TESTING DEVICE FOR BLADDER TISSUE ENGINEERING

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Aim:
To repair and regenerate diseased or malfunctioning bladder, a tissue-engineered vesical equivalent (VE) has to provide an effective blood-urine permeability barrier as well as good elasticity and compliance. In order to study the mechanical behavior of our VE, we developed a computer-controlled bioreactor allowing for the reproduction of a bladder duty cycle in vitro.

Method:
The self-assembly approach allows for the fabrication of a completely autologous bladder wall without the use of any exogenous scaffold. We designed a bioreactor to dynamically culture our VE using equibiaxial stress. The setup is composed of two independent chambers for the culture of the urothelium side and the muscular side of the VE in different culture media. The experimental setup is maintained in incubator-like conditions and is pressure-controlled using a LabView® Virtual Instrument. Over a 16h period, the urothelium side chamber is pressurized to reproduce 4 filling-emptying cycles. The increasing pressure gradient reaches 15 cmH2O in 4 hours and is followed by a pressure drop back to 0 cmH2O over 2 minutes. After the 4 cycles, the VE is kept in a no load state for the remaining 8 hours of the 24h cycle.

Results:
The VE produced by self-assembly resulted into an easy to handle tissue having a well differenciated and functional urothelium. VE cultured in the bioreactor showed no sign of deterioration over a 72 hours stimulation period. Histological analysis showed no sign of deterioration of the extracellular matrix.

Conclusions:
The validation of our system has shown that our VE is able to sustain physiologic pressures without experiencing mechanical failure. The setup has proven to be efficient to maintain the VE in sterile culture conditions and will be used to apply mechanical stimulation on the tissue over time to study the remodeling of our VE.

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PREPARATION OF CRYOPOLYMERS FOR BONE ENGINEERING

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The aim of this work is to test the ability of the cryopolymerization technique to produce scaffolds for bone engineering. Cryopolymerization is a processing procedure that generates highly hydrophilic and elastic porous materials. This study involves the manufacture and optimization of composite scaffolds based on 2-(Dimethylamino) ethyl Methacrylate (DMAEMA), 2-hydroxyethyl methacrylate (HEMA) and a bioactive ceramic -tricalcium phosphate (TCP). DMAEMA is both a pH-responsive cationic poly-electrolyte, and a thermo-sensitive polymer. HEMA is a non-ionic polymer with a reported good biocompatibility and haemocompatibility. TCP is a highly reactive ceramic that supports adhesion, differentiation and proliferation of mesenchimal stem cells and osteoblasts.

Cryogels were prepared by free radical copolymerization in an aqueous solution containing the ceramic component, the monomers, the initiator ($Na_2S_2O_7$), the activator (N,N,N´,N´-Tetramethylethylene diamine) and the croslinkers at -20ºC. Specimens with different monomer/water ratio (5-40), ceramic content (0-20%) and crosslinker concentration (0-2%) were prepared.

25 mm long and 13 mm width porous specimens were obtained for all the compositions assayed. Total monomer amount was optimized at 10%. Greater percentages yield specimens with isolated pores. Crosslinker/monomer ratio was optimized to a 1:300 ratio. The thawing rate was a factor that affected critically the properties of the cryogels. The density of the freeze dried specimens is a 28 % lower than the density of the specimens that were thawed before extracting the solvent suggesting that polymerization continues during the heating process. SEM images also show specimens with thicker, denser struts and smaller pore sizes for the thawed specimens than for the freeze dried specimens. A pore size distribution between 50 m and 1 mm was obtained.

DMAEMA/HEMA ratios up to 25/75 were studied. Greater porosity (75%) and pore size (1 mm) was obtained for a 75/25 monomer ratio. Swelling ability is also greater for 75/25 specimens, up to 400%, than for any other composition. However elastic modulus, 1.5 kPa, is not significantly lower than the modulus of denser compositions.XRD patterns of ceramic loaded specimens shows that the reinforcing phase is TCP. No transformation into apatite could be observed. 5% ceramic loaded specimens produce an increase in the elastic modulus of the specimens from 1125 to 1161 Pa for a 75/25 specimen while not affecting significantly the porosity of the specimens

As a summary, thawing/solvent extraction steps play a crucial role in the porosity of obtained scaffolds. Scaffolds with a 75% of porosity and pore sizes up to 1mm can be obtained with the cryopolymerization technique. It is possible to incorporate highly reactive TCP into these scaffolds.

Acknowledgements:
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THE VALUE OF 3D MULTISLICE CT IN FINE EVALUATION OF MAXILLARY BONE GRAFTING

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The study points out the importance of 3D Multislice CT-scan exam in the evaluation of the maxillary bone grafting.

We retrospectively reviewed the Multislice CT-scan imaging studies of 43 patients with maxillary bone grafting examined between January 2007 and December 2009 at The Imagistic Diagnostic Center Neuromed Timisoara using a 64 Somatom Sensation.

The 64 Multislice CT-scan exams offer a preoperative full map of the missing bone volume. Postoperative CT-exams detect the subtle local changes associated with block bone grafts harvested from mandibular symphysis and ramus buccal shelf and appreciate the integration of bone grafting at the recipient site .

The bone grafting investigated by CT-exams had mandibular location in 32,7% and maxillar placement in 67,3%. We evaluated the autogenous bone grafts for pre-implant alveolar ridge augmentation in 17%, for pre-implant maxillary sinus grafting in 15%, for reconstruction of post extraction small loss of buccal cortex in 15%, for filling the posttraumatic osseous defects in 10%, for filling the defects post resection of small odontogenic tumors in 28% and the correct alignment of alloplastic graft materials used for the same reasons in 15%. Some mild local edematous changes were reveled in early postoperative CT-exams in the donor and the recipient sites.

The Multislice CT-scan examination represents the elected method in current practice for the 3D evaluation of the the maxillary bone grafting with a special preoperative value in full appreciation of the osseous defect and postoperative assessment of well integration and complications eventually associated with the donor and recipient sites.

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LARYNGEAL REGENERATION USING TISSUE ENGINEERING TECHNIQUES IN A CANINE MODEL

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Aim:
We have previously reported that 3% collagen-coated polypropylene mesh wrapped with autologous fascia was a useful material for laryngeal regeneration after a partial hemilaryngectomy. However, a difference in the vertical level of the vocal folds was found. To increase the volume of the vocal fold, bone marrow-derived stromal cells (BSCs) were injected into the newly regenerated vocal fold 6 months after the operation. The aim of this study is to examine the efficacy of BSCs in repairing injured vocal folds at the chronic phase.

Method:
A partial hemilaryngectomy was performed on 8 adult beagles. Three percent collagen-coated polypropylene mesh was wrapped in fascia then fixed in place over the defect. Six months after the operation, either BSCs with 1% atelocollagen solution or only 1% atelocollagen solution were injected into the treated vocal fold. To measure the volume change of the vocal folds three-dimensional computed tomography was periodically performed. Vibratory examinations were also performed with excised larynges.

Results:
Three months after the injection, the volume was up about 10% compared to the volume before injection in both groups. Vibratory data of BSCs group were better than that of only atelocollagen group.

Conclusions:
This study suggests that the injection of BSCs with atelocollagen was useful forstaged regeneration of newly regenerated vocal fold at the chronic phase, but the efficiency of volume increase was limited. It was indicated that BSCs contribute to the improvement of tissue properties of the vocal folds.al models in our laboratory.

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A COMPARATIVE STUDY OF DIFFERENT MECHANICAL CONDITIONING REGIMES FOR THE DEVELOPMENT OF TISSUE ENGINEERED ANTERIOR CRUCIATE LIGAMENT

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Aim:
A comparative study is performed between three conditioning regimes of continuous levels (high and low intensity) and rehabilitative regime, with static culture as control.

Method:
Scaffolds of knitted silk fibroin with aligned electrospun silk fibroin (SF-AL) were seeded with rabbit bone marrow stromal cells (rBMSCs, P2, $1.5x10^6$/scaffold) and cultured statically for 3 days before loading into a customized bioreactor. Continuous low and high intensity, with different cyclic frequency (0.1Hz (4320cycles/day) and 0.5Hz (21600 cycles/day) respectively), were applied for the two groups ("Low" and "High") through the 28 days study. The group undergoing rehabilitative regime ("Rehab") would be exposed to "Low" (D3-D7) and "High" (D7-D28). All other parameters were constant.

Results:
Cell proliferation: "High" showed significantly lower proliferation rates than other groups from D3 to D7. "Rehab" showed a deviation from its proliferative phase from D14.

Collagen deposition: Significantly more collagen deposition in "Rehab" between D14 and D28 as compared to other groups, indicating the group undergoing a differentiative phase.

Mechanical properties: "Rehab" had significantly higher strength and stiffness than other groups by D21 with breaking load and elastic stiffness at D28 measured as 238$\pm$19 N and 44.4$\pm$ 2.8 N/mm respectively.

Histology: Sections in "Rehab" and "Low" showed aligned collagen deposition with "Rehab" having larger bands of collagen fibrils with crimp pattern at D28.

Conclusions:
Through this comparative study, a preliminary outlook of a suitable mechanical stimulation regime was presented. "Rehab" was shown to be a better stimulation approach of MSCs towards the regeneration of ligament/tendon tissue when compared to other continuous stimulation regimes.

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THE REGENERATION OF CHRONIC TYMPANIC MEMBRANE PERFORATION USING EGF BLENDED CHITOSAN PATCH SCAFFOLDS

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Aim:
The purpose of this study was to develop the novel chitosan patch scaffolds in which EGF was blended for healing chronic tympanic membrane perforation(CTMP) which is one of the most common otologic diseases.

Method:
EGF blended chitosan patch scaffolds (ECPS) was fabricated, and the morphological, mechanical properties of ECPS was assessed. The release properties of the ECPS were investigated to measure continuous drug release degrees, and so was *in vitro* test for measuring cytotoxicity and cell migration ratio. Finally, ECPS was used *in vivo* test to heal the CTMP

Results:
ECPS made of 3% chitosan and 3.5% glycerol and 0.7μg EGF had optimal characteristics, ~65μm in thickness, ~60° water contact angle, ~170% in elongation, ~16MPa in elastic coefficient. EGF blended in ECPS was released until 7weeks. *In vitro* study showed low cytotoxicity. *In vivo* study, CTMP model using ECPS was healed with higher success ratio. Healed tympanic membrane tissues using ECPS had lower thickness and less tophi

Conclusions:
Through experimental results, the developed ECPS could enhance the regeneration of CTMP.

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BRANCHED OLIGOMERIZATION OF CELL-PERMEABLE PEPTIDES MARKEDLY ENHANCES TRANSDUCTION EFFICIENCY OF ADENOVIRUS INTO MESENCHYMAL STEM CELLS

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Aim:
Cell-permeable peptides (CPPs) promote the transduction of non-permissive cells by recombinant adenovirus (rAd) to improve the therapeutic efficacy of rAd. However, their application was limited by low efficiency and high cost of CPPs. In this study, we investigated the effects of branched oligomerization of monomeric CPPs on the transduction efficiency of rAd into mesenchymal stem cells (MSCs).

Method:
To investigate the rAd transduction efficiency of various branched CPPs, *in vitro* transgene expression was analyzed in human MSCs derived from bone marrow or umbilical cord blood. In addition, the efficacy of *ex vivo* genetically modified MSCs was evaluated in two different animal models such as calvarial bone defect and spinal cord injury (SCI).

Results:
Tetrameric CPPs significantly increased transduction efficiency at 3000-5000-fold lower concentrations than monomeric CPPs. Furthermore, while only about 60% of MSCs were maximally transduced at 500 M of monomeric CPPs, greater than 95% of MSCs were transduced with 0.1 M of tetrameric CPPs. In a critical-size calvarial defect model, the inclusion of tetrameric CPPs in *ex vivo* transduction of rAd expressing bone morphogenetic protein 2 into MSCs promoted highly mineralized bone formation. In addition, MSCs which were transduced with rAd expressing brain-derived neurotrophic factor in the presence of tetrameric CPPs improved functional recovery in a SCI model.

Conclusions:
These results demonstrated the potential for tetrameric CPPs to provide innovative tool for MSC-based gene therapy as well as for *in vitro* gene delivery to MSCs.

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HEPATOCYTE SURFACE MODIFICATION FOR LIVER REGENERATIVE MEDICINE

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Aim:
Hepatocyte-based therapies have attracted attentions as a promising regeneration approach for the liver diseases. Because hepatocytes are prone to undergo phenotypic and functional alterations after cell isolation, it would be important to establish a method to modify cellular surfaces. Here, we describe a versatile method for modifying the surface of isolated hepatocytes by immobilizing synthetic polymers around the cells, yielding hepatocytes with a biocompatible ultrathin membrane.

Method:
Hepatocytes were isolated and purified from FVB/N mice by a two-step collagenase perfusion method. The surface of isolated hepatocytes was modified using a type of poly(ethylene glycol)-phospholipid conjugate bearing FITC (Lipid-PEG-FITC) according to the method we previously described (Bioconjug Chem. 2008;19:1389-95). After confirming the cell surface modification, we assessed the hepatocyte functions including cell viability, plating efficiency, protein productions (AAT and albumin), hepatocyte-specific gene expressions, and metabolizing activity of lidocaine and ammonia.

Results:
Among the types of phospholipids assessed in this study, 1,2-Dipalmitoil-*sn*-glycerol-3-phosphatidylethanolamine (DPPE) was found to be uniformly anchored to the hepatocyte cell membrane. Confocal fluorescence microscopic examination confirmed that more than 90% of the hepatocytes were coated with the polymer. We also confirmed that this cell surface modification procedure using the DPPE-PEG-FITC could be performed without affecting cell viability, cell attachment functions, gene expression levels and drug-metabolizing functions.

Conclusions:
We developed a novel method for modifying the cell surface of isolated hepatocytes without causing cellular damages. By replacing the FITC with functional proteins, e.g., an immunosuppressive or anti-coagulant agent, will lead to a new step toward to advancement of hepatocyte-based cell therapy.

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THE CREATION OF BRONCHIAL CHONDROCYTE SHEETS FOR AIRWAY RECONSTRUCTION

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Aim:
Tracheal / bronchial reconstruction is necessary in patients with extensive tracheal stenosis caused by congenital diseases, trauma, and neoplasms. Airway replacement after long-segment tracheal / bronchial resection remains a challenging because of the lack of a substitute conduit. Tissue engineering offers the potential to create replacement structures. Furthermore prosthetic or bioprosthetic materials lack growth potential and therefore in children require replacement as the children grow.

In this study, we create condrocyte sheets for airway replacement after long-segment tracheal / bronchial resection.

Method:
Lung tissues were excised from 1-day old luciferase positive rats. Lung tissues were finely minced and subjected to dissociation with 0.05% collagenase at 37°C for 30 minutes, under gentle shaking. Cell suspensions were then centrifuged, re-suspended in fresh collagenase solution, and treated in the same fashion, three additional times.

We observed the cell survival chondrocyte sheets transplantation in rat hemithorax by in vivo bioluminescence imaging. Luciferase positive neonatal rat chondrocytes were harvested as cell sheet from temperature-responsive culture dishes. Lung cells were then plated at an initial density of 1.0×10^5 cells/cm^2 on commercially available 30 mm diameter tissue culture dishes and cultured for 6 days at 37°C in a humidified atmosphere of 5% CO_2. At7 days, after cells had formed confluent monolayers, each cell was harvested by treatment with 0.05% trypsin-ethylenediamine tetraacetic acid for 5 minutes at 37ºC. For subculture, harvested cell suspensions were seeded on temperature-responsive culture inserts at an initial density of 1×10^5cells/cm^2. Cells were then cultured for an additional 7 days. At the time of surgery, cell sheets were non-invasively harvested by temperature-reduction to 20ºC, stacked to create threefold constructs, and used for transplantation. Histologic sections of chondrocyte sheets were stained with hematoxylin and eosin, azan, toluidine blue, and safranin-O.

Results:
Cultured chondrocytes were presented by toluidine blue stain. After transplantation of three chondrocyte sheets, these cell sheets attached rapidly to the surrounding lung pleura. By in vivo bioluminescence imaging, transplanted chondrocyte sheets were survival during re-thoracotomy. Histologic data of transplanted chondrocyte cell sheets showed the presence of both immature cartilage and mature cartilage after four weeks.

Conclusions:
This study demonstrates the feasibility of chondrocyte sheets for tracheal / bronchial reconstruction and recreating the cartilage.

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EFFECTS OF FLUORIDE APPLICATION ON PHOSPHORIC ACID ETCHING IN PRIMARY TEETH ENAMEL*

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Aim:
To examine the effect of fluoride application on 37% phosphoric acid (H3PO4)-etching in primary tooth enamel samples, based on the clinical protocol used in pediatric dental hospitals.

Method:
Enamel samples were prepared from thirty-six exfoliated and non-carious primary teeth. Each primary tooth was randomly assigned to one of four groups. Group 1 (control) received only acid-etching with 37% H3PO4 for 20 sec. Groups 2 to 4 received acid-etching with 37% H3PO4 for 20 sec at two weeks, one week, and immediately after acidulated phosphate fluoride (APF) treatment, respectively. Contact mode atomic force microscopy (AFM) images were obtained using a NANOstation II. The vestibular enamel surfaces of each primary tooth sample were scanned in air at a resolution of 512 × 512 pixels and a scan speed of 0.8 line/sec. Statistical analyses were performed to compare the mean values obtained from each group using the two-tailed Student's t-test and the Friedman's test ($p < 0.05$).

Results:
There were three main findings in this study: On the primary tooth enamel surface after APF treatment, substantial debris was observed, and the surfaces were smoother than those before APF treatment. Thus, APF treatment led to an insignificantly decreased surface roughness. Primary tooth enamel surfaces after acid-etching for 20 sec showed minute structures caused by the decreased hydroxyapatite nanoparticle space, indicating that acid-etching showed a significantly increased roughness ($p < 0.0001$, n = 9). As more time elapsed after APF pre-treatment, the surface roughness was decreased to a lesser degree, showing that the effects of APF pre-treatment progressively decreased with time ($p = 0.005$, n = 9).

Conclusions:
Primary tooth etching two weeks after APF pre-treatment used clinically in pediatric clinics may be an effective and promising process for obtaining proper etching effects on the enamel surfaces of primary teeth.

* This study was supported by a research fund from Seoul R&BD (grant #CR070054).

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EFFECTS OF NEUROPROTECTION BY ACUPUNCTURE IN A GLOBAL ISCHEMIC HYPERGLYCEMIA RATS*

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Aim:
We observed the effects of manual ACU on cerebral blood flow (CBF) throughout induction of ischemic and reperfusion conditions in hyperglycemic (HG) rats, using a ten-minute eleven-vessel occlusion (11-VO) rat model.

Method:
Male Sprague-Dawley rats (200-250 g) were used in the experiment. HG was induced by a single intraperitoneal injection of streptozocin (STZ), which was freshly dissolved in a 0.1 M citrate buffer at pH 4.5 and delivered at a dose of 60 mg/kg body weight. Seven burr holes were made for real-time measurements of brain temperature, two channels EEG, and two channels CBF. Ischemia was induced for 10 min and then reperfused for 40 min. ACU stimulation was applied throughout the ischemic period. We proposed 23 diagnostic parameters for a detailed analysis of changes in %CBF during conditions of ischemia/reperfusion. Statistical analysis was performed to compare changes in %CBF between control and ACU groups using the two-tailed Student's t-test. *p*-Values < 0.05 were regarded as statistically significant.

Results:
From CBF results, the five parameters including one (%CBFi) during the ischemic period and four ($\%CBF_{rep\text{-}max}$, $T_{rep\text{-}rise}$, $T_{rep\text{-}fall+base}$, and $S_{Crep\text{-}inc}$) during the reperfusion period, among the proposed diagnostic parameters of the ischemia-evoked %CBF response showed a significant difference between the two groups. A criterion of a > 70 % decrease in %CBF on the occluded side is frequently used to determine the success of the. These results showed a > 89% decrease in %CBF in both groups. In particular, ACU rats showed a significantly larger decrease ($p < 0.05$, n = 7) in %CBF (3.39%) during ischemia than control rats. Therefore, surgical and occlusion setups, supported by reduced %CBF in occlusion, are well established, and a real-time method is very useful for monitoring blood flow in the cerebral space.

Conclusions:
The decrease in %CBF during HG ischemia is responsible for the neuroprotective effects of ACU stimulation.

* This study was supported by a research fund from Seoul R&BD (grant #CR070054).

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THE EFFECT OF FORMALIN FIXATION ON EQUILIBRIUM PARTITIONING OF AN IONIC CONTRAST WITH MICROCOMPUTED TOMOGRAPHY (EPIC-µCT) IMAGING OF OSTEOCHONDRAL SAMPLES

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Aim:
EPIC-µCT is a non-invasive technique to quantify and visualise the three-dimensional distribution of glycosaminoglycans (GAGs) in cartilage. While this technique provides high-resolution data for fresh tissues, sample preparation and imaging time limit the experimental utility. Fixation may help overcome these issues; however, the effects of fixation on EPIC-µCT outcomes are unknown. This study aimed to determine whether formalin fixation of bovine cartilage affects x-ray attenuation, and therefore the interpretation of EPIC-µCT data.

Method:
Bovine osteochondral samples were incubated with ioxaglate for 22 hours prior to µCT scanning. Samples were scanned in fresh and formalin-fixed conditions, and samples scanned fresh were also re-scanned after fixation (n=8 per group). Wet weight, dry weight, and GAG content were determined for fresh and fixed samples (n=5).

Results:
The expected zonal distribution of contrast agent/GAGs was observed for fixed and fresh cartilage. However, the output range required for visualization of this distribution varied between fresh and fixed specimens. Correspondingly, average attenuation levels of fixed than fresh cartilage were significantly lower (14.3%, $p < 0.001$). Despite differences in attenuation, there were no significant differences in wet weight, dry weight, or GAG concentration between fixed and fresh samples.

Conclusions:
Formalin fixation reduces the attenuation of EPIC-µCT imaged cartilage. This reduction in attenuation is not due to changes in volume or GAG concentration following fixation. EPIC-µCT remains useful for studying GAG distributions in fixed tissues, such as archival samples, but the reduced attenuation should be taken into account when quantifying GAG for fixed cartilage.

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CARDIAC DIFFERENTIATION OF MOUSE ES CELLS BY PATTERNING CULTURE

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Aim:
Formation of ES cell aggregates promotes their differentiation into cardiomyocytes. ES cell aggregates are generally prepared via suspended spherical aggregates called embryoid bodies (EBs). Because the differentiation efficiency depends on the size of EBs, it should be controlled for the efficient differentiation. Although a hanging drop method is widely used to prepare size-controlled EBs, this method is unfavorable for the mass preparation because of its labor-intensive procedure. In this study, we developed a simple and scalable method to prepare size-controlled ES cell aggregates directly onto a substrate, by using cell-patterning technology.

Method:
Cell-patterning substrates were fabricated by a photolithography-based method. On the surface of the substrates, cell-adhesive circular domains (100-400 □m in diameter) were arrayed in the rest of non-cell-adhesive surface. Undifferentiated mouse ES cells were seeded onto the substrate and cultured for differentiation in □-MEM supplemented with FBS. The efficiency of cardiac differentiation was analyzed by flow cytometry.

Results:
ES cells seeded on the substrate proliferated within the circular domains and formed three-dimensional cell aggregates. Around day 7, some of the aggregates began to beat. Flow cytometry revealed that the optimal diameter of circular domains was 200 □m for efficient cardiac differentiation. We investigated culture conditions such as FBS concentrations for efficient cardiac differentiation. The percentage of cardiomyocytes reached about 7% at present.

Conclusions:
The cell-patterning method is useful for the simple mass preparation of cardiomyocytes from ES cells, and will strongly promotes the use of these cells in tissue engineering and transplantation experiments.

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ELECTROSPRAYING, A REPRODUCIBLE AND NON-TOXIC METHOD FOR PRODUCTION OF MICROSPHERES LOADED WITH GROWTH FACTORS

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Aim:
The ability of tissue-engineered constructs to guide cells towards differentiation into a specific tissue is a challenge. Growth factor (GF) delivery from scaffolds is one strategy to address this issue, however, many traditional methods of incorporation have been shown to denature the GFs and provide inhomogeneous release profiles. We aim to incorporate GFs into polymeric microspheres through electro-hydrodynamic atomization (electrospraying), with the intention of providing high loading yields, narrow size distribution, and reduced GF denaturation.

Methods:
Electrospraying was used to generate polycaprolactone (PCL) microspheres correlating changes in key parameters and with morphology, size and shape. Microspheres were viewed under SEM and the size distribution was determined with ImageJ. MC3T3 murine pre-osteoblasts were used to assess the biocompatibility of the microspheres based on cell viability/morphology (DAPI staining) and cell proliferation (PicoGreen) assays.

Results:
Preliminary data showed that the processing parameters of electrospraying influence the physical parameters of the microspheres. Spherical shape and narrow distribution were observed for feed rates ≤0.5mL/h and tip to collector distances ≥15 cm. PCL concentration and voltage were also shown to affect morphology. Cell viability and proliferation were observed consistently with the optimized electrospraying setup.

Conclusions:
We reproducibly produced biocompatible microspheres using electrospraying. The next stage of this work is loading of GFs, including bone morphogenetic proteins. After loading and optimization of the release, we hypothesize to combine this technology with electro*spinning* to provide a reproducible GF-delivery system integrated in a tissue-engineered construct: a nanofiber/microsphere composite able to mimic the *in-vivo* release profiles of GFs for bone tissue engineering applications.

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AN EXPERIMENTAL COMPARISON OF OVINE MANDIBULAR AND TIBIAL OSTEOBLASTS

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Aim:
Mandibular osteoblasts originate from the neural crest and deposit bone intramembranously, mesoderm-derived tibial osteoblasts by endochondral mechanisms. Bone synthesized by both cell types is identical in structure, but functional differences between the two cell types might exist. Thus, ovine mandibular and tibial osteoblasts of both juvenile and adult animals were compared regarding their molecular profile and mineralization potential.

Method:
The proliferative capacity of *in vitro* expanded, matched ovine mandibular and tibial osteoblasts was assessed using a PicoGreen assay. The osteogenic potential was analysed by ALP activity, alizarin red staining and calcium assay. Immunohistochemistry and sirius red staining were performed. Expression of osteogenic marker genes (e.g. Osteonectin) and others (e.g. genes of Wnt-pathway, Ihh) was evaluated using qRT-PCR. Osteogenic 3D cultures on mPCL-TCP scaffolds were examined for cell viability and morphology using confocal lasermicroscopy and SEM. The deposited calcium was measured with Wako Calcium-HRII assay and micro CT analysis. PMMa embedded specimens were sectioned and stained for haematoxylin-eosin and von Kossa.

Results:
The PicoGreen assay showed a higher proliferation rate for the tibial cells. However, mandibular cells exhibited higher ALP activity when cultured with osteogenic media. The alizarin red staining and calcium assay of mandibular cells showed a higher degree of mineralized matrix deposition. Differences observed were greater among juvenile cell types. Examination of the 3D cultures showed equally high viability for all cell types.

Conclusions:
The results suggest that neural crest-derived osteoblasts of the mandible represent a suitable alternative cell type for bone tissue engineering applications.

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NOVEL STRATEGY FOR DIRECTING THE DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS INTO THE MYOGENIC LINEAGE ON DENDRIMER-IMMOBILIZED SURFACE WITH D-GLUCOSE DISPLAY

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Aim:
The current study reports the cellular responses of human mesenchymal stem cells (hMSCs) cultured on the dendrimer-immobilized surface with D-glucose display. Based on examining morphological behaviors, moreover, fundamental mechanisms of cell and surface interaction are discussed in aspects of cytoskeletal formation and differentiation of MSCs.

Method:
Culture surfaces were designed by immobilizing dendrimer with D-glucose display, that is, 1st-generation (G1), 3rd-generation (G3) and 5th-generation (G5) dendrimer surfaces[1, 2]. For all experiments, hMSCs were seed on the surfaces and cultured for specified days in DMEM supplemented with 10% FBS and antibiotics. To evaluate cytoskeletal formation and differentiation, immunostaining and real time-PCR were performed using the standard protocol[2].

Results and Discussion:
To compare the dynamics of cell behaviors on different culture surfaces, the time-lapse observation of hMSCs on the PS, G1, G3 and G5 surfaces was conducted. The cells on the G5 surface showed more dynamic behaviors of temporal stretching and contracting associated with stimulated migration (such as “self-mechanotransduction”), as compared with the cells on the G1 and plain surfaces. On the G5 surface, moreover, a frequency of round-shaped cells increased, and spreading of the cells was appreciably suppressed. From the cytoskeletal staining of F-actin, it was found that the immature stress fibers were of significance in the cells on the G5 surface. In addition, the cells on the G5 surface expressed RhoA inactivation and Rac1 activation during the culture, indicating that the G5 surface permits the regulation of RhoA and Rac1 expression associated with altering in cellular morphology and migratory behaviors. It was also found that cardiac troponin T expression was, in particular, promoted within cell aggregates on the G5 surface, thus supporting the consideration that a balance of Rho family GTPases activation induces cardiomyogenesis in hMSCs.

Conclusions:
The current results suggest that the dendrimer surface can be a potential tool for the guided differentiation of hMSCs directing to cardiomyocyte-like cells in the absence of an aqueous cardiomyogenesis-inducing factor. These findings suggest that the dendrimer surface with D-glucose display offers a model of designing a substrate, based on the self-mechanotransduction in microenvironment, as a tool for guiding proper lineage specification in an *ex vivo* stem cell culture system.

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CULTURE SURFACE DESIGN FOR REGULATING CELL MORPHOLOGIES AND FUNCTIONS BASED ON CELL ANCHORING MECHANISMS

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Aim:
Many researchers reported that the regulation of surface adhesion ability of cells on a culture surface is reliable to gain morphological features of cells associated with cell phenotype. In the present study, we describe our novel strategies for creating a culture surface to control cell morphology and function, as well as approaches to their underlying mechanisms of action.

Method:
The dendrimer surface that can display D-glucose was prepared on plain surface of wells according to the previous procedure[1)]. Briefly, the surfaces with fourth generation of dendrimer were designed as structural templates by stepwise addition of tris (2-aminoethyl) amine during the procedure for synthesis of dendrimer. And L- and D-glucose was displayed as terminal ligands on the template dendrimer. For all experiments, several cell types including human fibroblasts and myoblasts were seed on the surfaces and cultured for specified days in DMEM supplemented with 10% FBS and antibiotics. To evaluate cell shape and function, immunostaining was performed using the standard protocol.

Results:
A culture surface was designed to regulate cell morphology in several cell types by changing the ratio of D- and L-glucose isomers displayed on plain surface. In addition, variation in the surface quantity of glucose transporter (GLUT)-mediated cell binding sites was investigated to be an important factor for morphological changes in several cell types. In the case of human fibroblast examined in present study, round-shaped cells dominantly appeared on the 100% D-glucose-displaying surface, while elongated cells prevailed on the 0% D-glucose-displaying surface. The cells on the former surface propagated with steric architectures of aggregation while monolayer growth with stretch-shaped cells occurred dominantly on the latter surface at 5 days, similar to the observations for a naked surface. Moreover, immunostaining of the cells provided evidence that collagen type II was produced exclusively around the round-shaped and aggregated cells on 100% D-glucose-displaying surface. A similar dependence of cell morphology on glucose-displaying surfaces was also seen in cultures of human myoblasts. Likewise in the case of fibroblasts, round cells on a 100% D-glucose-displaying surface showed active division to form cell aggregates, which were distinguishable from those formed by cells on 0% D-glucose-displaying and naked surfaces. Within 6 days most of the myoblasts in the aggregates had already completed their fusion into myotube-like multinucleated cells. These findings suggest that cellular morphology can be a physiological factor that dominates the phenotypes of cells in association with cell aggregates.

Conclusions:
The culture surface design in current study are based on the novel cell binding mechanism in which GLUTs are key elements responsible for regulating cell morphology and development of the cellular phenotypes. These findings provide new insights into mechanism controlling cell-substrate interactions for controlling the morphologies in various cell types associated with specific cell phenotypes.

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TRANSPLANTATION OF CARDIOGENIC CELLS DIFFERENTIATED FROM MESENCHYMAL STEM CELLS RESTORE ELECTROMECHANICAL FUNCTION IN THE INFARCTED RAT HEART

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Aim:
To achieve effective regeneration of injured myocardium, it is important to find a more effective way of improving the cardiogenic differentiation of MSCs in *in vitro*. In previous study, we showed that treatment of MSCs with a protein kinase C inhibitor suppressed the expression of cardiogenic markers. In this study, we hypothesize that the activation of protein kinase C induces the differentiation of MSCs into cardiogenic cells (CGCs) and the implantation of CGCs regenerates the infarcted myocardium more efficiently than intact MSCs.

Method and Results:
We have determined the effect of phorbol myristate acetate (PMA), a PKC activator, on cardiogenic differentiation of mesenchymal stem cells (MSCs) using sandwich ELISA assay. PMA treated MSCs showed the increased level of cardiac specific markers, such as cTnT, MLC, MHC, NKX 2.5, and MEF 2 with time dependent manner up to 9 days. In addition, β- and α- adrenergic, and muscarinic receptors, significantly increased and they were functionally reacted against norephinephrine stimulus. The mRNA levels of sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA 2a) and L-type Ca^{2+} channel (LTCC) in PMA treated MSCs were increased to similar levels in cardiomyocytes. Intramyocardial transplantation of CGCs into ischemic injured hearts significantly decreased interstitial fibrosis to 2.5±1.0% compared to MSCs control of 18±3.2%. TUNEL-positive cells caused by ischemic injury were significantly reduced by 45.0% in CGCs-transplanted group compared with MSCs group. Interestingly, though the mRNA levels of growth factors, such as HGF and VEGF were increased, inflammation cytokines like TNF-α, IL-1β, and IL-6 were significantly decreased in CGCs-transplanted groups compared to MSCs transplanted control. . CGCs-implanted rats exhibited restoration of conduction velocity, and reduction of ventricular tachycardia.

Conclusions:
These results suggest that regulation of protein kinase C activation could be a leading method for differentiation of stem cells into cardiomyocytes, and they may help to clarify the cascade of transcriptional activation that regulates differentiation into cardiogenic cells (CGCs) of MSCs. These results also contribute to a better therapeutic understanding of pre-differentiated MSCs on cardiac disease.

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CONSTRUCTION OF VASCULARIZED MYOCARDIAL TISSUE BASED ON A CELL SHEET ENGINEERING

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Aim:
Recently, researchers have challenged to construct three-dimensional (3D) myocardial tissues for treating failure heart. Tissue engineering is a novel method for regenerating damaged tissues. Our group has a special technology for making an intact cell dense
myocardial cell sheet from isolated myocardial cells. However, there is a problem that the scaling up of 3D myocardial tissue without vascularization results in cells death due to the lack of vessels supplying oxygen and nutrition, and removing waste molecules. Therefore, for constructing 3D myocardial tissues with a vascularization system, dense myocardial cell sheets were prepared in a bioreactor imitating an actual-living environment.

Method:
Rat myocardial cells including endothelial cells were cultured on a temperatureresponsive culture dish for 4 days. The harvested myocardial cell sheets were triplelayered and the tissues were repeated to put on a collagen gel culture scaffold with microchannels mimicking rat subcutaneous tissue. After the cultivation, histological analyses were performed for confirming the vascular development in the engineered tissue.

Results:
Histological observation demonstrated that the co-cultured endothelial cells migrated and connected to the microchannels resulting in well-vascularized tissues, furthermore repeated procedures realized 12-layered thick myocardial tissues (> 100 μm).

Conclusions:
For overcoming vascularization problems, 3D tissues with perfused capillaries were successfully prepared *in vitro*

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CALCIUM SULFATE COATED POLYMER-CERAMIC COMPOSITE PLUGS FOR ALVEOLAR CREST PRESERVATION AFTER TOOTH EXTRACTION: BIOCOMPATIBILITY ASSESSMENT

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Objective:
Bone defects after tooth extraction often lead to collapse of the alveolar socket. The width of remaining alveolar crest is often reduced making dental implant insertion impossible. Subsequent autologous bone augmentation procedures are required to allow for implant insertion then. Therefore, there has been great interest in developing new sponge like scaffolds with high osteoconductive properties that can be placed into the alveolar socket fimmediatly after extraction for preservation of the alveolar crest width. The purpose of this study was to investigate new sponge-like polymer-ceramic composite scaffolds suitable to act as alveolar plugs for their capability to host human primary osteoblasts. Boactive coatings with calcium sulfate were compared with non-coated surfaces.

Methods:
The behaviour of human osteoblasts on poly-L-lactic acid (PLLA)-based polymer-ceramic scaffold plugs was investigated. The effect of bioactive coatings of the osteoconductive material calcium sulfate ($CaSO_4$) on the scaffold surfaces was evaluated. Plugs coated with $CaSO_4$ (Version 1) were compared to $CaSO_4$-free controls (Version 2). Biocompatibility was assessed by scanning electron microscopy (SEM), fluorescence microscopy after staining for cell vitality with fluorescin diacetate (FDA) and propidium iodide (PI) and the MTT, LDH, and WST biocompatibility tests. Both versions were colonised by human osteoblasts.

Results:
The plugs showed a porous sponge like shape and handling would make them suitable for insertion into alveolar sockets. Both versions of plugs showed good biocompatibility according to the performed tests and were able to host human cells over several weeks. However, the biocompatibility of the $CaSO_4$-free scaffold was slightly superior *in vitro*. This was stressed by the cell vitality staining and LDH test.

Conclusion:
Both versions of PLLA -based polymer-ceramic scaffold plugs showed good biocompatibility for human osteoblasts. Alveolar socket preservation with this new type of plugs would be theoretically feasible. Future in vivo studies are needed to proof preservation of alveolar crest width in clinical settings.

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INCREASING THE POROSITY OF ELECTROSPUN SCAFFOLDS BY COLLECTING ONTO ELECTRO-CONDUCTIVE STRUCTURED SUBSTRATES

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Aim:
A melt electrospinning system incorporating an electro-conductive patterned collector will result in altered fibre deposition, leading to the fabrication of 3D polymer scaffolds for tissue engineering applications that are cell invasive.

Method:
A custom melt electrospinning device was constructed that allowed the introduction of various electro-conductive collectors which contained regular structures and patterns. Poly(-caprolactone) (PCL) was placed into a syringe and heated to 90°C, after which a potential difference of 7 to 12kV was applied. The collector distance was varied from 20mm to 60mm, and the effect of flow rate between 2 and 50 l/h investigated. In addition, an automated x-y stage was used to collect up to 20 samples from each batch, allowing sufficient samples for further *in vitro* and *in vivo* investigations.

Results:
Uniform diameter fibres were generated with melt electrospinning, ranging from 6m up to 40 m, depending on the electrospinning conditions. Such diameter fibres permitted an open scaffold structure that allowed cellular invasion that was increased by the structure of the patterned collector. The electrospun material therefore had a range of interconnecting pore sizes, the location of which depending on the structure of the collector. The automated stage allowed the collection of a large number of samples, with the electrospinning device often operated for well over 24 hr continuously.

Conclusions:
Structured electro-conductive collectors altered the deposition of the fibers, resulting in patterned electrospun fiber morphology.

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CELL ALIGNMENT AND ELONGATION IN UNIDIRECTIONAL POROUS COLLAGEN SCAFFOLDS

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Introduction:
Pore size and porosity effect on bioactivity has been hitherto fairly described. However, the influence of pore orientation on this parameter has been evaluated only in nerve and tendon regeneration. Previously, we have developed laminar scaffolds exhibiting unidirectional oriented pores. The present work intended to evaluate the alignment and elongation of fibroblasts seeded into unidirectional and multidirectional scaffolds.

Methods:
Scaffolds were manufactured using two collagen concentrations (2 and 5 mg/mL). Scaffolds were seeded with oral fibroblasts, incubated for a week and fixed. Transverse and longitudinal sections of each collagen concentration scaffold were H&E stained. Using ImageJ ten digital images of each section were analyzed. Linear intercept method was used to find both major and minor axis of the best fitted ellipse around the cells. Cell alignment was determined using the orientation index (OI). Ratio between major and minor axis was used to evaluate cell elongation.

Results:
More than 68% of cells of the unidirectional scaffolds had an OI between 0 and 1, meanwhile less than 50% of the cells of the multidirectional scaffolds has an OI between the same range. The ratio between cell major and minor axes was higher than 1 in the unidirectional scaffolds. Conversely, it was 1 in the multidirectional scaffolds.

Conclusions:
Fibroblasts seeded into unidirectional porous scaffolds are unidirectional oriented and elongated along the long axes.

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INHIBITION OF AORTIC VALVULAR INTERSTITIAL CELL DEGENERATION BY ECM MODIFICATION – IMPLICATIONS FOR HEART VALVE ENGINEERING

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Aim:
Recent data from our group showed a rapid *in vivo* re-endothelialisation of decellularized heart valves after modificatoion of the basal lamina by coating with bioactive substances. The enhancement of valvular interstitial cell (VIC) calcification by TGF-beta1 (TGF-ß1) and the endothelial inducing effect of vascular endothelial growth factor (VEGF) have been demonstrated. Based on these rationales we sought to investigate the modulating properties of extracellular matrix coating on VIC calcification in the presence of TGF-ß1 and VEGF.

Method:
Ovine aortic VIC cultured on collagen, fibronectin, laminin or uncoated surfaces were exposed to TGF-ß1, VEGF or left untreated for 7 days. Western blot analysis, live/dead assay, annexin V staining, alizarin red S staining and Real time PCR were performed after treatment.

Results:
Calcific nodule formation was dependent upon the ECM coating protein and growth factor exposition. VEGF significantly inhibited the formation of calcific nodules independent of ECM protein coating ($P<0.05$). TGF-ß1 exposition resulted in the formation of calcific nodules on collagen, laminin and uncoated control surfaces. In contrast, fibronectin coating resulted in significantly reduced nodule formation despite TGF-ß1 administration. Cell viability and apoptosis analysis showed a marked a increase of apoptotic and dead cells inside calcific nodules. Furthermore, these events correlated with the expression and not the gene amplification of the alpha smooth muscle actin gene as a marker of VIC activation and calcific nodule formation.

Conclusions:
An additive protective effect on VIC can be achieved by providing specific growth factors or a specific ECM environment. While certain soluble factors, e.g. VEGF are known for inhibiting calcification and apoptosis, the modification of culture substrate chemistry with TGF-ß1-binding ECM components like fibronectin offers the potential to alter availability and presentation of TGF-ß1 delivered to the cell surface receptors. ECM coating strategies, e.g. with fibronectin represent a promising and feasible tool for modification of heart valve scaffolds for tissue engineering purposes and preclinical trials.

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CONTROLLED TEMPORAL RELEASE OF A MODEL PROTEIN FROM PLGA / PLGA-PEG-PLGA MICROPARTICLES

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Aim:
Controlling the formulation of PLGA (poly (lactic-co-glycolic acid)) microspheres enables release profiles of encapsulated drugs and cytokines to be tailored. By manipulating the specific formulation of the PLGA it was hoped that temporal separation of dosing between different growth factors could be controlled.

Method:
Microparticles were formed using a double emulsion solvent evaporation process with Lysozyme as a model protein and Human Serum Albumin as a bulk carrier. Release profiles were modified by using two different lactide:glycolide ratios (85:15 and 50:50) and three different triblock (PLGA-PEG-PLGA) concentrations. Particles were sized using a laser diffraction method on a Coulter LS230. Protein release was measured by suspending particles in PBS, incubating at 37˚C on a rocker shaker and regularly sampling the release media. This release media was assayed for protein content and activity.

Results:
Particle size was tightly controlled within defined limits by optimizing the emulsion process. Mean particle diameters of 110-120µm were repeatedly obtained. The carrier protein and protein loading within the particles was tailored such that encapsulation efficiencies greater than 60% were obtained with very little denaturing of the protein. The formulation without triblock exhibited a typical three phase release profile however the addition of triblock favored zero order release kinetics.

Conclusions:
The obtained matrix of release formulations enables precise tailoring of release profiles giving controlled temporal dosing.

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ENHANCED OSTEOGENESIS USING CELL PERMEABLE TRANSCRIPTIONAL COACTIVATOR PROTEIN

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Aim:
Osteogenic fusion protein consists of small cell permeable peptide peptide and transcriptional coactivator of PDZ-binding motif (TAZ) was prepared to selectively direct osteoblastic differentiation of human hMSCs, thereby increasing osteogenesis.

Method:
The TAZ protein was conjugated with a cell permeable peptide, fragment of protamine termed as low molecular weight protamine (LMWP), in a bacterial expression vector to produce a LMWP-TAZ fusion protein. The fusion protein was examined translocalization efficiency, osteogenic- and adipogenic differentiation capacity.

Results:
The hMSCs treated with LMWP-TAZ exhibit increased osteoblastic genes and protein expressions, producing significantly higher quantities of mineralized matrix compared to TAZ. In contrast, adipogenic differentiation of the hMSCs was blocked by the treatment of LMWP-TAZ. The further study using rabbit calvarial bone defect presented that LMWP-TAZ protein significantly increased bone formation than unfused TAZ protein.

Conclusion:
The protein transduction of TAZ fused with cell permeable LMWP peptide was able to direct specific differentiation of hMSCs into osteoblastic cells. Taken together, the transcriptional cofactor protein delivery concept using cell permeable peptide was suggested as a novel strategy for enhanced tissue regeneration purpose.

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SIGNALING FOR NEOVASCULARIZATION OF AN ARTERIO-VENOUS LOOP-CONTAINING TISSUE ENGINEERING CHAMBER: ROLE OF NADPH OXIDASE TYPE 4

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Aim:
Angiogenesis, a process of new blood vessel formation, is a key process involved in normal development and wound repair as well as in the various pathophysiologies. Reactive oxygen species (ROS) function as signalling molecules in many aspects of growth factor-mediated responses including angiogenesis. In this study we investigated whether NADPH oxidase 4 is involved in the neovascularization process in the tissue engineering chamber.

Method:
We began by defining the relationship between neovascularization and Nox4 expression in the O'Brien arterio-venous loop-containing tissue engineering chamber. This was followed by evaluating the inhibition of Nox4 both *in vitro* and *in vivo* using NADPH oxidase inhibitors or small interfering RNA.

Results:
New blood vessels originating from the venous and the arterial ends of the loop could be identified after 3 days of implantation. The vessel density peaked after 7 days and was maintained for at least 14 days. This was accompanied by granulation tissue formation and concomitant increase in the mRNA level of Nox4 NADPH oxidase. Local treatment with the NADPH oxidase inhibitors apocynin significantly suppressed the vessel growth in the chamber. Inhibition of Nox4 expression using siRNA or treated with NADPH oxidase inhibitor, apocynin, treatment reduced NADPH oxidase activity which is associated with a reduction in angiogenic responses as assessed by tube formation and cell migration assays in human microvascular endothelial cells (HMEC). Assessment of the effect of knockdown of Nox4 by adenovirus carrying shRNA in the tissue engineering chamber is ongoing.

Conclusions:
NADPH oxidase 4-dependent redox signalling is important for neovascularization and boosting this signaling might be a new approach to extending vascularization and tissue growth for tissue engineering

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THE EFFECT OF HUMAN ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS ON AVASCULAR NECROSIS OF THE FEMORAL HEAD IN NUDE RATS

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Aim:
In this study, we evaluated the effect of human adipose tissue-derived mesenchymal stem cells(hATMSCs) on the treatment of rat avascular necrosis(AVN) of the femoral head.

Method:
Seven-month-old, 40 male athymic nude rats were operated by vascular-deprivation method under general anesthesia. At 2 weeks after operation, they were divided into 4 groups randomly and performed the core decompression. Under fluoroscopic control, hATMSCs (Treatment group, $2x10^5$, $2x10^6$, $6x10^6$ cells/rats) or saline (Control) were transplanted into the the lesion of osteonecrosis through transtrochanteric route via 21G syringe needle. The healing response was evaluated by X-ray, micro-CT, MRI, gross observation and histological assessment at 2, 4, 6, 8, 10 weeks after transplantation. To evaluate toxicological effect of hATMSC, we assessed systemic response according to guidelines for Nonclinical Laboratory Studies issued by Korea Food & Drug Administration.

Results:
In control group, we could observe empty lacunae at epiphysis with pale and fibrous bone marrow. hATMSCs-treated groups had better osteogenesis ability compared with control group. Although necrotic bone was not significantly changed in epiphysis and metaphysis, new bone formation were dose-dependently increased at metaphysis in hATMSCs-treated groups. In toxicological aspect, no significant changes were found in body weights, clinical signs, hematological/biochemical values, organ weights, histopathological findings. There was no inflammation, or lymphocytic infiltration at the site of transplantation.

Conclusions:
These results showed that transplantation of hATMSCs could have the potential for the partial improvement of osteogenesis in the treatment of AVN and no thATMSCs-related toxicity was observed in this model.

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THE ROLE OF HUMAN UMBILICAL CORD-DERIVED ENDOTHELIAL PROGENITOR CELLS AND MESENCHYMAL STEM CELLS INFUSION IN THE FUNCTIONAL IMPROVEMENT OF THE CHRONIC RENAL FAILURE IN THE NOD/SCID MICE

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[4] Laboratory of Developmental Biology and Stem Cell Differentiation/Transplantation, Seoul National University, Seoul, Korea

Aim:
We evaluated the beneficial role of human umbilical cord-derived endothelial progenitor cells(hEPCs) and mesenchymal stem cells(hMSCs) using the immunodeficient murine chronic renal failure(CRF) model.

Method:
CRF was surgically induced by resecting kidneys, leaving approximately 1/6 of the live kidney mass (5/6 nephrectomy). Mice in the treatment group received an injection of hEPCs or hMSCs ($1x10^6$ cells) via tail vein 1 day and 1week after nephrectomy. We injected PBS into Sham and CRF mice as control. Blood and urine samples were collected every 4 weeks after CRF model until 20 weeks for the assessement of the function of the kidney.

Results:
In CRF mice, the level of serum BUN and creatinine was higher as compared with the sham group. The BUN and creatinine levels in hEPCs group were lower than those of CRF group with time adjustment. In addition, the urine protein/creatinine ratio in hEPCs group were higher than those of CRF group. However, no significant differences in BUN and creatinine concentration were observed between hMSCs group and CRF group. The kidneys of mice were removed for histologic evaluation 20 weeks after CRF surgery. Histologically, global/segmental glomerulosclerosis and interstitial fibrosis were less apparent in the hEPCs group compared to the hMSCs and CRF group.

Conclusions:
These results showed that transplantation of hEPCs could have the potential for the partial improvement of kidney function and structure in the treatment of CRF.

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CELL PERMEABLE MAGNETIC NANOPARTICLES FOR EFFICIENT LABELING OF MESENCHYMAL STEM CELLS

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Aim:
Cell labeling has been a useful tool for tracking transplanted cells in cell-based repair/ replacement therapeutic procedure. Herein, we report that superparamagnetic iron oxide (SPIO) nanoparticles, with surface immobilized cell permeable peptide (CPP), were efficiently internalized into mesenchymal stem cell, which was almost equivalent to that by HIV TAT peptide-MRI imaging agent complex. We also presented preliminary findings of toxicity and effect on cellular function and differentiation capacity of magnetic labeling using the CPP peptide.

Method:
The CPP, here we used fragment of protamine termed as low molecular weight protamine (LMWP), was immobilized onto the surface of SPIO nanoparticles, followed by the incubation with MSCs for labeling purpose. To examine translocalization efficiency, LMWP-SPIO labeled cells were measured by using FACSCalibur, Confocal microscopy and Perl's reagent for Prussian Blue (PB) staining. The cell proliferation was measured after the MSCs labeling with LMWP-SPIO nanoparticles. The labeled cells were further examined their differentiation capacity to adipose or osteogenic tissue.

Results:
The LMWP was labeled on the surface of SPIO nanoparticles without causing aggregation. The cells labeled with LMWP–SPIO presented the highest iron content compared to those labeled with naked SPIO and the complex of SPIO with poly-L-lysine, which is currently used as a transfection agent. In addition to the iron content assay, Prussian staining and confocal observation demonstrated the highest intracellular LMWP–SPIO presence, and the labeling procedure did not alter the cell differentiation capacity of mesenchymal stem cells.

Conclusions:
The present study suggested that the cell permeable SPIO nanoparticles with LMWP were safe and useful tool in labeling MSCs for MRI based transplanted cell tracking purpose.

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IMPROVING THE BIOACTIVITY AND MECHANICAL PROPERTIES OF INJECTABLE CHITOSAN

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Injectable biomaterials are desirable for noninvasive bone tissue replacement to avoid open surgeries. Bioglass/chitosan composites are considered as promising materials for this application. These composites integrate the advantages of chitosan including biodegradability, ability to promote wound healing and osteoconductivity with the high bioactivity of bioglass. Lack of sufficient mechanical properties after injection is the main drawback of these composites. The objective of this study was to enhance the mechanical properties of composite using a non-toxic and biocompatible crosslinker.

Bioactive glass was synthesized by sol-gel method. Different ratios of bioglass and chitosan were prepared and *in vivo* tests were conducted on rat models. Prior to this test, the bioactivity of composites was assessed by *in vitro* test. Each composite was immersed in a buffer solution and growth of apatite on the surface was measured after 14 days using analytical techniques such as Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and scanning electron microscopy (SEM). The effect of using a crosslinker on mechanical properties of composites was investigated.

The results of XRD, FTIR and SEM analyses confirmed the formation of apatite layers on the surface of composites after incubation in buffer solution. Elevating the amount of bioglass in composites from 50 wt% to 70wt% resulted in increasing the bioactivity. Bone formation on the animal models was detected after four weeks. Addition of the crosslinking agent enhanced the mechanical properties of the samples. The fabricated injectable biocomposite can be used as bone tissue substitutes where the surgical sites are not freely accessible.

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A NOVEL FABRICATION PROCESS OF WOUND DRESSING CONTAINED ALOE

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The aim of this investigation is to improve the performance of wound dressing by adding the antioxidant and antibiotic aloe. Aloe Barbadensis was chose as the sample for the experiments. Aloe was extracted by the method of low temperature, pressure distillation in order to keep the effective ingredients. Extracted aloe was added into the temperature sensitive hydrogel as the wound dressing matrix. Biocompatible and high water content monomers, NIPAAm were polymerized with a crosslink agent, NMBA, a promoter, TEMED and a initiator, APS. The low critical solution temperature (LCST) of polymer could be changed by regulating the contents of compositions. The polymer could transform from gel into liquid at 20°C. Extracted aloe was mixed with the liquid polymer. The antioxidant of dressing temple was measured by using ESR. Besides, the water content and antibiotic ability were evaluated in the research.

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STRUCTURE AND MECHANICAL PROPERTIES OF PGA/GELATIN ELECTROSPUN NANOFIBERS SCAFFOLDS FOR VASCULAR TISSUE ENGINEERING

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Aim:
Various synthetic biodegradable polymers have been electrospun into thin fibers for generating scaffolds for tissue engineering purposes. In this study, gelatin was added to PGA and electrospun with different weight ratio to determine the effect of gelatin on structure and mechanical properties of scaffolds for vascular tissue engineering.

Method:
A series of PGA/gelatin solutions (0, 10, 30 and 50 wt% of gelatin) were prepared by dissolving them in HFIP and then electrospun. The morphology of scaffolds was investigated by SEM. The mechanical properties of scaffolds were measured by an uniaxial testing machine with a 50-N load cell under a cross-head speed of 1 mm/min.

Results:
SEM images showed that higher gelatin contents produced wide fiber diameters. Fiber diameters were calculated by analysis of SEM images. Fiber diameters were ranged from 80 nm to 1000 nm. The PGA with 30 wt% gelatin showed a very soft and flexible characteristic with low Young's modulus and the high elongation at break of 32%. PGA/gelatin 50 wt% showed an enhanced tensile strength, high Young's Modulus and the elongation at break of 6%.

Conclusions:
SEM analysis of the blended fibers revealed uniform fibers with no evidence for phase segregation. Our data indicate that with increasing the amount of gelatin there was an increase in the average fibers size and the tensile strength improved significantly. These fibers have acceptable mechanical characteristics that are being tested for vascular tissue engineering.

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THE STUDY OF PHOTOCROSSLINKED PEG-BASED POLYESTER HYDROGEL AS SCAFFOLD FOR BONE TISSUE ENGINEERING

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Polyethylene glycol and its copolymers with L-lactide or -caprolactone were synthesized, and acrylation was performed to add C-C double bonds at both ends of the polymers. The resultant polymers include PEG-DA, (PLA-PEG-PLA)-DA (denoted by PEL-DA) and (PCL-PEG-PCL)-DA (denoted by PEC-DA). These polymers can form hydrogel upon irradiation of UV in aqueous solution at the presence of initiators. The potential of these hydrogels to serve as scaffold for the induction of osteo-like cells from umbilical mesenchymal stem cells was investigated.

The mechanical properties of the hydrogels were evaluated. Swelling was reduced at higher concentrations of the polymers, while DSC results showed crosslinking degree and compressive modulus increased with the polymer concentrations. PEG-DA hydrogel had the highest compressive modulus, and PEL-DA hydrogel showed the fastest degradation rate (50% degraded in 28 days) and greater degree of acidification of the buffer solution (pH 3.7 in 28 days), among the three types of hydrogels, respectively. In vitro induction of stem cells to osteo-like cells showed that PEC-DA hydrogel had the highest amount and expression level of alkaline phosphatase of the encapsulated cells.

(I omitted the animal results which were not sufficient to be presented yet)

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TISSUE ENGINEERING AN ARTERIAL WALL MODEL

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Large arteries are complex, multilayered structures consisting of predominantly smooth muscles interspersed between thin lamellae comprised almost exclusively of elastin[1] and an intraluminal layer of endothelial cells. However, current tissue engineered blood vessels rarely incorporate sufficient elastin into the supportive scaffold. Recombinant human tropoelastin can be cross-linked into 3D synthetic elastin arrays[2]. We explored its potential as a biological scaffold for tissue engineered blood vessels on the basis that it more closely mimics the native structure.

Biological scaffolds were formed by electrospinning and crosslinking tropoelastin fiber to form thin insoluble scaffolds. Vascular cells were then seeded onto these scaffolds and cellular interactions investigated using a range of microscopic tests including scanning electron microscopy, histology and fluorescence microscopy. Cells attached and proliferated on the scaffolds for 7 days[3]. We directed the orientation of cell spreading by aligning electrospun fibers predominantly in one direction. Control of cell growth and orientation on these scaffolds point to the value of this system as an *in vitro* model for the vascular vessel wall.

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[2] Mithieux, S.M., Rasko, J.E.J. and Weiss, A.S. (2004) Synthetic elastin hydrogels derived from massive elastin assemblies of self-organized human protein monomers. **Biomaterials**. (25) 4921-27.
[3] Nivison-Smith, L., Rnjak, J. and Weiss, A.S. (2010) Synthetic human elastin microfibers: stable cross-linked tropoelastin and cell interactive constructs for tissue engineering applications. **Acta Biomaterialia**, 6(2) 354-9

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TISSUE-ENGINEERED BIOABSORBABLE SCAFFOLD USING INDUCED PLURIPOTENT STEM CELLS FOR TREATMENT OF PERIPHERAL NERVE DEFECT

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Aim:
The purpose of this study was to examine the adhesion of the iPS cell-induced neurospheres to the bioabsorbable nerve conduits and to examine their cellular characteristics.

Methods:
The nerve conduit has 2 layers. The outer layer is composed of PLLA mesh and the inner layer is composed of PLLA and PCL porous sponge. We generated primary and secondary neurospheres from iPS cells by a published protocol. The primary and secondary neurospheres were suspended in each conduit. After suspension, the conduits were placed in a cell incubator for 7 or 14 days. After the conduits were seeded with iPs cells, they were subjected to immunohistological analysis by using antibodies specific to the glial marker (GFAP), Schwann cell marker (S-100) and neuronal marker.

Results:
All the primary and secondary neurospheres that had differentiated for 7 or 14 days were found to have adhered to the inner surface of the conduits and migrated into the inner porous sponge. All neurospheres were positive for S-100 and GFAP but were negative for neurofilament protein. The cell adhesion and the immunostaining characteristics between the 7- and 14-day-differentiated neurospheres were not different.

Summary Points:
The neurospheres showed good adherence to scaffolds and their pluripotent abilities were also maintained. We believe that the methods and results obtained would be useful in designing artificial scaffolds using iPS cells not for the treatment of only peripheral nerve defect, but for other organs.

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CRYOPRESERVED GFP-LABELED HUMAN UMBILICAL CORD BLOOD DERIVED MESENCHYMAL STEM CELLS COULD BE USED AS SEED CELLS FOR BONE TISSUE ENGINEERING

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The osteogenic capability of human umbilical cord blood derived mesenchymal stem cells (UCB-MSCs) has been demonstrated both in vitro and in vivo, so cell labeling and storage are becoming necessary for researching their potential therapeutic use to tissue-engineer bone. The aim of this study was to determine the effect of cyropreservation on osteogenic differentiation of green fluorescent protein gene (GFP)-labeled UCB-MSCs in vitro. MSCs were isolated from full-term human UCB, expanded, infected with GFP gene, and then cryopreserved in liquid nitrogen for 4 weeks. After thawing, their cell surface antigen markers, GFP expression and osteogenic differentiation were analyzed. The results demonstrated that cryopreservation has no effect on the phenotype, green fluorescence expression or osteogenic differentiation of UCB-MSCs, showing cryopreserved human UCB-MSCs might be applied for bone tissue engineering.

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DEVELOPMENT OF AN ULTRA-THIN FIBROIN MEMBRANE FOR RPE CELL TRANSPLANTATION

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Aim:
One of the challenges associated with cell-based therapies for repairing the retina is the development of suitable materials on which to grow and transplant retinal cells. The present study was aimed at developing a porous, ultra-thin fibroin membrane that might better support the development and correct morphology of RPE cells.

Method:
Ultra-thin fibroin membranes were prepared using an optically flat casting table coated with Topas® (a cyclic olefin copolymer) and a 1:0.03 aqueous solution of *Bombyx mori* fibroin and PEO (M_v 900 000 g/mol). Following drying, the membranes were water annealed to make them water-stable, washed in water to remove PEO, sterilised by treatment with 70% ethanol, and washed extensively in saline. ARPE-19 cultures were seeded onto fibroin membranes pre-coated with vitronectin and grown for 7 weeks in medium supplemented with 1% fetal bovine serum. Primary cultures of human RPE cells were established from donor posterior eye cups seeded directly onto fibroin and maintained in medium supplemented with 10% serum and antibiotics. Comparative cultures were established on porous 1.0 µm pore PET membrane (Millipore).

Results:
The fibroin membranes had an average thickness of 3 µm and contained pores with average diameter of 3-5 µm. Cultures appeared to attach equally well to either fibroin or PET membranes. Over time cells on either material adopted a more cobblestoned morphology.

Conclusions:
Progress has been made towards developing a porous ultra-thin fibroin membrane that supports cultivation of RPE cells. Further studies are required to determine the degree of membrane permeability and RPE polarity.

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CHITOSAN-COLLAGEN COMPOSITE SCAFFOLDS FOR TISSUE ENGINEERED SKIN

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Aim:
A major challenge which still exists within the medial field is the therapy and treatment of patients with severe burns. This is due to an impaired barrier function which leads to chronic bacterial sepsis and increased morbidity. The ideal treatment of wounds resulting from severe burns would involve a full thickness skin graft with distinct dermal and epidermal morphology. This is traditionally achieved using grafts from the healthy region of the patient, however, at the cost of creating another major wound. The presented research investigates a scaffold-based full thickness skin graft as an alternative method of treatment based on a nano-fibrous chitosan scaffold combined with porous collagen to facilitate wound healing and re-epithelisation.

Method:
The chitosan nano-fibres were fabricated by electrospinning followed by cross linking, using glutaraldehyde and sodium-tri polyphosphate to retain the fibrous structure. The electrospun scaffolds were further freeze-dried with Type-I collagen to form a composite structure. Morphology of the scaffolds was assessed using scanning electron microscopy (SEM) while qualitative assessment for the presence of chitosan and collagen in the scaffolds was analysed by Safranin-O and Pircro sirius red staining. Cell attachment assays were performed using 3T3 fibroblast cells.

Results and Conclusion:
SEM micrographs reveal the presence of nano-fibers of around 50-100 nm in diameter. The effect of cross-linking of the nano-fibers was evaluated by the ability of the scaffold to retain the fibrous structures after treatment in the aqueous medium. Preliminary results using 3T3 fibroblasts show cell attachment to the scaffold indicating its non-toxicity as well its potential to be investigated further as a skin replacement.

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FIBROIN-BASED MATERIALS SUPPORT CO-CULTIVATION OF LIMBAL EPITHELIAL AND STROMAL CELLS

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Aim:
The silk protein fibroin (*Bombyx mori*) provides a potential substrate for use in ocular tissue reconstruction. We have previously demonstrated that transparent membranes produced from fibroin support cultivation of human limbal epithelial (HLE) cells (Tissue Eng A. 14(2008)1203-11). We extend this body of work to studies of human limbal stromal cell (HLS) growth on fibroin in the presence and absence of serum. Also, we investigate the ability to produce a bi-layered composite scaffold of fibroin with an upper HLE layer and lower HLS layer.

Method:
Primary cultures of HLS cells were established in DMEM/F12 medium with either 10% fetal bovine serum (FBS) or defined keratinocyte serum-free medium (DK-SFM, Invitrogen). Cultures grown in both conditions were subsequently passaged onto transparent fibroin membranes or within 3D scaffolds prepared from partially-solubilised fibroin. Primary cultures of HLE and HLS cells were also established separately in serum supplemented media and cultured together upon bi-layered silk fibroin or single-layered amniotic membrane (gold standard).

Results:
HLS cultures grown in 10% FBS were able to adhere to and proliferate on silk fibroin 3-D scaffolds and transparent films while those grown serum-free could not. HLE and HLS cells were able to be co-cultivated on composite fibroin scaffolds and amniotic membrane.

Conclusions:
HLS cultures can be grown on fibroin-based materials, but this process is dependent upon additional ECM factors such as those provided by serum. HLE and HLS cells can be co-cultivated in a 3-D scaffold of silk fibroin, however phenotypic analysis on the fibroin-cultured cells must be performed.

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SIMULTANEOUS EXPANSION AND HARVEST OF HEMATOPOIETIC STEM CELLS AND MESENCHYMAL STEM CELLS DERIVED FROM HUMAN UMBILICAL CORD BLOOD[1]

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Aim:
The simultaneous expansion and harvest of hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) derived from umbilical cord blood (UCB) were carried out within spinner flasks and a rotating wall vessel (RWV) bioreactor using glass-coated styrene copolymer (GCSC) microcarriers.

Method:
The medium used was composed of serum-free IMDM containing a cocktail of SCF 15ng·mL^{-1}, FL 5ng·mL^{-1}, TPO 6ng·mL^{-1}, IL-3 15ng·mL^{-1}, G-CSF 1ng·mL^{-1} and GM-CSF 5ng·mL^{-1}. Accessory stromal cells derived from normal allogeneic adipose tissue were encapsulated in alginate-chitosan (AC) beads and used as feeding cells. The quality of the harvested UCB-HSCs and MSCs was assessed by immunophenotype analysis, methylcellulose colony and multi-lineage differentiation assays.

Results:
After 12 days of culture, the fold-expansion of total cell numbers, colony-forming units (CFU-Cs), HSCs and MSCs using the RWV bioreactor were (3.7±0.3)-fold, (5.1±1.2)-fold, (5.2±0.4)-fold and (13.9±1.2)-fold respectively, significantly better than those obtained using spinner flasks. Moreover, UCB-HSCs and UCB-MSCs could be easily separated by gravity sedimentation after the co-culture period as only UCB-MSCs adhered on to the microcarriers. Simultaneously, we found that the fibroblast-like cells growing on the surface of the GCSC microcarriers could be induced and differentiated towards the osteoblastic, chondrocytic and adipocytic lineages. Phenotypically, these cells were very similarly to the MSCs derived from bone marrow positively expressing the MSCs-related markers CD13, CD44, CD73 and CD105, while negatively expressing the HSCs-related markers CD34, CD45 and HLA-DR.

Conclusions:
It was thus demonstrated that the simultaneous expansion and harvest of UCB-HSCs and UCB-MSCs is possible to be accomplished using a feasible bioreactor culture system such as the RWV bioreactor with the support of GCSC microcarriers.

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