chondrogenic phenotype of the MSC was assessed following short-term (1 week) culture.

Results: Addition of rAC to the culture media of rat articular chondrocytes led to elevated expression of Sox-9, collagen IIA1, FGF2, TGF beta-1 and several other chondrogenic markers. Similar results were obtained for cells grown in monolayer cultures or 3-D collagen scaffolds. Elevated Sox-9 and collagen IIA1 expression also was observed following rAC treatment of human chondrocytes from a patient with osteoarthritis. No changes in the total cell numbers were observed despite these positive changes in the chondrogenic phenotype, indicating that the primary effect of rAC was on the differentiation capacity of the cells, rather than on cell survival. We also evaluated the effect of rAC on rat bone marrow grown for 1 week, and found that the number of MSC in these cultures was increased ~2–3-fold as assessed by colony forming units (CFU-F) and flow cytometry, and that their chondrogenic phenotype was positively influenced as well.

Conclusions: Based on these results we suggest that AC is an important enzyme required to maintain the differentiated phenotype of primary chondrocytes, and that supplementation of media with rAC results in improved chondrocyte quality following ex vivo expansion. rAC also improves the yield of bone marrow-derived MSC from short-term cultures, and their chondrogenic phenotype as well. rAC may therefore be an important reagent that can be used to improve the production of chondrocytes for cartilage repair, either ex vivo or through direct administration to damaged cartilage sites.

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DETAILED EVALUATION OF CHONDRAL DEFECT REPAIR AND AUTOLOGOUS BONE MARROW DERIVED MESENCHYMAL CELLS TRANSPLANTATION. A NONHUMAN PRIMATE MODEL

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Purpose: Articular cartilage injury remains one of the major concerns in orthopaedic surgery. Although the repair process of articular cartilage defects has been studied in many species. The extent and time course of articular cartilage defect healing in humans are not well described.

Mesenchymal stem cells (MSCs) are an important cell source for cartilage regeneration. A number of successful results in transplantation MSCs into cartilage defects have been reported in animal models. However, Clinical studies of MSCs transplantation for cartilage repair were very few. Physicians not much know efficacy and safety of MSCs transplantation for cartilage repair in clinical.

Evaluation of the status of articular cartilage repair and MSCs transplantation for articular cartilage repair at different time points in a primate model may provide a healing process, efficacy, safety and postoperative activity recommendations in clinical.

Methods: Full-thickness osteochondral defects were created on the medial femoral condyles(5 mm in diameters and 5 mm in depth) and trochlea(3 mm in diameters and 5 mm in depth) of 18 cynomolgus macaques, and the animals were divided into three groups: MSC, Gel, and Defect. In the MSC group, the defects were filled with bone marrow-derived MSCs embedded in collagen gel. In the Gel group, the defects were filled with collagen gel without cells. In the Defect group, the defects were left empty and additional defect were created on the lateral femoral condyles(2 mm in diameters and 5 mm in depth). All groups were evaluated by gross and histologic examination at 6-, 12- and 24-weeks.

Results: In the defect group, Cartilaginous repair responses failed to occur in the lager 3 mm defects, which was covered only by fibrous scar tissue. In contrast, hyaline-like articular cartilage was regenerated by 24 weeks in 2 mm defects. In the MSC group, the border between bone and cartilage moved upwards, integrations between native cartilage and regenerated tissue were improved. After 24 weeks, histological scores of the MSC group improved and were better than those of two other control groups.

Conclusions: 2 mm diameter full-thickness cartilage defect whose size were critical for primate knees. In the primate animal model, significant improvements in the extent and quality of cartilage repair were observed from the 12- to 24-week time points after transplantation of MSCs.

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PROLIFERATIVE AND DIFFERENTIATION POTENTIAL OF STEM CELLS DERIVED FROM THREE MESENCHYMAL TISSUES IN LATE STAGE OSTEOARTHRITIC PATIENTS

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Purpose: To asses the presence and biological potential of stem cells in three mesehchymal tissues (subchondral bone, synovial layer, periarticular adipose tissue) in late stages osteoarthritic patients, subjects of total knee replacement (TKR)

Methods: Samples were collected from patients undergoing TKR, plastic adherent cells cultured in complete αMEM with FGF β for succesive passages: cell morphology and growth potential was recorded. Flow cytometric surface cell markers detection for stemness antibodies was performed. Differentiation assays for three mesenchymal lineages (osteogenesis adipogenesis chondrogenesis) was assesed by qualitative and quantitative method. Time lapse life cell imaging of nondiferentiated cells over 24 hours period was used to determine cell kinetics.

Results: Mesenchymal cells derived from all donors and tissue types displayed morphology and growth potential of MSCs, were positive for stemness related antibodies (CD 105, CD 73, CD 90), underwent differentiation toward three lineages with significant differences between tissue of origin and not between donors. Cell kinetics, recorded for one donor, was different for adipose derived, synovial derived and trabecular bone derived MSCs, a paralel could be made between growth kinetics and recorded cell speed.

Conclusions: Late stage OA derived human tissues (subchondral trabecular bone, extraarticular adipose tissue and synovial layer contain phenotypically different MSCs which have different growth and differentiation potential which could be related with the pathogeny and progression of OA, further to be investigated; these tissues can be conssidered as cell source for regenerative therapies.

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TGF-β TYPE II RECEPTOR AS A NOVEL INDICATOR OF CHONDROGENESIS IN EQUINE BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

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Purpose: Articular cartilage lacks the essential components for self-repair, often resulting in inadequate healing of isolated cartilage injury, which can lead to degenerative osteoarthritis (OA). Designing techniques to improve cartilage repair after injury may prevent or delay development of OA. Bone marrow-derived mesenchymal stem cells (MSC) are a promising cell source for articular cartilage regeneration. *In vitro*, MSC have been shown to undergo chondrogenic differentiation in the presence of transforming growth factor β1 (TGF-β1), via a signaling pathway known to play a key regulatory role in chondrogenesis. Various knockout studies suggest that the TGF-β type II receptor (TβR-II) is specifically important in skeletal muscle formation and bone morphogenesis, but its role has not been clearly defined in chondrogenesis. The purpose of this study is to test the hypothesis that levels of TβR-II can predict the cellular responsiveness of equine bone marrow-derived MSC to TGF-β1 and are related to subsequent chondrogenic potential.

Methods: Bone marrow aspirate was collected from the sternum of 9 horses and MSC were isolated and expanded in culture until passage two. To evaluate chondrogenesis, equine MSC were cultured as pellets in chondrogenic medium with or without addition of TGF-β1 (10 ng/ml). At Day 21, pellets were collected and pellet area was measured using a stereomicroscope. Pellets were paraffin-embedded, sectioned and stained with safranin-O/fast green for sulfated glycosaminoglycans (GAG). The Bern score was used for histological grading with a higher score indicating higher chondrogenesis. Presence of TβR-II was analyzed by immunofluorescence (K105, Cell Signaling Technology) and five to seven regions of each pellet were imaged with fluorescence microscopy at 40X. The TβR-II intensity of each region was quantified with Visiopharm image analysis software and normalized to the number of cells. Comparisons between groups were performed using paired t-tests and correlations were evaluated by Pearson's correlation. Statistical analysis was performed using SPSS and $p < 0.05$ was considered significant.

Results: Pellets cultured in chondrogenic medium supplemented with TGF-β1 had a greater pellet area than those cultured in chondrogenic medium only ($p < 0.001$). The addition of TGF-β1 to the culture medium led to positive safranin-O staining and higher Bern scores compared to pellets cultured without TGF-β1 ($p < 0.001$). TβR-II intensities were greater in pellets stimulated with TGF-β1 than in unstimulated pellets ($p = 0.043$). Overall, positive correlations were observed between TβR-II total intensity and measured pellet area ($p = 0.033$), as well as between TβR-II intensity and BERN score ($p = 0.037$).
Conclusions: The results from this study indicate that equine MSC are responsive to TGF-β1 and can undergo chondrogenic differentiation. This was observed by the increase in pellet area and higher Bern scores in the presence of TGF-β1. In addition to these commonly reported measures, this study also evaluated the intensity of TβR-II following *in vitro* chondrogenic differentiation and showed that it increased in the presence of TGF-β1. This increase suggests that TβR-II may be an indicator of cellular responsiveness to TGF-β1. The positive correlation observed between TβR-II levels and BERN scores indicates that TβR-II expression is important in chondrogenesis and may be a novel predictor of MSC chondrogenic potential. Using TβR-II as an indicator of chondrogenic potential thus warrants further investigation as it may lead to improved cell-based therapies for articular cartilage repair, which may delay or prevent the development of OA after injury.

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IS THE SECOND MICROFRACTURE STILL USEFUL FOR REPAIR OF ARTICULAR CARTILAGE DEFECT?

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Purpose: Microfracture (MFx) is considered as the first-line treatment for full thickness cartilage lesion because of its minimal invasiveness, technical easiness and high cost-effectiveness. Owing to its advantages, it can be postulated that MFx can be repeated in spite of time dependant poor long-term follow-up result. The purpose of this study was to evaluate whether the second MFx could effectively repair the cartilage defect which was generated at the spot with previously MF being bone.

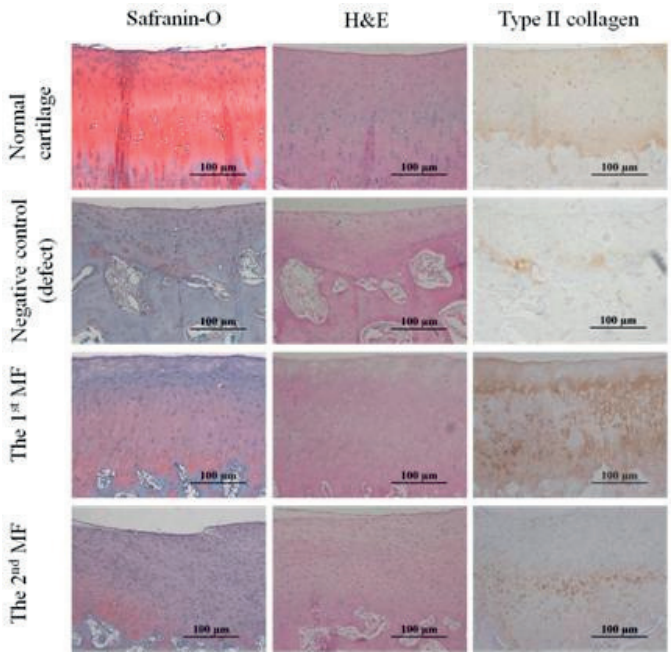

Fig. 1. Histologic evaluation of the repaired tissue 8 weeks after MFx. (A,E,I) normal, (B,F,J) control, (C,G,K) first MF, (D,H,L) second MF group.

Methods: Thirty-six New Zealand white rabbits were divided into 3 groups: (1) untreated full-thickness chondral defect, (2) treated with MFx and (3) the second MFx. The number of mesenchymal stem cells (MSCs) derived from bone marrow was evaluated by colony forming unit (CFU) assay. The repaired cartilage was evaluated 8 weeks after the first and second MFx. Cartilage evaluation was done by histology and biochemical assay and subchondral bone was analyzed by micro-CT.
Results: There was no significant difference in the colony formation between the first MFx and second MFx. The repaired cartilage after the second MFx was comparable with that after the first MFx in terms of histologic score and biochemical results even with slightly lower density of GAG and type II collagen. Subchondral bone remained severely damaged in both the first and second MFx.
Conclusions: The second MFx showed comparable results to the first MFx in spite of tendency to form lower quality of repaired cartilage in histologic study.

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CHONDROGENESIS DIFFERENTIATION OF MESENCHYMAL STEM CELLS FROM HUMAN AMNIOTIC FLUID CULTURED WITH TGF BETA 3

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Purpose: Recently, adult mesenchymal stem cells (MSCs) have been focused as an alternative source of cells for cartilage repair. It is accepted that amniotic fluid is a new source of MSCs, and has characteristics similar to embryonic stem cells which makes them a potential source for cell differentiation. Cellular condensation is a required step in the initiation of mesenchimal chondrogenesis. The aim of this study was to differentiate cells from amniotic fluid into chondrocytes in high density micromass culture to evaluate the expression of type II collagen.
Methods: Amniotic fluid from 44 pregnat women was harvested in the second semester. The samples were set in flasks and cultured. Adherent cells were selected and expanded until 4th passage to obtain the appropriate number of cells. The cells were analysed by flow cytometry and after this process, they were platted in high density micromass culture system, remaining under this condition by 3 weeks in Dulbecco's Modified Eagle Medium (DMEM) with high glucose and transforming growth factor-beta-3(TGF-beta 3)in a final concentration of 10 ng/ml in micromass culture. After 21 days, cell differentiation was verified by western blotting analysis of the secreted collagen II protein in the culture medium.
Results: Through flow cytometry, expanded cells showed typical cell surface antigens found in mesenchymal stem cells, such as positivity for CD90, and negativity for antigens found in haematopoetic lineage. After 21 days in high density micromass culture with TGF beta-3 containing medium, the expression of type II collagen was observed and confirmed by western blotting. fluid cells into chondrocytes.
Conclusions: The authors showed amniotic fluid MSCs can be differentiated in articular chondrocytes under the TGF-beta-3 stimuli, therefore can be used as a reliable source of MSCs.

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COMP BINDS TRANSFORMING GROWTH FACTOR BETA FAMILY LIGANDS TO ENHANCE THEIR ACTIVITY

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Purpose: Cartilage oligomeric matrix protein (COMP) is an important protein essential for the formation and maintenance of the structural integrity of cartilage matrix. COMP is a homo-pentamer in which each monomer comprises an N-terminal coiled-coil domain, epidermal growth factor (EGF) – like repeats, thrombospondin (TSP)-3 like repeats and a thrombospondin C-terminal domain. Owing to its repeated modular structure, COMP can assemble various extracellular matrix components like collagens and proteoglycans. We hypothesized that if COMP were to bind to growth factors, it could affect growth factor activity by controlling how and when the growth factors are presented to the cell surface. The aim of this study was to examine the mechanism and effect of binding