mal joints (6.1% vs. 2.2%), corresponding to the highest percentage of cellular expression to the co-expression of CD90/CD271 (9.8% vs. 2.6%). Spontaneous repair tissue contained cells positive for CD44 (12.3%), CD90 (10.4%) and CD271 (9.9%) antigens, but the CD105 antigen was absent.

**Conclusions:** These results suggest that during the OA process an increment of MSC percentage occur in synovial membrane. Absent expression of the CD105 antigen in the OA cartilage repair indicates that cells expressing this marker may be necessary for effective repair processes in OA cartilage.

This study was supported by grants from Secretaría Xeral de I+D, Xunta de Galicia PGIDIT05SAN08PR, PGIDIT04PXIC91602PN, PGIDIT04PXIC91601PN, FISICP03/00127 and FIS05/2495; Fundación Española de Reumatología.

**177**

**EXPRESSION ANALYSIS OF mRNA DURING DIFFERENTIATION FROM MSCS TO MATURE CHONDROCYTES**

C. Cicione 1, S. Diaz-Prado 1,2, E. Muñños 1, I.M. Fuentes 1,2, F.J. De Toro 1,2, F.J. Blanco 1

1Osteoarticular and Aging Res. Lab., CIBER-BBN, Rheumatology Div., INIBIC-Complejo Hosp. Univ. A Coruña, A Coruña, Spain; 2INIBIC-Univ. of A Coruña, A Coruña, Spain

**Purpose:** Mesenchymal stem cells (MSCs) are considered as a potential cell therapy to treat a variety of rheumatic diseases. MSCs are widely distributed in the body, including bone marrow (BM). Chondrogenic differentiation process involves the differentiation of MSCs towards cartilage or bone. Cartilage tissue engineering uses efficient protocols that suppress differentiation towards hypertrophic chondrocytes, providing a useful in vitro model for studying the gene regulation of chondrogenesis and cartilage development. This model may provide new molecular targets for rheumatic diseases treatment. The aim of this study was investigate the transcriptional regulation of genes implicated in the different steps of chondrogenesis in normoxia versus hypoxia conditions.

**Methods:** MSCs, isolated from 5 BM were expanded in monolayer. CD105+ and CD105- cells were separated from total BM by MACS Technology (Miltenyi Biotec, Spain) and their phenotype were characterized by flow cytometry. The three populations of MSCs (total BM, CD105+ and CD105- BM) were subjected to chondrogenesis by means of high-density 3D aggregate culture in normoxia and hypoxia conditions. Chondrogenesis was studied at different intervals of time: 0, 2, 4, 7, 14, 21, and 28 days and differentiation was confirmed by histochemistry (hematoxylin-eosin, Masson's trichrome, alcin blue, safranin O), immunohistochemistry (types II and I collagens, and aggrecan) and quantitative real Time PCR (qPCR) techniques. For qPCR experiments RNAs were isolated using Trizol reagent (Invitrogen, Spain). Different primers were designed to study mRNA expression of different genes implicated in the different stages of chondrogenesis (MSCs, condensation & chondroprogenitors, chondrocytes and prehypertrophic chondrocytes).These primers included Sox2, Nanog, OCT3/4, CDH2, TNC, Sox9, Agg, Col I, Col II, Col X and RunX2.

**Results:** At the starting point of the experiments, the stem cell phenotype markers (SOX2, Nanog and OCT3/4) showed higher expression levels in the CD105+ population. At 14-28 days in hypoxia and at 21-28 days in normoxia the expression levels of Nanog and OCT3/4 showed to be higher in the total bone marrow and CD105+ populations. Instead, the level of SOX2 at the same times of the experiment showed to be higher in the CD105+ population. These markers remained expressed until day 28, both in normoxia and in hypoxia, indicating that not all the stem cells were differentiated towards chondrocytes but some of them remained in a state of indifferentiation. With regard to cartilage specific markers, the CD105- population showed increased levels of SOX9 and AGG mRNA than CD105+ and total bone marrow ones, however the latter showed the highest expression levels of Col II followed by CD105- and CD105+ populations. In this way, the overexpression of Col II in hypoxia was accentuated between 7 and 21 days while in normoxia took place from 14 to 28 days.

**Conclusions:** Hypoxia accelerates the chondrogenic differentiation process from bone marrow-derived adult mesenchymal stem cells to mature chondrocytes. Isolation of CD105+ and CD105- populations reveals no improvement in chondrogenesis with regard to the total bone marrow population.In addition, while the CD105+ population seems to differentiate in the experimental conditions, the CD105- one shows to remain in an indifferentated state, maintaining an important expression of SOX2, NANOG and OCT3/4 genes.


**178**

**AN ORGAN CULTURE MODEL TO STUDY BIOLOGICAL REPAIR OF THE DEGENERATE INTERVERTEBRAL DISC**

L.A. Haglund 1, J. Moir 1, B. Jim 1, P.J. Roughley 2, T. Steffen 1

1Orthopaedic Res. Lab., McGill Univ., Montreal, QC, Canada; 2Shriners Hosp., McGill Univ., Montreal, QC, Canada

The current surgical procedures used to treat the degenerate intervertebral disc (IVD) are inadequate. Vertebral fusion can frequently reduce back pain, but does not preserve motion segmental mobility and thus may lead to subsequent degeneration in adjacent motion segments. In contrast, artificial disc prostheses preserve mobility, but in the long-term may result in serious complications due to wear, loosening, subsidence or mechanical failure. Biological repair of the degenerate IVD could provide a means of overcoming these problems. In vitro organ culture models using large IVDs have recently been described for this purpose, but they have suffered from the inability to achieve long-term NP cell viability, due to impaired nutrient supply because of endplate calcification and the postmortem formation of blood clots blocking the trans-endoplate nutrition path.

**Purpose:** To develop a bovine coccygeal IVD organ culture model of disc degeneration with long-term cell survival, in which induced biological repair can be studied.

**Methods:** IVDs were isolated from bovine tails by parallel cuts through the adjacent vertebral bodies, and remaining bone and most of the cartilage endplates were removed using a high-speed bone burr. The explants were maintained in culture in DMEM, supplemented with 5% FBS and antibiotics, with medium changed twice weekly. Degeneration was induced by injecting trypsin into the center of the NP. Cell viability was monitored in a 1 mm slice through the center of the disc, using a live/dead fluorescence assay (Live/Dead®). Tissue morphology was examined by histology. Annulus fibrosus (AF) and nucleus pulposus (NP) were extracted using 15 volumes 4M GuHCl containing protease inhibitors, and changes in the abundance and degradation of chondroadherin and fibromodulin were analysed by immunoblotting. GAG content was measured using the DMMB assay.

**Results:** Intact bovine discs maintained in free swelling culture with no external load applied demonstrated cell viability for up to 6 weeks. The amount of GAG retained in the tissue decreased slowly during the culture period and cell clustering reminiscent of cartilage and disc degeneration was found. As it is not practical to wait for 4-6 weeks before repair potential of various treatments remaining in a state of indifferentiation.
can be studied, a model to minimise the time needed to achieve a reproducible mild degeneration with maintained cell viability was developed. Degeneration was induced by injecting trypsin into the center of the NP after a 48 hours pre-culturing period. The discs were then maintained in culture for up to 14 days. The discs maintained cell viability, and showed depletion of proteoglycans as early as 4 days after trypsin injection. Histology revealed cell proliferation and clustering in areas of the NP as the proteoglycan content decreased. Only a minor effect was found on the collagen-binding proteins, chondroadherin and fibromodulin, at 14 days.

Conclusions: We have developed an aseptic method to isolate bovine coccygeal discs from tissue freshly procured from the slaughterhouse and maintain them in long term organ culture. We have also developed a trypsin-induced IVD degeneration model without compromising cell viability. The proteoglycans are largely depleted as early as 4 days post injection, and cell proliferation and clustering reminiscent of disc degeneration is apparent at later times. Only minor evidence of degradation of chondroadherin and fibromodulin was found, suggesting maintenance of the collage- nous framework of the tissue. This organ culture system has the potential to be a very useful tool to study the efficacy of IVD repair by biochemical stimuli.

179

DEMONSTRATION OF A NOVEL BIOSCAFFOLD SUITABLE FOR USE IN CARTILAGE TISSUE ENGINEERING THAT SUPPORTS CHONDROCYTE PHENOTYPE

A. Volk, M. Kim, G.R. Dodge
Univ. of Pennsylvania, Philadelphia, PA

Purpose: Producing an engineered biomaterial with native cartilage-like properties is crucial for functional cartilage repair. Many materials have been explored for their suitability for provid- ing a scaffold supporting cell growth, including various polymers, peptide hydrogels, collagen, and agarose. Microbial bio cellulose (BC), a natural polysaccharide that can be modified in both shape and resorbability, is of great interest for tissue engineering applications. We combined our self-aggregating suspension culture (SASC) approach with a free-floating BC scaffold and tested the ability of articular chondrocytes to interact and maintain their phenotype in culture. The scaffold was a biosynthesized form of cellulose produced by the bacteria Acetobacter xylinum, which provided a scaffold with significant fluid holding capacity, tensile strength, and shape retention. Such properties can provide an im- proved natural alternative to synthetic materials used for clinically relevant tissue repair applications. The objective of this study was to determine the biocompatibility of the BC with articular chondrocytes, as well as the sustained growth, viability and phenotype of the chondrocytes on and in various forms of BC.

Methods: Articular cartilage were removed from pigs or equine. Chondrocytes were plated at densities of 2 × 10⁶ cells/ml to 2 × 10⁷ cells/ml in poly-HEMA coated culture plates with free- floating bioscaffold as supplied by Xylos Corp. BC variables in- cluded matrix density and degree of chemical modification. Some material was physically modified to determine if the cellulose fibers/lamellae arrangement influenced cell growth. Cultures were maintained for 3 weeks, and uncultured chondrocytes were eval- uated as a control. Quantitative real-time PCR analysis was used to characterize expression (mRNA) levels of genes that are constit- utively expressed in the extracellular matrix of articular cartilage, type II collagen, aggrecan, and cartilage oligomeric matrix protein (COMP), as well as genes encoding proteins that phenotypically uncharacteristic, such as type I collagen. Analysis of relative gene expression used the 2^(-ΔΔCT) method.

Results: Chondrocytes attached to the BC and produced matrix around and in the scaffold. This occurred according to the density of the cellulose, in which case cells either attached around the scaffold in the densely packed material or filled in the loosely packed cellulose. The results showed the BC was supportive of cell growth and facilitates matrix interactions. The rounded appear- ance and extensive matrix surrounding the cells were consistent with the morphology of chondrocytes in hyaline cartilage. Using conventional and quantitative real time PCR showed levels of mRNA for type II collagen, Aggrecan and COMP in parallel with uncultured chondrocytes and consistent with hyaline cartilage. Levels expression for type II collagen and aggrecan were higher compared to scaffold-free cultures, and approached levels close to uncultured cells.

Conclusions: This study was to determine the compatibility of BC scaffold with chondrocytes and whether this unique and readily modified polysaccharide would support chondrocyte phenotype and gene expression. Results demonstrated chondrocytes quickly interacted with the BC and the biocompatibility of the BC scaffold was supported by the microscopic assessments, cell morphology, and gene expression. Gene expression profiles were consistent in chondrocytes cultured with the bioscaffold as compared to uncultured and our SASC model. Gene expression analysis did not show a change towards a hypertrophic chondrocyte or fibroblastic-like phenotype. These results provide a basis for developing BC as a biomaterial for cartilage repair.

180

PHENOTYPIC ANALYSIS OF CELL SURFACE MARKERS AND GENE EXPRESSION OF HUMAN MESENCHYMAL STEM CELLS DURING MONOLAYER EXPANSION

L. Galois¹, C. Cournil-Henriquet², Y. Wang³, C. Huselstein³, D. Mainard¹, D. Bensousan⁴, P. Netter², J. Stoltz³, S. Muller³, P. Gillot¹, A. Watrin-pinzano²
¹Dept. of Orthopaedic Surgery, nancy, France; ²UMR CNRS 7561, nancy, France; ³UMR CNRS 7563, nancy, France; ⁴UTCT, CHU Nancy, France

Purpose: Both chondrocytes and mensenchymal stem cells (MSCs) are the most used cell sources for cartilage tissue engi- neering. However, monolayer expansion to obtain sufficient cells leads to (1) the rapid dedifferentiation of chondrocytes and, con- comitantly, (2) the reduced ability of MSCs to differentiate into chondrocytes, thus limiting their application in cartilage repair. The aim of this study was (1) to investigate the influence of the monolayer expansion on the phenotype and the gene expression profile of both cell types, and (2) to find the appropriate compro- mise between monolayer expansion and the retaining structure of chondrogenic characteristics.

Methods: Human chondrocytes, isolated enzymatically from femoral head slice, and human MSCs, derived from bone mar- row, were maintained in monolayer culture up to passage 5. The expressions of cell surface markers (CD34, CD45, CD73, CD90, CD105, CD166) and several chondrogenic-related genes for each passage (P0 to P5) of those cells were then analyzed using flow cytometry and quantitative real-time PCR, respectively.

Results: Flow cytometry analyses showed that, during the mono- layer expansion, some qualitative and quantitative regulation occur for the expression of cell surface markers. Chondrocyte expression pattern is similar to those for MSCs. A rapid increase in mRNA expression of type I collagen and aggrecan occurs whereas a significant decrease of type II collagen and sox 9 was observed in chondrocytes through the successive passages. On the other hand, the expansion did not induced obvious change in MSCs gene expression.

Conclusions: In conclusion, our results suggest that passage 2 might be the up-limit for chondrocytes in order to achieve their subsequent redifferentiation in 3D scaffold. Nevertheless, MSCs