**P155**

**Differentiation capacity of mesenchymal progenitor cells from mild and severely affected osteoarthritic cartilage**

S. Fickert1, I. Sperling1, W. Schwab1; 1Department Of Orthopaedic Surgery, University Hospital of Mannheim, Mannheim, Germany, 2Department Of Orthopaedic Surgery, University of Dresden, Dresden, Germany, 3Dept. Of Anatomy, University of Dresden, Dresden, Germany

**Purpose:** Mesenchymal progenitor cells (MPCs) from bone marrow are able to differentiate in various phenotypes of connective tissue including cartilage, bone and adipose tissue (1-2). This led to a more precise characterization by analysis of cell surface markers and differentiation-related gene expression (3-4). In parallel it was recognized that MPC not only reside in bone marrow, but also in various other connective tissues like periost, adipose, or muscle tissue (5-8). Although in osteoarthritic cartilage cellular subpopulations with characteristics of MPC was described by fluorescence automated cell sorting (FACS). Aim of this study is to compare the chondro- and osteogenic capacity of chondrocytes from unaffected and severely destroyed regions of OA joints.

**Methods and Materials:** Cells of 6 patients with end stage OA that underwent total knee joint replacement were enzymatically isolated from unaffected, Grad 0 or 1 according to Outerbridge, or affected regions (Grad III). One aliquot was directly analyzed by FACS using various combinations of surface markers of MPC (CD9, CD44, CD54, CD90, CD166) and haematopoetic lineage (CD34, CD45, CD133). Remaining cells were cultivated, expanded over P1-3 and analyzed at each passage by FACS again. Of the cells isolated for their osteo- and at P3 for chondrogenic potential by using established differentiation protocols. The differentiation was analyzed by immuno-/histochemistry, by alkaline phosphatase (AP)/protein assay for osteogenic and GAG/DNA assay for chondrogenic differentiation.

**Results:** Using FACS analysis we could show that single staining of CD9, CD44, CD54, CD73, CD90, CD105 and CD166 positive cells within intact and severely degenerated cartilage from OA patients does not vary. The total quantities of single positive cells from both regions of OA joints decreased for CD 9 (fig. 1). The FACS analysis of CD34+, CD45+, CD133 (1 or 2)+ showed less than 1% staining. Under expansion and followed osteogenic differentiation the relative amount of AP activity markedly increased from passage 1 to 3 (fig. 2). The AP activity of cells from affected regions of OA joints was higher compared to unaffected. At passage 3 chondrogenic differentiation was performed for both with micromass culture over 3 weeks. The GAG content was higher in cells from intact cartilage (n=6, 270.9 microg/microg, +/- SD 82) compared to cells from severely affected cartilage (n=6, 245 microg/microg, +/- SD 55).

**Conclusions:** These results confirm that cartilage from OA patients contains cells that express typical combinations of MPC surface markers and have the potency of osteo- and chondrogenic differentiation. Besides this it seems that OA cartilage hostes cells which are different in their potential to osteo- and chondrogenic differentiation. This cartilage-derived mesenchymal progenitor cells may play different roles during regenerative process depending from the stadium of arthritic diseases.

---

**P156**

**Effects of autologous chondrocyte-seeded type II collagen scaffolds on repair of chondral defects in a caprine model.**

S. Vickers1, T. Gotterbarm2, D. Zhang3, T. Schmid4, H. Hsu5, M. Spector3; 1Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA, United States of America, 2Orthopaedic Surgery, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, United States of America, 3Division Of Biochemistry, Rush University Medical Center, Chicago, United States of America, 4Orthopedic Surgery, Brigham and Women’s Hospital, Harvard Medical School, Boston, United States of America

**Purpose:** The objectives of this study were to begin to test the effects of tissue-engineered cartilage implant compositional maturity (viz., glycosaminoglycan, GAG, content) on chondral defect repair in a caprine model, and to evaluate the presence of proteoglycan 4 (PRG4; referring to the lubricants, lubricin and superficial zone protein) in reparative tissue in the implanted and untreated defects.

**Methods and Materials:** Monolayer-expanded chondrocytes from 8 adult goats were seeded into type II collagen (Geistlich Biomaterials, Wolhusen, Switzerland) scaffolds and cultured in chondrogenic medium. By 2 weeks, GAG content in synthesized tissue within these constructs reached 30% of that in native caprine cartilage. Two chondral defects created in the trochlear groove of one stiffe joint of each animal were implanted with the autologous chondrocyte-seeded constructs (n=6 animals), or left empty as untreated controls (n=2 animals). After 16 weeks implant sites were analyzed histomorphometrically for type and amount of reparative tissue, and stained immunohistochemically for PRG4.

**Results:** Reparative tissue consisted primarily of fibrocartilage. Implantation of the cell-seeded constructs reduced the amount of fibrous tissue formation compared to untreated controls, but did not demonstrate statistically significant effects on other outcome variables. PRG4 was observed in the superficial zone of repair tissue from both implanted and untreated chondral defects.

**Conclusions:** Continuing investigations will evaluate effects of constructs with GAG contents 50% and 75% of normal cartilage on repair. Identification of PRG4 demonstrates the ability of cells within the repair tissue to synthesize this lubricating molecule, which may be of importance to the tribological function of the repair tissue.