Abstracts

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Collaboration for Better Wound Care: Industry, Scientists and Clinicians

The following compilation of abstracts represents a partial list of submissions received for presentation at the meeting.

ORAL ABSTRACTS

Signalling in the control of inflammation and tissue repair

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Proper leukocyte function at wound sites critically depends on adhesion molecules-mediated cell-cell and cell-matrix contacts. The involved membrane receptors and their downstream target molecules transducting signals to the cytoskeleton have not been clarified in detail yet. We here set out to define the signalling pathways which are relevant for leukocyte recruitment and for the intimate interaction between macrophages and apoptotic neutrophils during tissue repair. Using our previously generated β_2-integrin-deficient mouse model lacking the common β chain (CD18) of β_2-integrins and closely resembling the LAD1 syndrome in humans, we identified virtually abolished neutrophil recruitment and impaired formation of the phagocytic synapse between apoptotic neutrophils and macrophages. This resulted in reduced release of TGF-β_1 which was causally responsible for reduced myofibroblast-driven wound contraction and for delayed cutaneous wound healing. We now were able to identify the tyrosine-kinase Syk to relay signals from β_2-integrins to the cytoskeleton, thus coordinating β_2-integrin-mediated neutrophil migration to wounded skin. In addition, we have identified the guanine exchange factor Vav3 as a target molecule acting downstream of β_2-integrins and the Syk-kinase in macrophages. Similar to CD18, Vav3 was required for the proper phagocytic activation of macrophages to release TGF-β_1 and to drive the myofibroblast-dependent wound contraction, and if absent, resulted in impaired wound healing, as observed in Vav3-deficient mice.
Targeting signalling molecules responsible for leukocyte functions provides a valuable tool to specifically modulate and rebalance inflammation and tissue repair in vivo.

From bench to bedside: novel anti-inflammatory strategies

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Introduction: Scarring in the skin and at other body sites represents an area of clear medical need. We have evaluated the role of interleukin-10 (IL-10) in scarring and the scar improvement therapeutic potential of human recombinant IL-10 in pre-clinical models and in a within-subject, double-blind, placebo and standard care controlled phase II study in human volunteers.
Methods: Pre-clinical studies were performed in transgenic IL-10/Interleukin-4 (IL-4) double knockout mice and the effects of intradermal administration of IL-10 on scar formation (62.5–1000 ng/100 μL) was assessed macroscopically and microscopically in rats at 70 days post-wounding. Pre-clinical safety and toxicology studies were completed prior to initiation of the clinical study. The human trial consisted of 175 subjects and evaluated the effects of intradermal administration IL-10 (5–2000 ng/100 μL), at the time of wounding and 24 hours later, on safety and efficacy endpoints
Results: Deletion of IL-10/IL-4 resulted in delayed healing, increased inflammation and scarring. Interleukin-10 treatment of wounds in rats resulted in accelerated healing, decreased inflammation and reduced scarring ($p < 0.05$) which was associated with the regeneration of a dermal architecture more resembling normal skin. In humans administration of IL-10 was well tolerated and at concentrations of 5 and 25 ng/100 μL resulted in statistically significant improvements ($p < 0.05$) in scar appearance with multiple endpoints compared with controls at 12 months after wounding.
Conclusions: These data indicate that acute, local applications of IL-10 improve scarring both macroscopically and microscopically at the tissue level, and is another example of the new class of emerging therapeutics for the prophylactic improvement of scarring.

Inflammatory ulcers: topical and systemic treatment

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Treatments for chronic wounds include systemic and topical agents. A good balance between these two approaches has shown to produce a synergistic effect on tissue repair in certain type of chronic wounds. Recently new drugs have been developed and introduced in the field of wound healing. There are a large number of potential interventions for inflammatory ulcers such as vasculitis or pyoderma gangrenosum. The cornerstone of treatment is corticosteroids although use of immunosuppressive agents, immunomodulatory agents, plasmapheresis, intravenous immunoglobulin, and biological agents has also been described. Prolonged and high-dosage of these drugs are often required to attain and maintain disease control, but this comes at a high price considering side effects. Safety of interventions must be considered alongside efficacy, and we have responsibility to seek treatments with lower complication rates.

Consensus leading to evidence based guidelines

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The management of healing wounds is very complex because of the diverse aetiologies and comorbidities, the wide range of dressings, devices, drugs, surgery and the variety of advanced therapies available in wound healing.
A consensus is an absolute base and a starting point to develop guidelines that are based on scientific research. Therefore, high level evidence (meta-analysis of multiple RCTs or at least 2 RCTs) is needed, where possible, to underscribe or verify the statements. Evidence Based Guidelines can avoid improvisation, which can lead to poor wound healing, bad health economics and needless pain and suffering for the patient.
Only a few years ago, in our country, wounds were not routinely managed in a holistic manner, and often now, we still see too much focus solely on the wound instead of on the patient as a whole being. The principles of TIME, TIME-D, TIME-H, Care cycle and WoundBedPreparation were a great step forward in the right direction, but the aspect of a good diagnosis was still often neglected.

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The Evidence Based Guidelines are an excellent platform where nurses and doctors can collaborate with each other as a multidisiplinary team. Guidelines provide a tool, in which both doctors as nurses can follow a common pathway to obtain wound healing.
We are convinced that this common method shall result in golden standards for wound healing, which in turn will lead to a higher level of quality in our daily work. Nevertheless we must never forget that every patient is unique and that these guidelines have a general character, not fitting for each and every patient all of the time. Therefore we must stay alert to the uniqueness of each patient and avoid blindly following orders without looking at the clinical picture of the whole patient.
Furthermore, Evidence Based Guidelines also provide a scaffold in our educational programs to doctors and nurses and we have to convince the health workers to follow along in the same way of thinking and acting. CNC provides educational programs in Belgium following the latest consensus and guidelines.
At this moment we can say: There's still a lot of work to be done either by the experts to develop further on more guidelines as by us in teaching our colleagues!

Who needs the lab on a chip for wound healing?

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The visibility of wounds and wound science has grown markedly over the last 20 years with the formation and publication of Cochrane reviews, dedicated journals, publications and conferences. Various topics within this area are also subjects of doctoral research programs. This enormous growth in communications has facilitated a serious clinical challenge that is posed by chronic wounds. These communications have grown from very active hives where individuals have dedicated their efforts to improving diagnostic facilities, to understanding tissue repair at cell and molecular levels and so on. All this activity notwithstanding, clinically we continue to be dissatisfied with our abilities to manage chronic wounds and its complications.
Infection and oedema are the most common of complications. Investigative work up based on assessing perfusion, diabetic status etc will be more valuable if we possessed the tools to assess these complications. The big question is will one size fit all?

Beyond evidence-based guidelines: cost-effectiveness, impact on quality of life and translation of guidelines into every day's practice

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To ensure optimal wound care outcomes, clinicians must make wound management decisions based on systematically considered best practice information. Therefore it is necessary to 1/ identify and 2/ disseminate cost-effective, well-tolerated, evidence based wound care interventions that improve patient quality of life.
Just like in every field of nursing and medicine, evidence based guidelines are being developed in the field of wound care. As the number of evaluable RCT's and meta-analyses remains quite low in this domain, systematic review of literature only provides limited levels of evidence. This is especially the case for reviews concerning the clinical efficacy of specific treatment modalities. Therefore clinical guidelines on treatment of chronic wounds are not possible without the endorsement of expert opinions and/or consensus panel recommendations.
It is generally accepted that chronic wounds can be very costly to the health care system and can significantly impair the quality of life of those who suffer from them. On the other hand there are hardly any publications which have studied the finacial implications of a certain therapeutic option, nor the effect of a certain treatment on the quality of life. In a world where economical aspects and the well-being of patients gain importance, the relative lack of this kind of evidence makes it even more difficult to give recommendation of how to chose wound treatment strategies.
When evidence based treatment guidelines are compared with actual delivery of wound care practice, important inconsistencies are observed. This could be due to the fact that randomized controlled studies sometimes have a setup which is quite different from daily life practice. Clinical outcome studies and (local) clinical databases could be very useful to fill the gap between evidence based guidelines and daily practice in an objective way. Also studies to evaluate the best methods for translating evidence based knowledge into multiple wound care settings could be useful.

Collaborative working enhances wound care practice in tnp technology

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Introduction: As new TNP technologies emerge, it is important for clinicians to form collaborative relationships with manufacturers, during the design and final phases of product development, with formal pre-launch clinical studies assisting this process.
This research evaluated patients with a variety of complex wounds allocated a new gauze based TNP device for wound care (Wound*ASSIST*® TNP therapy system, Huntleigh Technology Limited) with three primary objectives:
1. Report clinical outcomes for a range of complex and chronic wounds
2. Evaluate ease of use with the device, and dressing pack components
3. Gather patient and clinician user satisfaction

Methods: A multi-centre prospective clinical outcome study was conducted across 3 specialist centres with varying levels of experience in using TNP; 'novice', 'aware', or 'experienced'. Data collected; demographic, past & present medical history, wound type, dimensions/progress and patient/clinician acceptability. Formal wound measurements and photography were conducted.
Results: 29 patients with chronic, complex or trauma wounds completed the study.
- Mean wound surface area was 30.2 cm^2 (1.9cm^2–76.6 cm^2) at the start of treatment
- Average duration of therapy was 8.9 days 92–17 days)
- Mean wound size reduction for all subjects was 23%

A consistent trend towards increased granulation tissue and epithelialisation was observed. Patients found the therapy comfortable and acceptable whilst clinicians reported a high level of satisfaction.
Conclusion: This study has shown positive and encouraging results in the treatment and management of complex wounds. Effective collaboration between manufacturers and clinicians can lead to enhanced wound care practices through the provision of products which promote practicality, ease of use and evidenced based outcomes.

The end of the affair: CXCR3 ligands stop wound repair and limit scarring

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The wound environment undergoes a series of changes that initially promotes cellular dedifferentiation and cell repopulation of fibroblasts, keratinocytes and endothelial cells. However, late in the regenerative phase, the environment switches to one that drives redifferentiation of these cells and organ maturation. Key regulatory signals that stop fibroblast migration and induce the involution of the excessive vascularization are the ELR-negative CXC chemokines CXCL10/IP-10 and CXCL11/IP-9/I-TAC; these ligands bind to their common G-protein coupled receptor (GPCR) CXCR3. Signaling through this receptor stops fibroblast immigration secondary to blockade of calpain2 activation and rear release of locomoting cells, channeling these cells towards wound contraction. At the same time endothelial cell migration is similarly blocked but concomitant activation of calpain1 and subsequent integrin beta3 cleavage leads to anoikis of nascent endothelial tubes reversing the exuberant angiogenesis of the regenerative phase. Wounds in mice lacking CXCR3 do heal but the resulting dermal tissue is hypercellular and hypervascular with pronounced cell turnover. The skin presents diminished tensile strength due to the excessive collagen presented in thickened but disorganized fibrils and persistent expression of components of the immature wound bed such as tenascin-C, collagen VII and fibronectin. Furthermore, the epidermis does not receive signals to limit its proliferation and by six months presents more layers of both nucleated and enucleated cells. Thus, the picture emerges of a wound that, while closed, refuses to stop healing, implicating the CXCR3 signaling system as key to wound resolution.

Fibroblasts are not equal in front of wound healing: new cell therapy strategies

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Immediately after injury, several events occur to repair the damaged tissue. Wound healing is a complex and dynamic process involving soluble mediators, blood cells, extracellular matrix components and resident cells, in particular fibroblasts. Briefly, three interactive phases take place during the wound healing process (inflammation, granulation tissue formation and remodeling). Thus, the ''quality'' of the healing, i.e. disappearance of scar and recovery of tissue functions, depends on a delicate equilibrium, and the remodeling phase, in which fibroblasts play a key role, is crucial for the rebuilding of the tissue as close as possible with origin.
When the injured area is too large, grafting becomes necessary, but in very large skin defects such as in burns, the amount of non-injured available skin is not sufficient. Skin substitutes are then alternative solutions.
The minimum requirement is to re-establish a barrier function obtained by the presence of the horny layer of the epidermis but there is a consensus as to the necessity of a dermal component. Various dermal substitutes have been developed. Some are acellular matrices, while others combine fibroblasts and extracellular matrix components. The presence of living fibroblasts has been shown to promote the rapid emergence of a functional dermis and consequently to permit efficient epidermal anchoring. Interestingly, efficiency of healing in adult depends on organs or tissues. Thus, the use of fibroblasts from sources other than the dermis is a promising approach in cell therapy strategies to tend towards embryonic healing without scar and fibrosis.

Cell-matrix interactions and scar formation. Does the scar microenvironment sustain the scar status?

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The connective tissue plays an important role in tissue function, not only does it serve as a scaffold to support the cells, but it also regulates cell function through all kinds of growth factors and hormones which are stored within the extracellular matrix (ECM). In addition the different components of the ECM themselves influence cell function. The cells bind to different molecules in the extracellular matrix via integrins/integrin-receptors.
The (biochemical) composition of the ECM depends on the function of the tissue and thus cells residing in the different tissues expose different phenotypes. The cells depositing ECM molecules, the fibroblasts, are at least for a large part responsible for their specific microenvironment. In normal skin, fibroblasts are surrounded by the ECM of the dermis and these cells posses a specific dermal spindle shape phenotype. In third degree burn wounds the normal dermal microenvironment is completely destroyed. During healing of these wounds, cells (including mesenchymal stem cells) are recruited from the surrounding tissues, such as subcutaneous fat, and the circulation. Once arrived at the wound site the cells start to repair the lost tissues and adept a phenotype promoted by the micro-environment. Because the dermal environment is lacking the cells are devoid of the proper control they acquire a phenotype of which the primary function is to close the defect. The presence of abundant inflammatory mediators most likely has additional effects on the transition into, for example, myofibroblasts. The micro-environment created by these myofibroblasts is distinctly different from normal dermal tissue and it is likely that unless interference takes place the cells will maintain this phenotype and deposit a defective ECM. Excessive collagen deposition, an altered collagen type I to III ratio, different fibril formation and altered crosslinking of collagen are only a few examples of differences in scar tissue in comparison with normal skin. It is very likely that the different microenvironment will maintain the ECM producing cells in a scar phenotype. In order to improve wound healing therefore it is important to guide the cells to the correct phenotype through the creation of a normal dermal microenvironment.

In vitro evaluation of a fibroblast seeded Collagen /Elastin matrix (Matriderm) – implication for in vivo use

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The collagen/elastin matrix Matriderm was tested in vitro for potential use as a vital skin equivalent. Therefore cell biological and immunohistological analysis were performed to determine the potential of Matriderm to allow and promote adherence, maturation and proliferation of keratinocytes and fibroblasts. Furthermore immunohistological analysis of basement membrane formation was performed.
Human keratinocytes and fibroblasts were established as primary cultures. Specific differentiation states of fibroblasts, mitotic progenitor type fibroblast (MF), or mitomycin –C induced post mitotic fibrocytes (PMF) were used.
Adhesion rate of primary keratinocytes in acellular Matriderm was 68%. Fibroblasts adhered at about 73% after 10 hours of culture. Pre-seeding of Matriderm with fibroblasts enhanced keratinocyte take on Matriderm. Interestingly pre seeding of the Matriderm inhibited keratinocyte migration deep into the material.
Fibroblast seeded composites showed enhanced expression of keratinocyte differentiation makers. The composite (keratinocyte -fibroblast seeded Matriderm) showed an enhanced basement formation (laminin and collagen IV expression) as compared to non-fibroblast- seeded Matriderm. No clear benefit was observed in vitro when pre-seeding with MF: PMF in a 2:1 ratio as observed in normal undiseased skin.In summary the fibroblast-seeded collagen/elastin matrix Matriderm exhibits enhanced capacity for keratinocyte promotion and basement membrane formation. Seeding modalities for clinical use have to be discussed.

Cross-linking affects performance of collagen scaffolds in dermal repair

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Introduction: Collagen scaffolds are increasingly used to treat full-thickness wounds. Cross-linked as well as non cross-linked matrices are currently commercially available. In this study, two commercially available scaffolds were compared to 5 different cross-linked and 5 different non-cross-linked experimental scaffolds in a porcine full-thickness surgical wound model.
Materials and Methods: Ten experimental scaffolds (NP1 till10) and two commercially available scaffolds (a non-cross-linked (NX-L) and a cross-linked control (X-L)) were transplanted, on full-thickness excisional wounds on the flanks of Yorkshire pigs. Subsequently a meshed split skin autograft (SSG) was applied on top in the same procedure. Control wounds were transplanted with the gold standard treatment; meshed SSG alone. Biopsies were taken on day 7, 14, 21 and 56.
Results: After 7 days the structure of all cross-linked scaffolds was clearly present, where only remnants of the non-cross-linked scaffolds were visible. After 21 days, no remnants were found for both scaffold types. Giant cells were found for both scaffold types. However, the cross-linked scaffolds showed much higher numbers than the non-cross-linked. The cross-linked scaffolds showed more contraction in comparison to SSG, but less contraction compared to X-L. Three prototypes of the non-cross-linked experimental scaffolds showed less contraction than the commercially available NX-L. Two of these closely approached the quality of healing seen for the SSG-standard.
Conclusion: When used for dermal wound repair, experimental cross-linked scaffolds showed less contraction than the commercial X-L, but still lead to wound contraction. The non-cross-linked scaffolds degraded fast, but with respect to contraction, closely approached the gold standard treatment.

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Preliminary evaluation of a double-layered skin construct in a porcine full-thickness wound model

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Introduction: To improve the healing of large wounds, a double-layered skin construct (DLSC) was prepared, consisting of:
— 1. a porous scaffold of type I collagen and solubilised elastin, containing dermatan sulfate and heparin, fibroblast growth factor 2 and vascular endothelial growth factor
— 2. a dense film of type I collagen, containing heparin and FGF-7
In this study, the performance of the DLSC was evaluated in a porcine full-thickness wound model and compared with the commercially available Integra DRT and split-skin.
Materials and Methods: DLSCs (6x), Integra (6x) or split-skin (2x) were transplanted on full-thickness wounds (3×3 cm) on the back of two female Yorkshire pigs. At day 7, the silicon layer of Integra was replaced by a split-skin, the DLSC was not covered with split-skin.
Biopsies for H&E staining were taken on day 7, 14, 21 and 55.
Results and discussion: In the split-skin, a differentiated epidermis on a thin dermis containing mainly fibroblasts, newly formed extracellular matrix and some blood vessels were found.
In the DLSC, newly-formed blood vessels and a major cellular response, consisting of fibroblasts, macrophages and neutrophils was observed, diminishing from day 14 at places where the scaffold was degraded. From day 14, an epidermis was formed, which may be due to the added FGF-7.
Integra showed, in comparison with the DLSC, a slower cellular response and scaffold degradation. On day 7, mainly fibroblasts and some blood vessels were present, from day 14 accompanied by neutrophils, macrophages and the formation of an epidermis.
Conclusion: Preliminary evaluation of the DLSC in a porcine full-thickness wound model showed formation of an epithelium. In comparison to Integra, an increased angiogenesis and cellular response at early time points was observed.

Raising standards in orthopaedic wound care – a prospective, comparative evaluation of a modern dressing design

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Introduction: Prevention of infection in orthopaedics remains a constant challenge. In spite of increasing evidence to support use of modern dressings many units use traditional adhesive dressings, which can cause wound healing problems. Our modern dressing design hydrofibre/hydrocolloid (HF/HC) was highly effective following hip and knee replacements in a national arthroplasty unit. The aim of this study was to evaluate this dressing in a district hospital to see if similar clinical outcomes could be achieved.
Methods: Prospective evaluation of the modern dressing involved 89 consecutive elective and trauma patients from October 2007–January 2008. Outcome measures included blistering, wear time, dressing changes, delayed discharge and SSI rate. Results were compared to a traditional dressing currently used.
Results: 65 patients HF/HC group, 24 Traditional group. CUSUM tool set an upper alert limit of 5% blister rate. Traditional group breached upper alert limit after 8 cases. It was decided unethical to continue after 24 cases. Comparison of HF/HC with Traditional showed blistering 1.5% versus 16.7%, wear time 4.8 versus 1.4 days and dressing changes 0.9 versus 2.8. Delayed discharge 0% compared with 4.2%. SSI rates 0% versus 4.2%. All outcome differences were statistically significant except delayed discharge ($p < 0.05$, Mann Whitney, chi-square tests).
Conclusion: Implementation of evidence-based modern wound care into a district general orthopaedic department achieved improved clinical outcomes with elective and trauma patients, similar to those of a national arthroplasty unit. This supports the use of the dressing and highlights the ongoing concerns of traditional dressings used in orthopaedics.

Characterisation of biofilms on epithelial surfaces by confocal microscopy

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Historically, microbiology research has tended to focus on single microbial species cultured in liquid media. Such a 'free living' mode of growth is known as the 'planktonic' growth phase and it is now recognised that such an existence is rarely encountered in the general environment and clinical situations. A biofilm is defined as a microbial community growing in a coaggregated form, often attached to a solid substrata which may be abiotic or a living tissue, and encased within an extracellular polymeric substance. The significance of biofilms is that the organisms present can differ greatly from their planktonic counterparts with regards to resistance to environmental pressures such as those exerted by host defence mechanisms and administered antimicrobials in clinical infections. As a result it is now evident that biofilms are implicated in over 65% of hospital acquired infections. Furthermore, extensive physiological differences often manifest as enhanced expression of virulence factors resulting in greater damage to host tissue.
In order to combat biofilms, research into their composition and structure is required to identify the presence and interactions of significant organisms. In recent years, analysis of biofilm structures has been greatly aided by the development of new molecular biology techniques and imaging methods. In particular, the coupling of fluorescent *in situ* hybridisation (FISH) with confocal laser scanning microscopy (CLSM) has allowed the identification and spatial location of microorganisms with a 3D-image of biofilms.
This presentation presents results of the *in vitro* production of two separate but clinically important biofilms involving *Candida* from the oral cavity, and also chronic wound bacterial isolates. Through the use of CLSM and specific staining for respective organisms, strain and species variation in terms of biofilm construction will be demonstrated. Preliminary findings involving application of these techniques to clinical specimens will also be shown. It is envisaged that extrapolation of these approaches to clinical samples will allow biofilm management techniques to develop by more targeted therapeutic measures.

A new antimicrobial enzyme system for the control of wound bioburden

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Aim: A new, optimized, antimicrobial enzyme system was developed for the control of wound bioburden. This Glucose oxidase – Lactoperoxidase – Guaiacol (GLG) enzyme system, incorporated in an alginate gel dressing, was analyzed for antimicrobial activity against antibiotic-resistant bacterial strains and for cytotoxicity towards keratinocytes and fibroblasts.
Methods: The susceptibility of a wide range of antibiotic-resistant bacterial strains (clinical isolates) to the GLG enzyme system was analysed using Minimal Inhibitory Concentration (MIC_{90}) determination. Further, challenge tests and cytotoxicity tests were performed with Flaminal® Forte, a new hydroactive alginate gel dressing with antimicrobial activity, obtained by the presence of 1.5% GLG enzyme system.
Results: The MIC_{90}- concentrations for most of the tested strains were in the range of 0.015%–0.06% GLG enzyme system and for all Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains $\leq$ 0.004%. Challenge tests show that for 11 strains all the bacterial cells are killed within 3 hours of incubation. For the other 3 strains all the bacterial cells are killed within 6 hours of incubation. Cytotoxicity determined by measuring metabolic activity using MTT assay on cells incubated in a 20% Flaminal® Forte solution showed no decrease in metabolic activity compared to the control cells. In contrast, other antimicrobial wound care products showed a high degree of cytotoxicity.
Conclusion: With the increasing concern of bacterial resistance towards antibiotics, this study clearly shows that low concentrations of the GLG enzyme system are successful in killing antibiotic-resistant bacterial strains. Furthermore, results show that Flaminal® Forte combines strong antimicrobial activity with non-cytotoxicity, promoting optimal wound healing.

Cadaveric donor skin: prediction for success of a split skin graft?

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A split skin graft is usually only performed if the culture swab of the graft site is negative. Unfortunately the graft site swab often shows bacterial growth. This means that the split skin graft has to be postponed until the bacteria are no longer present (for example after antibiotic therapy). The main disadvantage is that the patient has to stay in the hospital for a much longer period of time. (It can take weeks before the swab cultures are negative).
Our hypothesis is that bacterial growth will not determine whether a split skin graft of cadaveric donor skin will be rejected immediately by the body.
Materials and Methods: Of all 17 patients included in this study, a culture swab of the wound bed was taken. Then a split skin graft with human cadaver donor skin was performed. If the donor skin was not rejected immediately, the patient received an autologous split skin graft. However, if the donor graft was rejected, the patient was not grafted with an autologous graft. In the mean time if the culture swab showed bacteria, antibiotics were prescribed.
Results: 13 patients received donor skin. None of these patients rejected the donor skin. 4 patients were not treated with donor skin, since their wounds had healed before the start of the treatment. After the donor skin treatment, all 13 patients received an autologous graft. In 12/13 patients (92.3%) the autologous graft was received without any complications. This is the same success rate as reported in the literature (90%).
However, the culture swabs showed that in most cases more than one bacteria was present; most prominently Pseudomonas and the S. aureus. Only 2 patients had a negative culture.
Conclusion: This study shows that donor skin grafting can predict if an autologous split skin graft will be a success, even if the wound has bacterial growth. This could mean that it is not necessary to delay split skin grafting in order to remove bacteria.

Characterisation of biofilms within medical devices

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Introduction: The concept of bacteria as a solely planktonic life form is rapidly becoming outdated. In actuality, the planktonic phenotype, seems to merely represent a phase in the life cycle of bacterial colonies, with permanent adherent microcolonies called Biofilms, representing the ''natural'' state of many species. Biofilm presence has long been recognised in water treatment facilities and in dental plaque.
More recently biofilms have been identified on many medical devices including, contact lenses, breast implants, hip prostheses and venous cannulas.
Colonisation of endotracheal tubes (ETTs) with bacterial flora is thought to be a source of recurrent ventilator associated pneumonia and as a medical device in contact with known biofilm-forming dental flora; ETTs represent an ideal environment to investigate biofilm micro-diversity.
Objectives: The aim of this work was to identify the presence of biofilms with endotracheal lumens, and to characterise the microflora therein.
Methods: 26 ETTs were obtained from 21 patients in a General ICU. Sampling involved the physical removal of biofilms within the airway lumens and from the dressings. Bacterial cell culture techniques were used to quantify and identify microorganism populations present. Colonies were further characterised using molecular methods such as Denaturing Gel Gradient Electrophoresis (DGGE) and PCR. Confocal Light Scanning Microscopy (CLSM) was used to elucidate the three dimensional nature of the biofilms.
Results: Molecular analysis shows multiple bacterial genotypes within each endotracheal tube, and consisting of a range of microorganisms including dental flora, such as Streptococcus mutans and Pseudomonas gingivalis, as well as recognised pathogens like Pseudomonas aeruginosa. Bacterial culture methods similarly identify many cohabiting phenotypes, and using cultural assays, counts of up to 210 million colony forming units within each centimetre length of the ETTs. Cultural and structural analysis revealed the presence of fungal hyphae adding to the biofilm, identified as Candida albicans.
Conclusion: Endo tracheal tubes have been shown to harbour polymicrobial biofilms, consisting of dental flora and pneumonia-causing pathogens. This has important consequences for intubated patients, and the role of a non-adherent or bactericidal coating in ETTs needs to be evaluated. These techniques will now be applied to tracheostomies and burn wounds.

Analysis of extracellular matrix proteins during wound healing processes

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During wound healing, the extracellular matrix has to be remodelled. Especially, to obtain the mechanical stability of the scare tissue, collagens have to be deposited. Collagens are a rather heterogenic group of molecules, defined by their Gly-X-Y repeats. In addition to the fibril forming collagens, other collagens termed FACIT collagens, are fibril associated collagens with interrupted triple helices. Two members of this family, collagen XII and XIV are expressed in skin. The domain structure of collagen XII and XIV consist of two small C-terminal interrupted collagenous domains and a large non-collagenous NC3 domain that comprises 90% of the protein mass. FACIT collagens are thought to function as a modulator of fibril formation and/or as a bridging molecule between the fibrils and the surrounding extracellular matrix. In this study, we investigated the localization of these proteins in adult skin, during skin development, and in healing and non-healing wounds, e.g. in adult mice skin, collagen XII is deposited close to the basement membrane around the hair follicle. To further study the function of collagen XII *in vivo*, a collagen XII knockout mouse line was generated and the skin phenotype was analyzed.

The placenta growth factor in wound repair

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Cutaneous wound healing is strictly dependent on an efficient angiogenic response and vascular defects often underlie impaired repair and chronic ulcer formation. Improving the angiogenic response has been, therefore, considered as a therapeutic approach in ulcer treatment. Placenta growth factor (PlGF), a member of the vascular endothelial growth factor (VEGF) family acting through the VEGFR-1 receptor, is expressed during the angiogenic phase of wound healing. The analysis of PlGF knockout mice has revealed that in the absence of this factor granulation tissue vascularization is reduced and the healing process retarded. With respect to VEGF, PlGF displays weaker pro-angiogenic activity, but the capacity to induce non leaky, mature vessel formation, without compromising lymphatic functionality makes it a promising therapeutic tool. In diabetic wounds, PlGF expression is strongly reduced, and local PlGF overexpression accelerates diabetic wound closure by enhancing granulation tissue vascularization. Moreover, diabetic wound treatment with an adenovirus vector carrying the PlGF gene (Ad.PlGF) accelerates wound healing by improving granulation tissue formation and vascularization. A key effect of PlGF activity resides in its ability to chemoattract VEGFR-1 expressing cells. Adenovirus-mediated PlGF gene transfer associates with increased local macrophage recruitment, while VEGFR-1+ hematopoietic stem cell mobilization into the peripheral blood is not enhanced due also to progenitor cell lack of responsiveness to PlGF in the diabetic condition. Cultured normal dermal fibroblasts also express VEGFR-1 and PlGF treatment promotes their function by improving motility and the capacity to invade matrigel. Moreover, PlGF recovers migration impairment of diabetic cultured fibroblasts. Such findings reveal a possible direct effect of transduced PlGF in inducing granulation tissue formation in diabetic wounds. The analysis of the effect of PlGF administration to diabetic wounds shows that this factor has therapeutic potential for ulcer treatment by potentiating different aspects of the repair process.

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The impact of proteases on wound angiogenesis

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VEGF family members are key mediators in vascular remodeling. Structure-function analysis of these protein families is not complete and a detailed analysis is mandatory for a comprehensive understanding of this essential growth factor family in processes such as tissue repair and tumor development. A common structural feature of VEGF proteins is differential mRNA splicing of a single gene, which gives raise to different protein isoforms that differ primarily in the absence or presence of a carboxyl-terminal domain of highly basic-amino acids. This so called heparin-binding domain (HBD) has been identified as the epitope for the cell surface glycoprotein neuropilin and proteoglycans, both of which are central receptor molecules to control vascular growth. However, it is still unclear how cell functions are specifically modulated by neuropilin/proteoglycan and HBD interactions of different VEGF protein members. We performed systematic structure-function analysis of different proteins of the VEGF family which will be discussed.

Systemic transplantation of progenitor cells accelerates wound epithelialization and neovascularization in the hairless mouse ear wound model

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Impaired wound healing is a major clinical problem. Recent, studies have shown that endothelial progenitor cells (EPC), from the peripheral blood, accumulate in granulation tissue at the site of neovascularization causing secretion of growth factors and cytokines. To investigate these findings, we transplanted systemic EPC and then measured epithelialization and neovascularization in the hairless mouse ear wound model.
Standardized full thickness dermal wounds (2.25 mm diameter) were created on the dorsum of the mouse ear. Wound epithelialization was measured every third day until wounds were completely healed, using intravital microscopy and computerized planimetry. CD31, CD90, cytokine stromal cell-derived factor 1α (SDF1α) and vascular endothelial growth factor (VEGF) were measured on days 3 and 6 using immunohistochemistry.
The systemic transplantation of heterogeneous EPC had a high significant ($p < .0001$) effect on wound healing with resurfacing occurring by 6.5 days $\pm$ 0.12 days compared with that after PBS injection by 10.5 days $\pm$ 0.32 days. Immunohistochemically, the EPC-transplanted group showed a significant increase in vascular density relative to the PBS-treated group indicated by CD31 staining ($15.03\,mm^2 \pm 2.44\,mm^2$ *vs.* $6.72\,mm^2 \pm 0.85\,mm^2$ at day 6; $p < .001$). Furthermore, the transplantation of EPC increased the expression of the SDF1α (5.1 ± 1.69 *vs.* 1.05 ± 0.26 at day 3, $p < .01$; 11.83 ± 3.86 *vs.* 1.8 ± 0.35 at day 6, $p < .001$; 18.34 ± 3.55 *vs.* 2.97 ± 0.59 at day 12, $p < .001$).
These findings demonstrate that transplanting systemic EPC into "normal" healing wounds promotes epithelialization and neovascularization and thus could be a useful method for accelerating wound healing.

Identifying a Gene signature for the wound healing continuum

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Introduction: There is a deleterious effect upon wound healing functionality associated with increasing age with a continuum between old and young. Oral mucosal fibroblasts (OMFs) display rapid, scarless, wound healing and represent a young 'enhanced' wound healing phenotype. At the other extreme, 'aged' chronic wound fibroblasts (CWFs) display an impaired wound healing phenotype. Between these extremes lie normal dermal fibroblasts (NDFs) which have an intermediate healing phenotype.
Aim: To identify a signature group of genes whose pattern of expression correlates to the wound healing continuum.
Methods/Results: Primary OMFs and CWFs were compared to patient matched NDFs ($n = 3$ and $n = 4$ respectively). Early passage cultures were 'serum starved' for 48 hours and re-stimulated with serum, modelling an *in vivo* wound response. RNA isolated 0 and 6 hours after serum stimulation was analysed by *Affymetrix*™ cDNA Microarray.
Comparison of serum modulated genes in OMFs, NDFs and CWFs to ascending/descending continuum patterns identified 221 genes with continuum-like expression characteristics including genes which were serum induced or repressed to descending or ascending levels across the sampled continuum. For example, BMP2 expression displayed high levels of serum induction in OMFs (684), which was reduced in NDFs (350/197) and further reduced in CWFs (71).
Conclusion: This analysis demonstrated that there are a number of genes displaying a continuum-like pattern of expression and may help in the identification of gene expression signatures for wound healing.

Lympho-hematopoietic stem cells and their ageing

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The lympho-hematopoietic system is largely composed of cells with short lifespans (days) and thus requires continuous replenishment of the cells lost through hematopoietic stem and progenitor cells in a process called hematopoiesis. Experimental evidence from several laboratories clearly demonstrates that hematopoietic stem cells harvested from young and aged animals show functional differences that are intrinsic to hematopoietic stem cells, implying that also stem cells in the hematopoietic system can not defy aging. We will thus discuss the cellular phenotypes and the possible molecular mechanisms associated with aged hematopoietic stem cells with respect to the specific properties stem cells are endowed with, and will investigate whether stem cell aging is inevitable or whether some of its aspects can be reverted or at least ameliorated.

The ROS connection in ageing and tissue repair

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Chronic venous leg ulcers represent the final outcome of lower extremity chronic venous insufficiency in many patients. Chronic ulcers of the lower leg have a prevalence of 3 to 5% in the population over 65 years of age with an increase of up to 12% in the population over 70 years of age. The incidence of ulceration is rising as a result of our ageing population. As healing may be slow or may never be achieved, ulcers create persistent and substantial demands on resources. Since the ageing trend of the world's population is unbroken and the prevalence of chronic venous diseases is correlated with age, it can be predicted that the financial load of the society for the treatment will increase in future.
Great efforts have been made to accelerate tissue repair in chronic venous leg ulcers with only limited success. This may at least be partly due to the finite knowledge of the pathophysiology of chronic wounds and the role of their microenvironments. Factors that play a role in impaired wound healing, in addition to local and systemic factors like venous insufficiency, ischaemia and malnutrition, are an increase in the concentration of reactive oxygen species (ROS) due to persistent macrophage activation, increased proteolysis with a decrease in concentrations of growth factors, and the presence of senescent cells in the tissue of the ulcer base and edge. The exact underlying mechanisms driving chronically impaired wound healing are still unknown. An emerging sequence of events with toxic concentrations of reactive oxygen species has been identified, in conjunction with increased free iron and a hostile proteolytic microenvironment being the key events of tissue destruction and localized premature ageing in chronic venous leg ulcers. Based on these data, a therapeutic algorithm has been established.

Ageing in clinically relevant settings of tissue repair

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By the year 2050, one in five Americans will be over the age of 80, an increase from 12% in the year 2000. Although people are living longer, many have chronic diseases and many more will be diagnosed with conditions that require surgical treatment. Although current literature suggests that acute wound healing is not impaired by age alone, elderly patients are more susceptible to perioperative complications that can impede wound healing. Malnutrition, a major risk factor for the development of non-healing wounds, has been reported to be present in 61% of elderly patients in the perioperative period. This high complication rate may be due to aberrations in the stress response that occur with aging. The majority of chronic wounds occur in older adults with multiple comorbidities. Contributing factors include impaired scavenging of reactive oxygen species, increased expression of cellular inflammatory factors, reduced inflammatory cell function and impaired angiogenesis. Alterations in the healing response due to age alone may not be sufficient to prevent healing, but the additional stress of chronic diseases such as diabetes and peripheral vascular disease tips the balance to a state of 'acute on chronic' oxidative stress.
We have used a rat model to evaluate the effect of age and ischemia on wound healing. In this model we have demonstrated that ischemia impairs wound healing in aged rats more than young. Despite elevated superoxide dismutase activity, ischemic wounds of the aged rats had markedly elevated levels of 3-nitrotyrosine. Our hypothesis is that there is a relative deficit of glutathione resulting in the inability to convert hydrogen peroxide to water. Further elucidation of this mechanism will lead to therapies that improve wound healing in the elderly.

Cellular senescence and tissue homeostasis

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Normal cells can undergo only a limited number of doublings and then became permanently growth arrested. This, so-called replicative senescence, is a consequence of a progressive telomere shortening that is perceived by the cells as a DNA damage leading to the activation of p53 tumor suppressor and thus to cell cycle arrest. In addition, the cells can senesce prematurely after exposure to various types of stress. Interestingly, the overexpression of several oncogenes leads also to premature senescence, indicating that senescence represents a potent anticancer mechanism. We have shown that this stress-induced senescence is due to a DNA damage response, suggesting common mechanisms underlying the various types of cellular senescence. Beyond their inability to proliferate, senescent cells express a pro-inflammatory phenotype, implying that they are locked in an activated state mimicking the early remodeling phase of wound repair. It is believed that due to this phenotype senescent cells can contribute to the ageing process and to the development of various pathologies, including implications in wound repair. We have shown that p53 is responsible for the senescence-associated overexpression of inflammatory molecules such as ICAM-1, indicating that the various features of senescence (cell cycle arrest and the pro-inflammatory phenotype) are, at least in part, linked with the same molecular mechanisms. In addition, although senescence is considered to be an anticancer barrier, it has been proposed that senescent cells, due to their specific inflammatory phenotype, create a permissive environment for the growth of cancer cells. In this vein we have shown that senescent stromal lung fibroblasts enhance the growth of cancer cells, both in vitro and in vivo, supporting the idea that the role of senescence in tissue homeostasis is antagonistically pleiotropic.

An in vitro fetal wound model

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Introduction: Early gestation fetal wounds heal without scar formation. Understanding the mechanism of this scarless healing may lead to new therapeutic strategies for improving adult wound healing. The aim of this study is to develop a human wound model in which scarless wound healing can be studied.
Methods and Materials: A burn wound (10×2 mm) was made in human *ex vivo* fetal skin and in *ex vivo* adult skin under controlled and standardized conditions. Subsequently, the skin samples were cultured at the air-liquid interface for 7, 14, and 21 days.
Results: During culture, re-epithelialization of the wound took place in both the fetal and adult skin samples. However, the neo-epidermis covered a higher percentage of the burn wound in fetal skin. Incubation with bromodeoxyuridine showed that cells of the epithelial tongue and fibroblasts in the wound area were proliferating. More proliferating cells were present in fetal skin than in adult skin. Similar to *in vivo* early gestation wound healing, α-smooth muscle actin was observed only in the blood vessels and not in the fibroblasts.
Discussion: This *in vitro* fetal skin model can be used to examine different aspects of scarless wound healing. The model contains all cell types normally present in human fetal skin and can easily be studied. Our model can be manipulated to study the role of certain growth factors or other elements in scar formation.

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A search for embryonic effector molecules for a skin construct by exon array analysis of developing skin

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Introduction: The current treatments of skin lesions do not result in full skin regeneration. To get closer to true regeneration of damaged skin, one approach may be to release those growth factors/morphogens that are involved in embryonic development, thus mimicking embryonic skin generation. A first step in this approach is to identify appropriate effector molecules. Using microarray analysis, we have selected two growth factors/morphogens essential for embryonic skin development. In future studies, these effector molecules will be incorporated in a collagen-glycosaminoglycan construct for sustained release and evaluated for their potential to induce skin regeneration.
Materials and Methods: Using mice, four time points were selected: E14, E16, P1 and P90. RNA was isolated from dorsal-lateral skin. The expression levels were analyzed using Affymetrix GeneChip Mouse Exon 1.0ST Arrays. The obtained data set was screened using three selection criteria: 1) the effector molecule must have growth factor and/or morphogenic properties; 2) the effector molecule must show embryonic specific expression; 3) the effector molecule must be able to bind to glycosaminoglycans. The gene expression levels of the candidate genes found with the exon array were validated by Real Time-Quantitative PCR.
Results and discussion: Based on these criteria, we found several candidate genes of which insulin-like growth factor 2 (IGF2) and sonic hedgehog (SHH) are most promising. IGF2 expression was found at E14, E16 and P1, but not at P90. IGF2 stimulates cell proliferation in both epidermis and dermis and may additionally stimulate angiogenesis via increased VEGF expression. SHH expression was found predominantly at E16 and P1, supporting the observation that SHH may stimulate appendage formation. Both molecules can be incorporated in collagenous scaffolds via the glycosaminoglycan heparin.

Myofibroblasts in the liver: fibrosis, cirrhosis and the stromal reaction to liver cancers

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Fibrosis, defined as the excessive deposition of extracellular matrix in an organ, is the main complication of chronic liver damage. Its endpoint is cirrhosis, which is responsible for significant morbidity and mortality. The accumulation of extracellular matrix observed in fibrosis and cirrhosis is due to the activation of fibroblasts, which acquire a myofibroblastic phenotype. Myofibroblasts are absent from normal liver. They are produced by the activation of precursor cells, such as hepatic stellate cells and portal fibroblasts. These fibrogenic cells are distributed differently in the hepatic lobule: the hepatic stellate cells resemble pericytes and are located along the sinusoids, whereas the portal fibroblasts are embedded in the portal tract connective tissue around portal structures. Differences have been reported between these two fibrogenic cell populations, in the mechanisms leading to myofibroblastic differentiation, activation and "deactivation", but confirmation is required. It is now widely accepted that the various types of lesion (e.g., lesions caused by alcohol abuse and viral hepatitis) leading to liver fibrosis involve specific fibrogenic cell subpopulations. The biological and biochemical characterisation of these cells is thus essential if we are to understand the mechanisms underlying the progressive development of excessive scarring in the liver. Moreover, in liver cancers, the myofibroblast is the main cell involved in the formation of the tumoral stroma. Usually, in hepatocellular carcinoma, tumoral stroma is scanty; often, the tumoral stroma is mixed with the fibrous stroma of the surrounding cirrhosis. In contrast, in cholangiocarcinoma, the stroma is abundant, sclerous, sometimes with calcification and may be extensive. We can assume that, in these two types of cancers, the myofibroblasts involved in the stroma derived from different subpopulations of liver fibroblasts. All this information is required for the development of treatments specifically and efficiently targeting the cells responsible for the development of fibrosis/cirrhosis, and of tumoral stroma.

The myofibroblast – friend or foe in regenerative medicine?

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Excessive myofibroblast contraction impairs tissue function as in hypertrophic scars, in fibrotic diseases, and in the stroma reaction to epithelial tumors. Moreover, bioengineering approaches must prevent myofibroblast formation, either arising from mesenchymal stem cells that are used to repair tissues/organs of mesodermal origin or from fibroblasts at the interface between implant and host tissue. The contractile apparatus of myofibroblasts represents an important therapeutic target to reduce fibrosis and to increase bioengineering success rates.
Myofibroblasts not only exert force but adapt to the stress in their microenvironment. I will present various strategies to modulate myofibroblast tension, such as innovative polymer materials of different stiffness and control over cell size and adhesion by surface microstructuring. As a general rule, releasing myofibroblasts from stress leads to the loss of contractile features and ultimately to de-differentiation and/or apoptosis. We propose therapeutically interfering with their stress-perception apparatus as novel strategy to eliminate fibrogenic cells.

Alpha – smooth muscle actin (aSMA) expression in foetal skin fibroblasts

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Introduction: Early to mid-term foetuses heal deep skin wounds without the formation of a scar. Determination of the differences between adult and foetal wound healing might yield insights about how we could achieve scar-less healing in adult skin. The goal of this study is to investigate the behaviour of foetal and adult skin fibroblasts in dermal matrices.
Material and Methods: Foetal and adult skin fibroblasts were cultured on glass slides, Matriderm, Integra and De-Epidermised Dermis (DED). Cells on glass slides were fixed in formalin upon confluence. The dermal matrices were cultured for three weeks and contraction was determined using planimetry. The matrices were fixed and αSMA expression was assessed with immunohistochemistry.
Results: Foetal skin fibroblasts were able to contract Matriderm scaffolds while Integra and DED did not show any significant contraction. Histological analysis showed strong αSMA expression in fibroblasts cultured on glass and in Matriderm, while in DED and Integra almost no αSMA expressing cells were found.
Discussion: Foetal skin fibroblasts appear to be able to contract certain matrices and express high amounts of αSMA when cultured on glass or an artificial collagen matrix. This indicates that the absence of αSMA activity in foetal wounds is not an intrinsic property of these cells but rather is caused by the cell's surroundings. Interestingly the glycosaminoglycan containing scaffold showed reduced contraction and αSMA expression. Whether this is due to the glycosaminoglycan needs to be studied further.

Controlled mechanical stimulation of skin cell proliferation in vivo

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Introduction: Cells respond to mechanical cues from the environment by increased proliferation and matrix organization. Although physical devices are currently used in clinics, the forces applied are empirically decided and scientific results have not been recollected. In this study, a custom designed stretch device was used to systematically investigate the role of mechanical stimulation in vivo.
Methods: A stretch device was applied on the intact (n = 18) or wounded (n = 10) skin of mice. Using this device, the skin received 0.5 N continuous or 0.5 N cyclical (2 min on/1 min off) stretching forces for 1 or 4 hours. Sham devices were used as controls. After 2 and 10 days, skin was harvested for immunolocalization of cell proliferation (Ki-67), vascularity (PECAM-1), and inflammation (CD45).
Results: Both continuous and cyclical forces stimulated cell proliferation, with cyclical patterns being the fastest triggers. The vascular area and perfusion of stretched tissues increased in parallel. Ten days post stretch, when signs of extracellular matrix reorganization started to appear, cell proliferation returned to baseline. Wounds receiving cyclical stimulation showed maximal histological response.
Conclusion: In light of the disappointing results obtained with growth factors, physical stimulation appears to be a promising strategy to stimulate wound healing. The proliferative response to mechanical stimulation of epidermal cells was higher compared to cells in the dermis, a mechanically shielded environment. Cyclical force regimens appeared to be more effective in both intact tissues and wounds.

The role of $\alpha_v\beta_6$ integrin in diabetic wound healing in mice

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Introduction: Integrins are cell-specific heterodimeric receptors that bind to extra cellular matrix components, thereby stimulating intracellular signaling pathways necessary for cell proliferation, differentiation, and migration. During wound healing, expression of $\alpha_v\beta_6$ integrin is induced by keratinocytes. In the present study, we compared wound healing rates between β6 integrin-deficient ($\beta6^{-/-}$) mice and wild type (WT) under normal conditions, and in animals challenged with streptozotocin (STZ)-induced diabetes.
Methods: Twenty 12-months-old male FVB mice were enrolled in this study. Half of the animals were made diabetic using daily i.p. injections of citrate-buffered STZ (55 mg/kg) for six consecutive days. Control animals were injected with citrate-buffer only. After five weeks, four full-thickness 4 mm excisional wounds were created on the dorsal skin of each mouse. Wound healing rates were quantified by measuring wound areas over 10 days using NIH ImageJ analysis software (http://rsb.info.nih.gov/ij/).
Results: No significant difference in wound healing rates between WT and $\beta6^{-/-}$ control mice was observed, and these wounds all closed at day 9. In general, diabetes-induction yielded a 3 day healing delay for both WT and $\beta6^{-/-}$ compared to controls. Within the diabetic group, a significant delay in day 1–3 ($p < 0.05$, Student's *t*-test) and day 4–5 ($p < 0.01$) was observed in the $\beta6^{-/-}$ mice compared to WT.
Conclusion: The $\alpha_v\beta_6$ integrin does not affect wound healing rates under normal conditions. However, when challenged with diabetes, $\alpha_v\beta_6$ integrin depletion results in a dramatic delay in wound healing. This finding might gain novel insight into the molecular pathophysiology of impaired diabetic wound healing.

Bioengineered tendon: from tenocytes culture to ASCs (Adipose Derived Stem Cells) culture

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Background: After a tendon injury, the healing process results in formation of a fibrotic scar. The structural organization and mechanical properties of this healed tissue are insufficient since tendon has a limited regeneration capacity. This is the reason why obtaining tendinous tissue by means of tissue engineering becomes a clinical necessity. Recently medical research stressed the importance of ASCs which can differentiate in vitro toward the osteogenic, adipogenic, myogenic, and chondrogenic lineages.
Objective: The aim of the present study is the reconstruction of a tendon structure using ASCs undergone mechanical stress.
Methods: ASCs were isolated from lipoaspirate, expanded to obtain an adequate cellular density and placed on the biomaterial of hyaluronic acid (HYALONECT®). Utilizing an experimental bioreactor, traction conditions were maintained. Samples were analyzed with histological studies, immunohistochemical studies and electron microscopy analysis. Specific gene expression was studied with RT-PCR.
Results: After 18 days of culture, cells were found adhering to the fibres of the scaffold and synthesis of the extracellular matrix was observed. The application of mechanical stress allowed the alignment of cells and the extracellular matrix, parallel to the traction lines. RT-PCR showed maintenance of the differentiated phenotype.
Discussion and conclusion: Taking our stand on these observations, we can conclude that ASCs cultivated in dynamic conditions on a three-dimensional support made up of hyaluronic acid, are able to reconstruct a tendon tissue. Thus we can hypothesize that ASCs are able to differentiate and organize themselves in tendon structures, opening therefore new surgical prospects for therapy of tendon injuries.

A NOSF (Nano-Oligosaccharide Factor) lipido-colloid dressing inhibits MMPs in an *in vitro* dermal equivalent model

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Matrix metalloproteinases (MMPs) activity increases in chronic wounds, leading to a metabolic imbalance slowing down the healing process. Mechanisms of action of a new compound, Nano-Oligosaccharide Factor (NOSF), on wound healing was studied *in vitro*. The aim of this work was to compare the effects of lipido-colloid dressings containing or not NOSF on the activity of MMPs.
Normal human dermal fibroblasts were incorporated within a collagen matrix, i.e. dermal equivalent (DE). Dressings were applied on DE, and the culture medium was analysed at days 2, 4 and 8. MMP-2 and -9 (Gelatinases), MMP-3 (Stromelysin) or MMP-1 and -8 (Collagenases) activities were analysed using zymography techniques with gelatin, casein and collagen gels, respectively.
At Day 4, NOSF clearly inhibited gelatinases (MMP-2 et -9) and collagenases (MMP-1 et -8). Kinetic analysis of NOSF effects demonstrated that the inhibition of gelatinases was slightly (MMP-9) or not (MMP-2) detectable at Day 2, while the effect existing at Day 4 persisted at Day 8. In contrast, concerning collagenases, the inhibitory effects of NOSF were detected as soon as Day 2 but disappeared at Day 8.
Using a dermal equivalent made it possible to study the effects of NOSF *in vitro* but at tissue level, in a close to *in vivo* dressing application situation. The effects of NOSF on collagenases appeared rapidly but did not persisted while those on gelatinases were delayed but were maintained longer. This study contributes to explain the efficient results of NOSF observed *in vivo* on chronic wounds.

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Complications in the venous system in patients with diabetes

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It is commonly stated that venous insufficiency is a risk factor for the development of diabetic foot disease. Analysis of the literature yielded little objective evidence to guide us in this context. As common complaints of the venous system are deep vein thrombosis (DVT) and venous insufficiency, we have studied the point prevalence of these issues in our centre.
In a study on diabetics at risk of DVT, we recruited 175 patients who were grouped as follows:
Group 1: Diabetics 'at risk' of DVT (n = 33)
Group 2: Diabetics 'not at risk' of DVT (n = 15)
Group 3: Non-diabetic patients 'not at risk' of DVT (n = 127).
The point prevalence of DVT in Group 1 was 12.5% (90% CI) (6.6%–22.4%).
The point prevalence of DVT in Group 3 was 22.8% (90%CI) (17.3%–29.5%).
None of the diabetic patients 'not at risk' had DVT.
This study permits the conclusion that the diabetic patient is at less risk of getting DVT than the cohort group.
A second study was done on the point prevalence of venous incompetence. Diabetics (n = 75) with and without foot ulcers were tested for above knee venous incompetence in both deep and superficial venous systems, venous refilling times (VRT) as a measure of haemodynamic disease and arterio-venous response as a measure of venous incompetence. The results yielded a prevalence of deep venous incompetence of 64% and 70.7% in the right and legs respectively, which is significantly higher than a report on the general population ($p < 0.05$). 42.7% and 49.3% of subjects had reduced VRT in right and left legs respectively. 30.7% and 33% of subjects had reduced AV response on right and left legs respectively. These data permit the conclusions that venous incompetence is high in the diabetic population who also exhibit haemodynamic dysfunction.
These finding provoked the question: should we investigate and treat the venous aspect of the peripheral circulation in diabetics to improve our understanding and perhaps management of diabetic foot disease?

Posttraumatic osteomyelitis: current treatment options and future directions

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Introduction: Chronic infected wounds, with little tendency to heal spontaneously, pose a continuing challenge in medicine. Despite various options for treatment of such infected wounds, such as repeated surgical debridement and antibiotic therapy, the condition cannot always be treated effectively and can result in prolonged and multiple hospitalizations. In some cases a final solution to cope with such wounds, for example severe posttraumatic osteomyelitis, may be a disabling amputation. The process of wound healing is complex and normally proceeds through at least three stages. The first, catabolic stage in a traumatic wound lasts from day 1 to day 5. The second stage lasts about two days, from day 5 to day 7, and is called the proliferation or anabolic phase. During this phase, fresh granulation tissue grows into the wound area. The last stage of wound healing, the recovery stage, occurs when the ingrowth of fibroblasts creates scar tissue. This final stage may last until day 21 of the recovery. The normal process of wound healing may be disturbed at each stage by local or systemic infection, be delayed by underlying conditions such as diabetes mellitus or by exogenous factors such as smoking. If the wound becomes colonized by bacteria or even infected, production of lytic enzymes and toxins by microorganisms and the host inflammatory reaction impairs wound healing and spontaneous wound closure is delayed even further. Many of these factors play a role when infection progresses to involve underlying bone, resulting in osteomyelitis. For better knowledge for treatment of the infection, posttraumatic osteomyelitis should be separated in acute onset osteomyelitis (clinical signs of infection observed within 6 weeks after initial trauma or orthopaedic procedure) and late, chronic osteomyelitis (clinical signs of infection after 7 weeks or more after trauma or orthopaedic procedure.
Diagnostic Approach in posttraumatic osteomyelitis: In case of suspected post trauma osteomyelitis, clinical examination for local signs of infection such as painful swelling of soft tissues should be performed. Bacterial culture by sterile aspiration of soft tissue fluids or fistula is needed to confirm the diagnosis and to identify the bacteria(s) causing the infection. Furthermore X Ray examination of the affected bone in combination with CT-PET scan and/or MRI is needed for visualization the extension of the soft tissue or bone infection.
Therapeutic approach in Osteomyelitis: Although the current standard surgical approach to osteomyelitis, i.e., repeated debridement of necrotic tissue, lavage, sequestrectomy or bone resections, complemented by antibiotics, can cure acute post trauma osteomyelitis the infection in most cases, especially in chronic osteomyelitis many cases can not be treated successful in curing the infection or prevent prolonged or recurring hospitalization. Even after wound closure, a risk of recurrence of infection remains, which may lead to re-hospitalization(s) and a prolonged time of healing. In severe cases segmental bone resection followed by callus distraction can be useful to cure severe osteomyelitis but patients compliance should be checked carefully in advance before such treatment can be performed. Furthermore during the period of callus distraction many minor (e.g. pin tract infections) or major complications (e.g. delayed or failed callus formation) can be observed. Therefore new therapeutic approaches like Vacuum Assisted Closure therapy, the new VAC Instill therapy, and experimental Maggot Debridement Therapy (MDT) are used to today to reduce the risk of invalidating amputations, especially in younger patients.
Conclusion: Despite modern concepts orthopaedic treatment for injured patients, there is still a risk on acute posttraumatic infection and osteomyelitis. Although early surgical debridement and lavage in acute osteomyelitis can cure the acute infection in combination with antibiotic therapy, late complications can occur. If a patient suffers chronic osteomyelitis, there is a risk for recurrent exacerbations, depending on contributing risk factors which can cause recurrent hospital admissions and, in worst case scenario's disabling amputations. Therefore consequent diagnostic and therapeutic approach is needed to reduce these risks.

Novel lipase activity detected in induced *Lucilia sericata* excretions/secretions

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Introduction: Maggot debridement therapy (MDT) is a controlled application of commercially produced sterile larvae from the green bottle fly *L. sericata* to a wound in need of debridement. Many patients with chronic wounds are ideal candidates for MDT. The maggots gently remove necrotic tissue by proteolytic digestion during the 3 day application, leaving the healthy granulation tissue unharmed. The composition of the maggot's excretions/secretions (ES) is subject to worldwide research and an elucidation of the mechanisms behind MDT will allow supplementations into other treatment regimes.
Methods: ES were collected from 1st, 2nd or 3rd instar level maggots subjected to the different inductions prior to ES collection. Starvation, feeding on a sterile food source, external bacterial challenge with *Staphylococcus aureus* and for 3rd instar maggots a 3 day patient treatment induction. ES samples from different inductions with total protein amounts of 50 μg and 100 μg was subjected to enzyme activity screening assays using a Crystal violet fatty acid lipase activity assay.
Results: Lipase activity was detected in all samples however, the highest activities compared to the addition of a purified commercial lipase were detected in the 3rd instar and patient induced ES.
Conclusion: To the authors knowledge this is the first report of differentially induced lipase activity in ES. Lipolytic activity may play a role in the mechanisms of larval feeding in MDT, especially in the late stages of the therapy; however whether this activity originates from the excretions or secretions of the maggots is subject to further research and identification.

The use of acellular, radiation-sterilized amniotic membrane increases healing rate of hard-to-treat chronic venous leg ulcers

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The amnion is a tissue forming the innermost layer of fetal membranes and placenta. Due to specific composition, amniotic membrane is an ideal surface

for cell migration and differentiation. Moreover, amnion components may display anti-inflammatory/immunosuppressive activity. The properties of the amniotic membrane make it an attractive dressing material. Its current clinical applications include treatment of severe skin burns and ocular surface, pleura or pericardium reconstructions. Also, some attempts to use the freshly isolated amnion for chronic wound management have been undertaken. However, the use of freshly isolated material is limited. Therefore, various procedures of preparation, sterilization and storage of amnion samples have been developed, but their clinical effectiveness requires further verification.
The aim of study was to assess the influence of acellular, gamma-irradiated amnion samples on hard-to-treat chronic venous leg ulcer in 38 patients. The study procedure included once-per-week Bates-Jensen questionnaire-based wound assessment, digital planimetry, and application of the respective dressing and two-layer compression of the affected leg. After four weeks of treatment individuals displaying re-epithelialization rate $< 10\%$ per week were qualified for a further treatment with the amnion dressing ($n = 22$). The remaining 16 patients (control group) received previous treatment. After next four weeks in amnion-treated group the re-epithelialization rate significantly increased (4.89 ± 1.07 to 11.86 ± 1.70; $P = 0.00001$), whereas in control group it remained unchanged (12.24 ± 1.42 to 12.55 ± 2.18; NS). The observed difference may result from biologically active factors in amnion samples, as verified by western-blot.
The acellular, radiation-sterilized amnion could be a valuable dressing supplement especially useful for hard-to-treat chronic venous leg ulcers.

A novel vitronectin: growth factor complex for treatments of wounds

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Topical administration of growth factors has displayed some potential in wound healing, but variable efficacy, high doses and costs have hampered their implementation. Moreover, this approach ignores the fact that wound repair is driven by interactions between multiple growth factors and extracellular matrix proteins. We have discovered, however, that complexes comprised of insulin-like growth factors (IGF) and IGF-binding proteins bound to the extracellular matrix (ECM) protein vitronectin (VN) significantly enhance cellular functions relevant to wound repair in human skin keratinocytes in 2D and 3D *in vitro* cell models and are active, even in the presence of wound fluid. Moreover, these responses require activation of both the IGF receptor and the vitronectin-binding α_v integrins. Further, we have assessed the complexes as a topical agent in the treatment of deep partial thickness burns in a porcine model. This has revealed that the complexes hold promise as a wound healing therapy. Critically, the significant responses observed *in vitro* and the encouraging data *in vivo* were obtained with nanogram doses of growth factors. This suggests that coupling delivery of growth factors to ECM proteins such as VN may ultimately prove to be a more effective strategy for developing a wound healing therapy. In light of this encouraging data we have manufactured the components to GMP standard, completed toxicity testing and a clinical trial examining the potential of these novel complexes as a treatment for venous and diabetic ulcers will commence in March. Our progress towards this trial, as well as the development of new second generation growth factor: VN chimeric protein mimetics, will be presented

Controlling sprouting angiogenesis – a tale of leaders and followers

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Endothelial cells within the angiogenic sprout need to coordinate their behaviour and cellular functions during sprout induction, elongation and lumen formation, as well as during subsequent branching and anastomoses. Initial sprout induction involves selection of a leading tip cell, which will then guide the growing sprout by reading attractive and repulsive guidance cues provided by the surrounding tissue environment. A regular vascular patterning is achieved by coordinating the balance of tip-cell migration and stalk cell proliferation. The graded distribution of heparin-binding isoforms of VEGF-A is instrumental for this balance: The steepness of the gradient regulates the speed of tip cell migration, whereas the local concentration reaching the stalk-cells gauges their proliferation[1,2]. Recent studies illustrated that VEGF-A is responsible for induction of the sprouting response, whereas Dll4 signalling through Notch1 regulates which cell will become the tip cell and which cells will constitute the trailing stalk cells[3,4]. Genetic and pharmacological inhibition of endothelial Notch signalling results in excessive filopodia formation and tip cell activity in all endothelial cells exposed to the inductive stimulation through VEGF-A. Conversely, ectopic activation of Notch signalling leads to rapid reduction in filopodia formation and sprouting activity, resulting in reduced vascular density in the ensuing plexus. These studies illustrated that the tip cell response is the default answer to VEGF-A stimulation, whereas the stalk cell phenotype is an acquired function dependent on cell autonomous Notch activity. Computer simulation of the tip-cell selection process indicated that the VEGF-A gradients facilitate stable selection and that the filopodia extension confers robustness to this system[5]. These observations on the regulation of tip and stalk-cells establish two fundamental principles in angiogenic sprouting: vascular patterning can be achieved 1) by balancing the migration of tip-cells to the rate of proliferating stalk-cells through VEGF-A gradients, and 2) by regulating the numbers of tip- versus stalk-cells through modulation of the Dll4/Notch signalling pathway involved in tip-stalk selection.

The meaning of skin injuries and their surgical reconstruction: first long time experiences with the collagen-elastin matrix Matriderm as a dermal substitute in severe burn injuries of the hand

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Skin has a important concrete physiologic role, but beside its physical functions, it has also a massive psychological meaning: skin is the part of us that comes into contact with what is outside of us and it gradually enables us to get to know the world, but it also sets the boundary between us and the other objects in the world. Think, then, what happens to a person who suffers a sudden skin injury: The sense of invasion or leakage in the part that holds, protects, unites and distinguishes the individual as a unique human being is liable to create a whole set of feelings of disintegration and existential fear.
In burn injuries this is often accompanied by a real danger due to the serious physical condition, which includes loss of fluids, risk of infections, the need for artificial respiration, repeated surgical intervention and much more, all resulting in helplessness and fear of death. In addition, because injury to the body cover also damages the person's appearance, that is, his body image, the negative feelings are also accompanied by confusion and problems with the self-identity. This is particularly severe when visible parts of the body, especially the face and hands, are involved, because self-perception and physical image are a result of continuous mutual reflections between us and the social world. It can be said, that a person with a burn injury not only experiences a sense of disintegration and confusion regarding identity, but also feels anxiety regarding his or her place in the social world. A very important part in the healing process is the patient orientated surgical treatment. One of the main surgical objectives is to achieve an earliest possible, durable and mechanic stable coverage of the burn wounds, but due to the improvements in burn wound treatment, not only survival, but also regained quality of life plays an important part of the final outcome.
Great efforts have been made in the past to develop artificial replacements of dermis and epidermis to overcome the problem of poor skin quality and scar contraction in order to achieve optimal function and aesthetic appearance of the restored and reconstructed skin. Thereby, optimal skin reconstruction plays a very important role in regaining quality of life after burn injuries. Thereby the reconstructed pliability of the grafted areas is of utmost importance for good hand function. The collagen elastin matrix Matriderm was evaluated as a dermal substitute for the treatment of severe hand burns.
In a series of 10 patients, mean age 43 years, TBSA 22.8%, an early debridement and immediate grafting with the matrix and unmeshed skin graft was carried out in a one-stage procedure. In the early postoperative follow up an overall take rate of 97% was observed. In contrast to conventional skin grafts, the colour of the skin grafts over the matrix appeared pale in the first few days, but after 2 weeks no difference was observed. After three months, pliability of the grafted area was excellent, (mean VSS 3.2 ± 1.2). Full range of motion was achieved in all hands, no blisters and no unstable or hypertrophic scars occurred. These good functional and aesthetic results remained stable, even after the one year follow up.
Matriderm has proved to be a dermal substitute suitable for the treatment of hand burns. We therefore consider Matriderm as a promising dermal substitute for the treatment of severe hand burns.

A 15 year landscape on culturing skin cells for burns patients

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Since the early 90s my group have been delivering laboratory expanded cultured autologous keratinocytes for the treatment of patients with extensive burns injuries, initially as cultured epithelial autografts (CEA) and more recently on a carrier membrane. A 10 year audit of the clinical use of CEA identified major problems in timing the production of these integrated cell sheets to the needs of the patients. Accordingly we then developed a chemically defined plasma polymerised surface containing 20% carboxylic acid groups from which to deliver the cells to the patients. Taking this approach forward so that it was sustainable rather than dependent on research funding was tackled by establishing a spin out company, CellTran Limited in 2000.
The first product (available in the UK since 2004 as Myskin) was used to deliver cells for burns patients and for patients with chronic non-healing diabetic foot ulcers. Supplying the cells in easy to handle polymer discs obviates the necessity of the surgeon or nurse handling spray-on cells or fragile sheets of cultured cells. Crucially it also gives a much more flexible timing of delivery of cells to patients as cells are now expanded in the laboratory and then only transferred to the discs 2 days prior to application of the cells on discs to the patients. More recently the company in May 2008 received approval from the Department of Health to supply allogeneic screened keratinocytes on a plasma carrier dressing available from frozen for treatment of patients with partial thickness burns.
From 1994 we have also been involved in the bigger challenge of making 3D reconstructed skin to replace or augment split-thickness skin grafts. Initially this used human allodermis and more recently we have progressed to a biodegradable synthetic electrospun scaffold. Early pilot clinical results identified problems of delayed angiogenesis and tissue engineered skin graft contraction. Accordingly in recent years, our *in vitro* research has concentrated on getting a better understanding of the problems of neovascularisation and of reducing contraction in tissue engineered skin. These remain major challenges in developing tissue engineered skin to benefit burns patients.

Development of a novel dermal substitute based on glycerinised allograft

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Introduction: The Quest remains for an optimal and cost-effective dermal substitute (DS) which improves the outcome of split skin grafted (SSG) defects. Skin restoration with a dermal substitute and a thin SSG significantly reduces scarring, contracture and donor morbidity compared to SSG alone. Currently available dermal substitutes remain controversial due to variable take rates, susceptibility to infection and high costs. No valid monitoring modality has yet been reported to gage the ingrowth of the DS and elucidate the optimal time for SSG application. We developed a prototype derived from human glycerinized allograft by processing in low concentrations of NaOH and assessed it's efficacy in a porcine wound model and an initial phase 1 clinical study in 12 patients with full thickness burn defects.
We initiated monitoring of the DS with Laser Doppler Imaging (LDI is a validated tool for burn depth assessment) that can delineate vascularization of the DS and optimal time for SSG engraftment.
Methods: 120 full thickness wounds of 4×4 cm were created on the flanks of 15 pigs. One-Stage Procedures: wounds were engrafted with NaOH-DS and SSG and controls engrafted with SSG only or in combination with AlloDerm or an artificial collagen matrix. Two-stage procedures: wounds were randomly engrafted with NaOH-DS or Integra and subsequently engrafted with SSG after 7 days. Outcome was assessed by digital imaging, planimetry and punch biopsies. Clinical Study: 12 full thickness burn wounds in 12 patients were engrafted in a two stage procedure (one week interval). DS ingrowth was monitored by LDI.
Results: Wounds with prototype and SSG showed less contraction compared to controls with a higher quality neodermis. SSG survival in one stage procedure approached only 65% for wounds engrafted with prototype or Alloderm, due to delayed vascularization as seen on histology. Two stage procedure takes of NA-OH DS approached 85% comparable to controls engrafted with SSG only or Integra. Contraction rates were significantly lower in wounds with DS and SSG ($p < 0.05$) than those with SSG alone and comparable to Integra and SSG engraftment. Clinical take rates of the prototype approached 95% in 12 patients.
Discussion: A viable, cost-effective Dermal Substitute can be procured from decellularised, glycerinized allograft. Most favorable results were obtained in a two stage procedure. Emphasis is placed on initial clinical application of the prototype including the use of LDI for monitoring purposes. These promising results prompt us to advocate Glyaderm for dermal substitution. Ongoing comparative clinical trials and multicentre protocols are discussed which include web-based multicentre interactive database, objective scar assessment and paired intra-individual comparison.

Characterization of the engraftment of autologous keratinocytes cultured on hyaluronan scaffolds on deep burns: clinical-histological correlations

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Background: Among the treatment options available for deep and extensive burns, autologous keratinocytes cultured on hyaluronan scaffolds (CEA-HYAFF-11®) are clinically used yet their engraftment is sill poorly characterized.
Objective: To characterize the engraftment of CEA-HYAFF-11® on deep burn wounds.
Methods: In this clinical trial, CEA- HYAFF-11® sheets were grafted on deep second degree-third degree burn wounds. After early excision, wounds were left (1) spontaneously granulating, (2) grafted with autologous skin mesh grafts or (3) grafted with allogenic cadaver skin followed by dermoabrasion. Each area was treated with (experimental) and without (control) CEA- HYAFF-11®, and clinical and histological descriptions were made.
Results: Although CEA- HYAFF-11® grafted wounds showed an early clinical improvement compared to non treated wounds, histological specimens did not show signs of epithelial engraftment during the first week. Evidence of engraftment of CEA- HYAFF-11® first appeared two weeks post grafting with a newly synthesized, linear basal lamina while non grafted wounds were still mainly raw. From the results we identified distinct phases in CEA- HYAFF-11® engraftment starting from survival to adhesion and terminating into terminal engraftment. CEA-HYAFF-11® engraftment increased re-epithelialization compared to controls also on meshed autologous skin grafts and allogeneic, dermoabraded skin, with the latter being the more reliable substrate for keratinocyte taking.
Conclusions: CEA- HYAFF-11® appeared to be a reliable resource for the treatment of burns wounds especially when limited donor sites make autologous skin transplantation difficult. Areas of engraftment showed linear basal lamina as a distinctive feature compared to spontaneous re-epithelialization.

Update on clinical wound care and burn treatment

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Improved resuscitation techniques and optimal treatment in specialized burn centres have resulted in increased survival of patients with extended and deep burns.
Nowadays, emphasis in burn treatment is shifting towards reducing scar tissue formation.
Anti-scar therapies in burn patients should not only be started after the healing of the burn wound, but already be considered from the time of the injury on. Indeed, cooling of acute burns can make the difference between a superficial and a deep second degree burn, thus avoiding scar formation. Adequate systemic fluid therapy can prevent secondary deepening of burn wounds by improving microcirculation in zones of stasis. An exact assessment of burn depth (and the indication to operate) can be provided with a Laser Doppler Imaging (LDI) thus avoiding unnecessary scar formation caused by grafting superficial wounds or prolonged conservative treatment of deep burn wounds.
The local treatment of burn wound should provide an optimal environment for wound healing and reduce all inflammation while additional (and new) anti-infection therapies (silver, iodine, honey) might be required to avoid infection and the risk of deepening of the burn wounds.
Surgical therapy can influence the resulting scar through a precise indication for escharotomy and grafting, by the judicious use of meshed grafts and Meek expansion and by the application of dermal substitutes or flap surgery. Physical therapy can influence early scar formation and local anti-scar therapies such as pressure garment and silicone application are applied in various forms to improve the final scars. Skin hydration and moistening can help to keep the scars supple and prevent itching while sun blocks protect against UV radiation and secondary pigmentation.
An update will be provided on all recent strategies to further improve function and aesthetics of scar formation after burns.

Effect of different therapies on inflammation, itch and nerve regeneration in a porcine scald wound model

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Effects of several treatments for partial thickness scald burns have been described but there are few data available on the mechanisms of action. Especially nerve regeneration and itch have only occasionally been studied although data emphasizing the role of the nervous system in wound healing are increasing.
We have studied the effects of 3 different treatments in a standardized model using Duroc pigs; Silver Sulfadiazine cream (SSD), Glycerol preserved allogeneic skin (GPS) and a hydrofibre dressing (Aquacel). Wounds were made by applying hot water (80 °C) for 20 sec on the flanks of the animals and were covered with the different dressings (N = 9 wounds per type of dressing). The inflammatory response and nerve outgrowth were studied on sections of biopsies of day 3, 7, 14 and 56 after wounding. Contraction was measured using planimetry at day 56. Itching behavior was recorded using a digital camera.
The macrophage influx was the highest at day 7 but there were no significant differences between the treatments. The outgrowth of the epidermis was significantly faster in the wounds treated with GPS. There were no significant differences in wound contraction at day 56 but the wounds treated with SSD had thicker scars compared to wounds treated with GPS or Aquacel. The latter wounds showed also hyperkeratosis. The animals treated with SSD had up to 35% higher scores for itching behavior. First results using specific antibodies for nerve fibres suggest a delay of outgrowth of the nerve fibres in the wounds treated with SSD.

Biodegradable poly-n-acetyl glucosamine (pglcnac) nanofibers enhance wound healing by activating cell migration, angiogenesis and cell proliferation

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Introduction: The clinical usage of tissue regenerative templates to treat tissue defects is still limited because of delayed revascularization of matrixes leading to infection and rejection. We have recently shown that biodegradable, diatom-derived, haemostatic poly-N-acetyl glucosamine (pGLCNAc) nanofibers induce a marked increase in cell proliferation and angiogenesis in diabetic wounds in mice. Here we developed a modified biodegradable short fiber Poly-N-acetyl glucosamine (sNAG) to overcome present limitations of clinically used skin substitutes.
Objective: We test the cellular mechanism of action of sNAG on cells in vitro and the ability of sNAG-scaffolds to lead tissue regeneration in an experimental diabetic wound healing model.
Methods: sNAG effects on cell metabolism and migration where tested *in vitro* assays. In vivo, full thickness wounds of db/db mice (n = 15 per group) were treated either with a NAG-scaffold (NAG-scaffold), or were left untreated (NT). Macroscopic wound healing kinetics, granulation tissue formation, cell proliferation (Ki67), angiogenesis (PECAM-1), migrative activity (MAP-Kinase), keratinocyte migration (p63) were monitored over a study period of 28 days. mRNA levels related to migration (uPAR), angiogenesis (VEGF), inflammatory response (IL-1b) and ECM remodeling (MMP3 and 9) were measured in wound tissues on day 10.
Results: sNAG enhanced cell metabolism, endothelial and fibroblast migration *in vitro*. NAG-scaffold produced faster wound closure compared to controls mainly by re-epithelialization. sNAG treated wounds showed increased keratinocyte migration, granulation tissue formation (2.8 fold), cell proliferation (4fold) and vascularization (2.7fold) compared to NT wounds. mRNA levels related to angiogenesis (VEGF), cell migration (UPAR) and ECM remodelling (MMP3, MMP9) were upregulated in NAG-scaffold treated wounds compared to NT wounds.
Conclusions: The nanofiber pGLcNac based scaffold enhanced wound healing mainly by re-epithelialization and angiogenesis. This effect was likely lead by specific cell - fiber interactions leading to revascularisation and integration of the scaffold. The introduced polymer nanofiber material is a potential candidate to overcome existing limitations of regenerative templates for the treatment of tissue defects.

POSTER ABSTRACTS

POSTER 1

New therapeutic approach in the treatment of severe radiation burn: surgery and local stem cell therapy

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The therapeutic management of severe radiation burns remains today a challenging issue. The conventional surgical treatment (excision and skin autograft or flap) often fails to prevent unpredictable and un-controlled extension of the radiation necrotic process.
We report here our second experience of therapeutic management of a radiation accident victim combining surgery and cellular therapy using autologous Mesenchymal Stem Cells (MSC).
The patient presented a very severe arm radiation burn which was treated by several surgical times: iterative excisions, skin graft, latissimus muscle dorsi flap and forearm radial flap). Local autologous MSC were administrated as an adjuvant to improve the surgical approach. The clinical evolution (radiation pain and healing progression) was favourable and no recurrence of radiation inflammatory waves was observed during the eight month patient's follow-up suggesting that MSC act as "cell drug" in modulating radiation inflammatory processes.
These results open new prospects in the medical management of severe radiation burns.

POSTER 2

Cell regeneration therapy as an adjunct to healing in recalcitrant wounds

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Background: Chronic wounds have for many centuries represented a major source of morbidity and mortality in patients. In the modern era we realise that these wounds also produce a major psychological impact to the patients, and a financial burden to the healthcare systems around the world.
Ever since the first skin grafts were attempted in India around 2000bc for the reconstruction of punitatively amputated noses, the surgical profession and medical sciences have been searching for the panacea to treat chronic wounds. Yet as our understanding of the aetiology of chronic wounds deepens we realise that the varied nature of the wound pathogenesis means there will never be a "one cure for all" treatment. In this way we can see that a split skin graft is certainly not the optimal option for many patients, and that alternatives must be found.
Method: In two selected patients with chronic static laparotomy wounds we demonstrate the successful use of a biological tissue regeneration therapy (Apligraf®) in the management of these recalcitrant wounds.
Conclusion: In carefully selected patients under the care of a Multidisciplinary Wound Healing Team, the use of cell based tissue regeneration products may provide a valuable adjunct to standard wound healing regimens.

POSTER 3

Deep venous incompetence in patients with diabetes mellitus: a case control study

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Introduction: Venous hypertension has been suggested to be a risk factor for diabetic foot disease. This is considered to the effect of autonomic neuropathy though this appears to be based on clinical evidence. It has also been reported that diabetics with neuropathy have impaired venous function and haemodynamic dysfunction. Deep venous incompetence causes venous hypertension and haemodynamic changes. The prevalence of deep venous incompetence in the diabetic population is largely unknown. This aim of this study was to determine the prevalence of deep venous incompetence in the diabetic population in Southampton since as diabetic foot disease is a major problem.
Methods: This study was a case-controlled study at the Southampton General Hospital. The study included two groups of subjects recruited from Diabetic Foot Centre that is a referral point for diabetic care including foot disease. Patients were subdivided in to groups.
- Group 1 – The diabetic patient with frank ulcers or healed ulcers.
- Group 2 – The diabetic control subject with no foot ulcers.

Each patient underwent a duplex ultrasound scan to measure venous reflux for deep venous incompetence and laser Doppler flowmetry (LDF) scans. LDF scans were done to determine the arterio-venous response (AVR) and the venous refilling time (VRT) respectively. The data were analysed using the Pearson Chi square, Fisher's exact and the Mann Whitney U tests. The study was conducted with Ethics approval and prior informed consent of patients.
Results: 75 subjects were recruited and tested.
64% and 70.7% of diabetic subjects had deep venous incompetence in their right and left legs respectively which is statistically significantly greater ($p < 0.05$) than a previous report.
42.7% and 49.3% of subjects had a reduced VRT in the right and left legs respectively.
30.7% and 33.3% of subjects had loss of the arterio-venous response in the right and left legs.
The presence of symptoms was also recorded during the study.
Discussion: These data permit the conclusion that here is a high prevalence of deep venous incompetence in subjects with diabetes mellitus in our practice. Given that venous incompetence is reported to be a risk factor for diabetic foot disease and the argument that venous disease impairs wound healing, should we be treating this aspect of the circulation in the diabetic population who are at known risk of foot disease?

POSTER 4

Intensive treatment of wounds in lower limb amputation surgery can save a leg!

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Trans- tibial amputations have less co- morbidity than trans- femoral amputations. There is more stability when sitting, rehabilitation is easier and, most important of all, patients will preserve more of their self esteem.
However, trans- tibial amputations wounds have a higher risk of infection than trans- femoral amputations. Infection of the trans- tibial wound results in longer hospital stay, another operation and can even lead to a trans- femoral amputation.
In our clinic we have a very intensive treatment scheme for amputation wounds. This includes, amongst other techniques, frequent inspection of the wounds, Maggot debridement therapy and Vacuum Assisted Closure therapy (VAC). Using this scheme, our hypothesis is that we prevent patients from undergoing a trans- femoral amputation.
In a prospective study, we investigated all lower limb amputations from January 2003 until January 2007. During this period, 67 patients underwent a transtibial amputation. 51 of these 67 patients (76%) went home with a healed wound, 6 patients died within 30 days of surgery and in 10 patients conversion to a trans- femoral amputation was necessary.
When comparing these outcomes to the literature on lower limb amputations, we find that our intensive treatment scheme pays off. In our clinic we relatively perform more trans- tibial amputations than primary trans- femoral amputations. Furthermore, our trans- tibial amputation wounds had a significantly higher healing rate than the wounds described in the literature (76% versus 44-62%). Also, a smaller percentage of trans- tibial amputations is converted to a trans- femoral amputation.
These results demonstrate that it is better to use an intensive treatment scheme in order to save a leg, since it has a significantly higher success rate!

POSTER 5

Platelet Rich Fibrin (PRF) for hard to heal ulcers in patients with diabetic feet

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Platelets play two important roles in wound healing: hemostasis and initiation of wound healing. After platelet activation and clot formation, growth factors are released. In ordinary blood, the number of platelets is 0.2×106/ul. In contrast, it is $> 1.0 \times 106$/ul in platelet-rich plasma.
Autologous growth factors from concentrated platelet suspensions have been used to treat wounds for years. The use of platelet-derived wound healing formulae delivered in a crystalline collagen carrier was first published in 1986.
The Vivostat® System is a medical device for thepreparation of an autologous fibrin sealant from 120 ml of thepatient's blood. The system is fullyautomated and microprocessor controlled and is made up of threecomponents: an automated processor unit, an automated applicatorunit, and a disposable, single-patient–use unit, whichincludes a preparation set and a Spraypen applicator.
This pilot study of very hard-to-heal ulcers investigated whether treatment with autologous platelet-rich fibrin is feasible and to see if wound healing is improved. We would like to discuss our results of application of PRF (Vivostat PRF, Birkeroed, Denmark) in 8 treated patients with a diabetic foot ulcer.
Between September 2006 and November 2007 we recruited 18 patients with chronic, hard to heal ulcera. All wounds were located on the lower limb. In our population of 18 patients, there were 8 patients with a diabetic foot ulcer. 9 wounds were included in this study. (One of the patients had 2 wounds on the lower limb which were treated simultaneously). The population consisted of 6 males and 2 females with a mean age of 55.3 years. (range 38–70). The mean wound duration before treatment was 7.5 months (range 1–24).
The patients received a total of 23 treatments. In total, with a short follow up, (1–24 months), over 60% of the wounds have closed. 2 wounds were smaller than 1/3 of the initial size, although these are estimates only.

POSTER 6

A comparative study of the influence of different pressure levels combined with various wound dressings on negative pressure wound therapy (NPWT) driven wound healing

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Introduction & Aim: To present date, although a number of studies have been conducted to support the important role of NPWT in wound healing, none have investigated the optimal pressure level intensity and manner of delivery of negative pressure together with various combinations of existing dressing technologies. This study compared different commercially available dressing types in combination with a NPWT system used at various pressure levels. Wound healing progression from these various parameters was subsequently assessed.
Methodology: Five pigs (Landras type) were allowed to acclimatize to the housing conditions prior to all procedures. The animals were sedated, shaved and scrubbed for surgery and transported to the operating room. Full thickness wounds extending to the fascia over the deep back muscles ($3.5\,cm \times 3.5\,cm$, maximal 10 per animal) were excised on each pig. Different commercially available materials (foam, gauze, silver, textiles) were trimmed to the exact size of each wound. Each wound site was sealed with a thin air-permeable adhesive film. The dressing system is then connected to a vacuum source which was set to a different level of negative pressure (10, 20, 30 kPa).
Result & Conclusion: Morphological and histological observations of our wound porcine model indicate that healing is taking place independently from the type of dressing used. Thus, providing insights that there is currently not one type of dressing which is clearly outperforming the others when used in combination with negative pressure therapy on porcine wound models.

POSTER 7

Correlation of physical properties and dressing design

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Aims: Wound problems such as blistering are common following total knee replacement (TKR) surgery. The physical properties of the wound dressings may be responsible for this. They may not stretch to accommodate skin movement and consequently transmit high levels of shear force to the skin.
Skin movement of TKR wounds was quantified and compared with the material properties of a hydrocolloid, adhesive and two occlusive film dressings.
Methods: TKR wound measurements were taken at 0°, 30°, 60° and 90° knee flexion in 85 patients. Percentage change in wound length at each flexion level was calculated.
The material properties of the dressings were quantified using an Instron 5800 uni-axial testing machine. Strain was calculated using grip to grip measurements. Three samples of each dressing were tested.
Results: The traditional dressing showed very high loads for less than 5% strain. The other three dressings extended to at least 25% strain with less than 10N load.
The hydrocolloid and both occlusive dressings extended the required amount to accommodate skin movement (28% strain) with loads of 7.7N, 8.9N and 6.5N respectively. The adhesive dressing extended to $< 10\%$ of the required amount before reaching the limit of the 100N load cell.
Discussion: TKR wounds demonstrate dynamic morphology and strains of over 20% with normal knee flexion. Hydrocolloid and some occlusive dressings exhibit suitable material properties to accommodate this skin movement but traditional adhesive dressing do not. This may explain the high blister rates with some dressings.

POSTER 8

LASER stimulation on fibroblasts WI-26 based on photodynamic processes

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Phthalocyanines are interesting compounds for use in Photodynamic Process (PDP) considering their high absorbance (680 nm), with optimal tissue penetration by light. Chloroaluminium-phthalocyanine (AlClPC) is a photosensitizer drug applied in the treatment of cancer and others no-oncological diseases by PDP assay. The main goal of this work was to develop and study an AlClPC-nanoemulsion to be used in combination with low energy LASER to modulate cellular growth of fibroblasts.
WI-26 fibroblasts were used *in vitro* biostimulation studies in monolayer after light application. The cells were incubated with AlClPC-nanoemulsion for 30 minutes in the dark, with later irradiated at 670 nm; at different energy doses (24 hours and 48 hours) after nanocarrier treatment. Cellular viability was measured 3 and 5 days after irradiation using a MTT classical assay. Scratching experiments to mimic the wound repair have been done using the same protocol.
Irradiation by low energy (1.0 to 5.0 J) stimulated cellular growth in a dose-dependent manner, while the energy of 10 J showed a negative response for this biostimulation. The results also showed that the biomodulation was time-dependent, higher with a delay of 48 hours between dye and light application.
This protocol of PDP will know be used on *in vitro* reconstructed tissues to better assess its effect on the wound healing process.

POSTER 9

BITECIC (Biomaterials and Tissue Engineering Centre of Industrial Collaboration) – A collaborative approach for creative partnerships

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Aim: To provide an insight into the BITECIC model and its methodology for fostering productive collaboration.
BITECIC (Biomaterials and Tissue Engineering Centre of Industrial Collaboration) was established in recognition of the need for a collaborative approach to enhance the development of advanced clinical treatments and medical devices in wound care and other medical specialisms. Developing creative partnerships by bringing together diverse expertise from an academic, clinical and commercial perspective is at the heart of BITECIC's way of working and contributes to the development of novel therapies that meet clinical and commercial expectations.
This collaborative approach will be of particular value in wound care, which faces diverse and complex clinical and commercial challenges. The effectiveness of working in partnership with industry, clinicians and academics was illustrated by a recent project to determine the needs and opportunities in the area of chronic wound infection. This example will be described in order to provide an insight into the BITECIC model and its methodology for fostering productive collaboration.
BITECIC is committed to the development of solutions to real clinical needs. In order to support this, BITECIC is working in collaboration with the Healthcare Technologies Knowledge Transfer Network (Healthtech KTN) to develop the Statements of Clinical Need (SOCN) initiative. This provides the infrastructure to connect the industrialists, healthcare professionals and researchers who comprise the UK Health Technologies community and to facilitate communication between these specialist groups. The SOCN project will be described and delegates encouraged to participate.

POSTER 10

The effects of nicorandil on wound healing (case series)

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Introduction: We would like to report a series of nicorandil induced non-healing wounds seen over the past year.Nicorandil is a nicotinamide ester, and derivative of synthetic nicotine. It functions as a potassium channel activator. Uniquely Nicorandil has a nitrate group in its structure, adding venodiliatation to its effects. Thus causing increased blood flow in the coronary arteries and decreasing preload, the effects combining to form a highly effective anti anginal. The association of nicorandil use and oral/anal ulceration is now fairly well recognised. Less well known are nicorandil's association with para-stomal ulceration, and indeed its association with ulceration of the GI tract from mouth to anus. More recently there have been increasing reports of nicorandil-induced ulceration in parts of the body distant to the GI tract. We report a series of cases seen in our wound healing clinics, consisting of, patients, with lesions ranging from post surgical wounds to leg ulceration.
All these ulcers had a common clinical appearance: there was no visual evidence of healing, all lesions were extremely painful, and all healed rapidly following withdrawal of nicorandil.
Objectives: The aim of this work was bring to a wider audience our experiences of nicorandil's association with non healing wounds.
Methods: Patients included in the study were those who have presented to the Wound Healing Outpatient Clinics' of the Wound healing research unit at Cardiff university over the last 18 months. All patients were taking Nicorandil, and had typical non healing painful wounds, which have resolved or moved rapidly towards resolution following exclusion of Nicorandil from their treatment.
Results: The mechanism and incidence of the non-healing associated with Nicorandil is currently unknown, and may or may not be associated with the properties of arterial and venous dilatation. It had previously been assumed that the nicorandil itself caused the ulceration being seen in the GI tract. We would hypothesise that the effects of nicorandil (either directly or via metabolites) is not in causing Ulceration/Wounds, but in preventing the normal healing mechanisms of healing in a pre-existing wound. Thus explaining why initially non-healing GI tract ulcers were observed (as this is a common site of injury/ulceration), but since then non-healing wounds, in other sites, and even post surgically have been observed.
Conclusion: As the use of nicorandil increases these painful, debilitating, costly, and yet easily treatable wounds will become more common and a high index of suspicion is needed in any patient taking nicorandil, with any non healing wound.

POSTER 11

A prospective trial of near-infrared spectroscopy (NIRS) as a continuous non-invasive method of flap monitoring following breast reconstruction

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Background: In the UK, following mastectomy for Breast cancer, patients are offered a choice of reconstructions, up to and including myocutaneous flap reconstruction. Arterial or venous embarrassment represents a major cause of complications, often requiring intervention or resulting in total flap failure. A system to recognise impending problems would improve salvage rates. Near Infra-red spectroscopy (NIRS) has previously been shown to be a reliable indicator of tissue status in trauma.
Objective: This pilot study examines the use of NIRS as a tool to assist the clinician as a means of monitoring myocutaneous flaps following oncological reconstructive surgery.
Methods: Nineteen patients having myocutaneous flap reconstruction, either as an immediate or delayed reconstruction, were enrolled into the study. Measurements were made using the Inspectra™ $St0_2$ monitor (Hutchinson®), measurements of tissue oxygen saturation ($St0_2$) and Total Haemoglobin Index (THI). Measurements were taken prior to, during, and after the surgery; with post-operative monitoring occurring continuously for 72 hours. Data were correlated with clinical outcome measures.
Results: Out of the nineteen patients, there were two complete flap failures and four complications requiring intervention. In these six cases, problems were identified by either a significant rise in the THI or drop in the St02. One patient's data were unrecordable in recovery, and the patient was therefore returned to theatre immediately for a salvage procedure, which was ultimately successful.
Conclusion: The Inspectra™ $St0_2$ monitor (Hutchinson®) has proved to be a useful aid in the clinical setting. While not replacing the need for experienced and rigorous nursing care, we believe it can be a valuable and objective means of assessing flap viability.

POSTER 12

Wound diagnostics: can a single molecular marker concur with an expert's multifactorial assessment of wound healing status?

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Aims: Diagnostic tests are needed to improve outcomes and health economics in wound care. Ideally, a healing status indicator should provide the highest level of diagnostic accuracy (normally available only from specialised centres of excellence), regardless of the carer's expertise.Mologic has developed a novel rapid protease assay with an indicator molecule biased towards MMPs 9 and 8, to indicate wound healing status. The aim of this study was to assess correlation between the test result and expert clinician judgement.
Methods: Fluids from 10 randomly selected wounds judged to be healing were collected onto swabs, which were stored and shipped to the UK at $-80\,^{\circ}C$. Fluids from another 9 randomly selected wounds judged to be non-healing were processed identically. Samples were coded to ensure blinding to wound identities. Extracts of the samples were assayed by conventional procedures for total protein, MMP 8 and MMP 9 (commercial immunoassays) and with zymography, as well as by the non-optimised prototype novel rapid assay. The results of all the tests were compared with each other and with the expert clinical judgement.
Results: The prototype rapid assay results did not correlate with conventional tests, but they did correlate with the expert judgement. 7 of 9 ''non-healing'' wound samples were protease positive. 7 of 10 ''healing'' wound samples were protease negative and another 3 had reduced protease activity. Under these particular prototype conditions/thresholds and clinical assessment criteria, a sensitivity of 77% and specificity of 80% were achieved.
Conclusion: Appropriate assays can be tuned to correlate well with expert clinical judgement. Further developments will improve correlation.

POSTER 13

Development of a novel growth factor formulation to maintain serum-free and feeder cell-free culture of human embryonic stem cells

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To realise the full clinical potential of human embryonic stem (hES) cells, culture methods that do not involve animal products or purified/non-defined factors must be utilised. This requires removal of animal feeder-cells, serum and any other animal-derived products.
We have utilised a subtractive proteomic approach to analyse the components in culture medium that are produced by the feeder cell layer in the presence and absence of hES cells. We have subsequently used this information to add select candidate factors in recombinant form to develop a serum-free and feeder cell-free culture system for hES cells. We have successfully cultured hES cell lines (BGV01 and HUES2) for over 30 passages using this new serum-free and feeder cell-free formulation. The medium contains low osmolarity DMEM, glutamax, LiCl, β-mercaptoethanol, Lipid concentrate, Trace elements, Activin-A, bFGF, Albucult™ (recombinant human albumin, Novozymes Biopharma UK Ltd.) and an in-house recombinant chimeric protein called VitroGro® (composed of domains of vitronectin linked to IGF-I).
We have shown by immunofluorescence and FACS that hES cells cultured in this serum-free and feeder cell-free culture system express the undifferentiated cell-surface markers SSEA4, TRA1-60 and TRA1-81 and the intracellular marker Oct4. Real-time PCR analysis has revealed that the expression of UTF1, SOX2, FOXD4, OCT-4 and Dppa by these cells does not change more than two-fold compared to cells cultured in the presence of serum and feeder cells. Interestingly, hTERT is down-regulated four-fold and REX1 is down-regulated 75-fold in the cells grown in the serum-free and feeder cell-free system. REX1 has recently been linked to increased differentiation potential; hence the down-regulation of REX1 in these cells may demonstrate their stable pluripotent status. We have recently injected the cells into SCID mice to observe their ability to form teratomas in-vivo and will also present data from these studies.
In conclusion, our serum-free and feeder cell-free media formulation supports the undifferentiated growth of hES cells for over 30 passages and allows hES cells to be cultured in xeno-free, fully defined, synthetic media. The media developed in our laboratory contains very few growth factors and these are used at concentrations much lower than those previously published by others. This in turn substantially reduces the cost of culturing these cells and makes large-scale hES culture for therapeutic applications a realistic possibility for the first time.

POSTER 14

Exploring the application of ultrasound technology in pressure ulcer prevention – an international multidisciplinary team study

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Introduction: High frequency ultrasound (HFUS) can create real-time two-dimensional images of internal structures to examine the first few centimetres depth of sub-dermal soft tissue, thus may have the potential to detect tissue changes in pressure ulcer prevention. However, there is no evidence on the repeatability of HFUS. This study aimed to investigate the inter- and intra-rater repeatability of HFUS scanning.
Methods: In a laboratory based study, 24 healthy subjects (21 females, 3 males; 32.08 ± 12.25 years; BMI 24.94 ± 5.57) participated on two occasions, one day apart. Consenting subjects had points marked (day 1 only) on both heels (lateral, posterior and medial aspects) and seating interface (coccyx, and left and right ischial tuberosities) and scanned by a researcher, then repeated by a second researcher using a HFUS scanner. Both researchers were blinded to each other's scanning. HFUS images were quantitatively and qualitatively analysed blindly by two assessors.
Results: Qualitative visual analysis showed almost perfect agreement between two assessors (0.88 kappa co-efficient). Intraclass correlation coefficients (ICCs) conducted on pixel intensity summation quantitative results showed low inter- and intra-rater repeatability (25% moderate or high ICCs; ICC ≥ 0.6).
Conclusions: Although quantitative analysis showed low inter- and intra-rater repeatability, qualitative analysis showed better agreement. In practice, clinicians qualitatively read the images, and visual analysis appears to be the gold standard in HFUS interpretation. Quantitative methods of analysing HFUS images require further exploration by industry, scientists and clinicians.

POSTER 15

The effect of topical analgesics on human keratinocyte and fibroblast behaviour

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Background: The application of topical analgesics to the donor site of split-thickness skin grafts has been proven to be an effective method of pain management but little is known about their effects on wound re-epithelialisation. This study compares the effect of four analgesics on human keratinocytes and fibroblasts and whole skin explants *in vitro* to determine whether skin cell behaviour and subsequent donor site wound repair is affected by topical application.

Methods: The effect of diclofenac, bupivacaine, lidocaine and ketorolac were studied at concentrations between 10 mM and 1 nM. Their effects on skin outgrowth were investigated using an *ex-vivo* skin explant model. The *in vitro* proliferation of cultured primary human keratinocytes and fibroblasts was also measured.
Results: Keratinocyte outgrowth from the explant model was most inhibited by diclofenac with a significant reduction at 100 μM ($p = > 0.001$). Diclofenac also exhibited the strongest inhibitory effect on cell proliferation especially keratinocytes. Ketorolac was the most cytotoxic. Bupivacaine showed cytotoxicity in a dose-dependent manner with only the very highest concentrations having a significant inhibitory effect. Lidocaine showed no evidence of cytotoxicity at the concentrations tested in either the *in vitro* cell studies or the *ex vivo* explant model.
Conclusions: It is important to ensure that topical analgesics are not used for a prolonged period at concentrations which inhibit cell growth as this may result in a altered cell behaviour and decreased wound healing.

