P205

Development of an ANIMAL FREE culture medium

J. van der Lee[1], **C.n Bengtsson**[2], A. Lindahl[3];
[1]Clinical Chemistry And Transfusion Medicine, Institution of Biomedicine, Gothenburg, Sweden, [2]Department Of Clinical Chemistry And Transfusion Medicine, Institute of Biomedicine Sahlgrenska Academy, Gothenburg, Sweden, [3]Clinical Chemistry And Transfusion Medicine, Biomedicine, Gothenburg, Sweden

Purpose: The need of defined and animal free products in the tissue engineering production is increasing. New regulations in Europe will require pharmacopoeia products. The aim was to produce an animal free culture medium totally based on pharmacopoeia graded chemicals.
Methods and Materials: A culture medium was designed from salt and amino acid composition present in the knee joint, in collaboration with Nidacon International AB. The proliferative capacity of human chondrocytes was tested in monolayer cultures and results compared with standard DMEM/F-12 culture medium. The proliferation rate was assessed by calculating the number of cells at every passage for 4 weeks. The redifferentiation capacity of the cells was tested by culturing the cells in a 3D pellet mass system at different passages. After 14 days, the pellets were processed in order to determine the histoarchitecture.
Results: The new medium resulted in much higher proliferation rate compared to the standard medium, i.e. a ten fold increase in total number of cells after 4 weeks in culture. No difference was seen in redifferentiation capacity of the cells cultured in the new medium compared to the standard medium.
Conclusions: The new pharmacopoeia based medium resulted in much higher proliferation rate compared to the standard medium. The reason for this may be that the new medium is better optimized compared to the media that were developed in the 60´s when the cell culture technique was still under development.

P206

New possibilities of allogeneic chondrocyte transplantation for cartilage repair: an in vitro study of immunological properties of human articular chondrocytes derived from osteoarthritis joints.

S. Abe[1], H. Nochi[2], T. Matsuno[3];
[1]Orthopaedics, Asahikawa Medical College, Asahikawa, HOKKAIDO, Japan, [2]Orthopaedic Surgery, Asahikawa Medical College, Asahikawa, Japan, [3]Orthoopaedic Surgery, Asahikawa Medical College, Asahikawa, Japan

Purpose: Previously we demonstrated that both juvenile and adult healthy human chondrocytes did not stimulate allogeneic reaction, instead, they inhibited the mitogenic activity of poly-clonally activated CD4+T cells (2004, 2006 ORS). Based on our previous findings, the immune privilege is not dependent on only a lack of B7-1/-2 co-stimulatory molecules on chondrocytes surface or low MHC class II expression, but it is also dependent on expression of some inhibitory signals to T cell proliferation. The purpose of this study is to determine whether these immunological properties of articular chondrocytes change by aging or degeneration and whether allogeneic chondrocyte transplantation is immunologically safe.
Methods and Materials: Human articular chondrocytes were isolated from osteoarthritic knee joints aged 61-85. To determine the allogeneic reaction of chondrocytes, a MLR assay was performed. To clarify the reaction of CD8+T cells against chondrocytes, a cytotoxic assay was performed. Lastly, a proliferation assay in mitogenic activating CD4+T cells was performed with or without chondrocyte contact.
Results: The chondrocytes failed to stimulate the proliferation of allogeneic PBMCs in the MLR assay. The chondrocytes expressed MHC class I antigen in the flow cytometric analysis, but they did not elicit allogeneic reactions of MHC class I restricted CD8+T cells, as well as class II restricted CD4 T cells. The chondrocytes inhibited the proliferation of activating CD4+T cells by cell-to-cell contact.
Conclusions: This study indicates chondrocytes keep their immunological properties independent of their aging and osteoarthritic condition. As regards recent reports, chondrocytes seem to have immunological properties similar to mesenchymal stem cells. These data translate into possibilities for allogeneic chondrocyte transplantation derived from osteoarthritic joints.

P207

Repair of large full-thickness articular cartilage defects by transplantation of autologous uncultured bone-marrow-derived mononuclear cells

F. Chang[1], T. Ishii[2], T. Yanai[2], H. Mishima[2], H. Akaogi[2], K. Ogawa[2], N. Ochiai[2];
[1]Orthopaedic Surgery, Institute of Clinical Medicine, Graduate School of Human Comprehensive Sciences, University of Tsukuba, Tsukuba, Japan, [2]Orthopaedic Surgery, University of Tsukuba, Tsukuba, Japan

Purpose: To investigate the feasibility of autologous uncultured bone-marrow-derived mononuclear cell transplantation in large full-thickness cartilage regeneration.
Methods and Materials: After fixing with a hinged external fixator, the entire surface of the left tibial plateau was resected and large full-thickness cartilage defects were formed in 48 rabbits. Animals were divided into four groups: autologous uncultured bone-marrow-derived mononuclear cells with fibrin gel (BMC), autologous uncultured peripheral-blood-derived mononuclear cells with fibrin gel (PBC), fibrin gel alone (GEL), or nothing (CON) transplanted to the articular cavity 7 days after the operation. The rabbits were killed 8 or 12 weeks after the operation. The repair of defects was investigated histologically and scored using a histological and histochemical grading scale that was modified from the International Cartilage Repair Society Visual Histological Assessment Scale. To evaluate the regenerated cartilage, we also morphometrically measured the staining area positive for Safranin-O or type-II collagen and calculated the percentages of the positive staining areas with respect to the regenerated soft tissue area.
Results: Histological findings showed that the BMC group had superior cartilage repair compared with the other groups and that the PBC and CON group showed better cartilage repair than did the GEL group. Histological scores and morphometrical measurements also showed the same results quantitively.
Conclusions: The transplantation of autologous uncultured bone-marrow-derived mononuclear cells contributes to articular cartilage repair. The easy and safe method used in this study is potentially viable for clinical application.

P208

Histological and biomechanical assessment of cartilage repair by a scaffold-free 3D bioengineered tissue (3DBT) derived from synovial MSCs

W. Ando[1], K. Tateishi[2], D. Katakai[3], M. Imura[4], Y. Take[5], K. Nakata[2], H. Fujie[6], J. Hashimoto[2], K. Shino[2], H. Yoshikawa[7], N. Nakamura[8];
[1]Department Of Orthopedics, Osaka University Graduate School of Medicine, Suita, Osaka, Japan, [2]Department Of Orthopedics, Osaka University, Suita, Osaka, Japan, [3]Biomechanics Laboratory, Department Of Mechanical Engineering, Kogakuin University, Tokyo, Japan., Hachioji, Japan, [4]Biomechanics Laboratory, Department Of Mechanical Engineering, Kogakuin University, Hachioji, Japan, [5]Orthopedics, Osaka University Guraduate School of Medicine, Suita, Japan, [6]Biomechanis Laboratory, Department Of Mechanical Engineering, Kogakuin University, Hachioji, TOKYO, Japan, [7]Department Of Orthopedics, Osaka University, Suita, Japan, [8]Orthopaedics, Osaka University Graduate School of Medicine, Osaka, Japan

Purpose: We have developed a novel scaffold-free 3D bioengineered tissue (3DBT) composed from MSCs and their synthesized extracellular matrix. The 3DBT is free of xeno-derived materials and has good adhesive properties to cartilage matrix and chondrogenic differentiation potential. The purpose of this study was to quantitatively assess the quality of repaired chondral lesion with the 3DBT from morphological and biomechanical aspects.
Methods and Materials: Porcine synovial MSCs cultured with ascorbic acid were detached from the substratum by the application of shear stress at the cell-substratum interface. The detached cell/matrix complex was allowed to contract to develop the 3DBT. The 3DBT was implanted at a 8.5mm diameter, 2mm deep chondral defect at medial femoral condyle of four-month-old pig without any reinforcement with fixation device including suture. In the control group, the defect was left untreated. The repaired tissue was evaluated histologically including the modified ICRS score, and biomechanically by static compression test and friction test, at 6 months postoperatively.
Results: Implantation of a 3DBT without ex vivo chondrogenic manipulation prevented progression to osteoarthritic changes with repair by a chondrogenic-like tissue, as well as secure biological integration to the adjacent cartilage. Histologically, most area of repaired tissue was positively stained with Safranin O and collagen II. In histological scoring, all criteria of the 3DBT treated group were significantly better than those for the untreated group. Biomechanical evaluation revealed that repaired tissue exhibited similar mechanical properties to normal cartilage.
Conclusions: Based on these results, scaffold-free, 3DBT technology could be a unique and promising method to promote MSC-based cartilage repair.