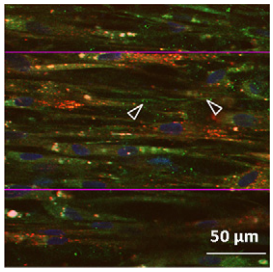

Fig. 1. Human MSCs were cultured for 21 days within collagen channels of 100 micrometers. Immuno-reactivity for collagen type II (green), 4′,6-diamidino-2-phenylindole (DAPI) counterstain of the nuclei (blue) Dil stain for cell membrane (red).

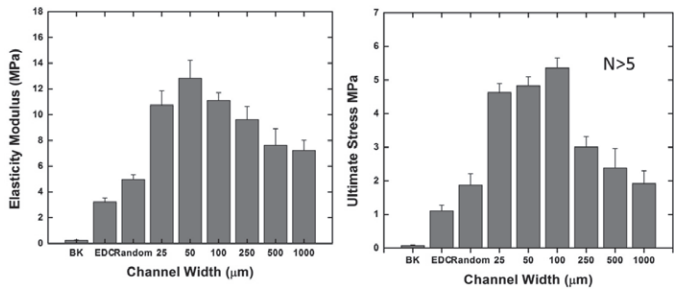

Fig. 2. Effect of microscale guidance on mechanical properties. hMSCs were cultured in collagen channels for 21 days in membranes containing channels of various dimensions (25–1000 μm). Unseeded (BK), unseeded crosslinked (EDC) and randomly seeded (Random) membranes were used as controls. Mean values of modulus of elasticity and ultramate stress are shown (n = 5/7 samples/condition).

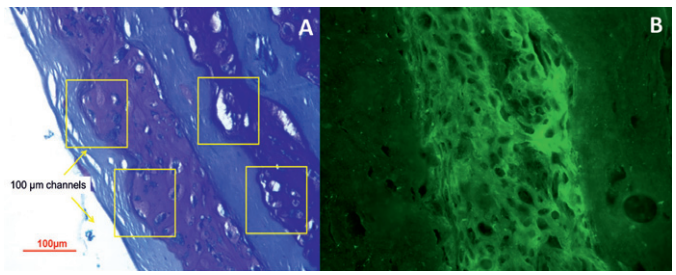

Fig. 3. Histology (toluidine blue staining, 3A) and immunohistochemistry (type II collagen staining, green, 3B) of constructs fabricated with microscale guidance channels.

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ISOLATION AND CHARACTERIZATION OF MULTI-POTENTIAL MESENCHYMAL CELLS FROM MOUSE SYNOVIUM

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Purpose: Human synovial mesenchymal stem cells are shown to have a higher capacity for proliferation and greater chondrogenic potential than those from other cell sources, such as bone marrow cells (BMCs) and muscle derived cells (MDCs). Thus, synovial mesenchymal stem cells are considered to be one of the appropriate candidates of cell sources for articular cartilage repair. However, numerous basic research questions related to the molecular mechanisms of tissue repair from these cells are also still largely unanswered. Mouse primary cell culture, in general, enables us to proceed the research for elucidating the molecular mechanisms of target phenomena because of a relatively easy for gene manipulation that is indispensable for the molecular analysis. However, one of the obstacles we are currently confronting is that mouse synovial mesenchymal cells (SMCs) are not available for basic research, whereas the rabbit, cow, and rat SMCs are available in addition to human mesenchymal stem cells.
The aim of this study was to establish methods to harvest synovium and to isolate and culture primary mesenchymal stem cells in mice.
Methods: Synovium was harvested from the infrapatellar fat pad of 10-week-old female Balb/c mice. The tissue was minced well to small pieces and given collagenase processing. Digested cells were filtered and cultured for 14 days. For flow cytometry, antibodies against CD29, CD34, CD45, CD117, and Sca-1 were used. As examination of the proliferation potential, growth kinetics and colony forming assay were performed. For *in vitro* chondrogenesis, 3×10^4 cells were placed in a polypropylene tube and centrifuged. The pellet was cultured in chondrogenic medium containing BMP7. For osteogenesis or adipogenesis, the cells were cultured in either calcification or adipogenic medium. For qRTPCR analysis the following primers were used: RUNX2 for osteogenesis, PPARγ for adipogenesis, Sox9 and Col2a1 for chondrogenesis. BMCs and MDCs were used as controls.
Results: As the mouse SMCs were not able to be harvested using the same protocol for human SMCs (Sekiya et al. Arthritis Rheum, 2006), the protocol for human needed the modification for mouse SMCs. The major modifications were the changes of collagenase treatment conditions, such as lesser concentration, shorter reaction time, and supplementation of DNase in collagenase solution. The proliferative potential of mouse SMCs was superior in comparison to that of both BMCs and MDCs. The colony-forming potential of mouse SMCs was similar to MDCs and superior compared to that of BMCs. The positive ratios of CD29, CD34, CD45, CD117, and Sca-1 in mouse SMCs were similar to those in both BMCs and MDCs. The positive ratios of CD44, CD106 and CD140a in mouse SMCs were higher than that in both BMCs and MDCs. The mouse SMCs were differentiated into cartilage *in vitro*. The pellets from mouse SMCs were larger and had amount of cartilage matrix shown by toluidine blue staining than those from both BMCs and MDCs. The osteogenic potential of mouse SMCs was similar to that of MDCs and superior to that of BMCs. The adipogenic potential of mouse SMCs was superior to that of both BMCs and MDCs.
Conclusions: These synovial mesenchymal cells harvested by the established method are expected to enable us to analyze the complex network of signaling pathways that very likely regulates their proliferative and differentiation potential of synovial mesenchymal stem cells by conducting *in vivo* analysis of genetically modified experimental models. In conclusion, we established primary mouse synovial cell culture method by determining the condition for isolation of the cells. The cells derived from mouse synovium demonstrated proliferation ability and multipotentiality, both characteristics of mesenchymal stem cells.

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EARLY INJECTIONS OF ADIPOSE-DERIVED STEM CELLS (ASCS) PROTECT AGAINST CARTILAGE DAMAGE AND LOWER SYNOVIAL ACTIVATION IN EXPERIMENTAL OSTEOARTHRITIS

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Purpose: Synovial activation is evident in a substantial subpopulation of patients with early osteoarthritis (OA) and has been associated with pathophysiology and clinical symptoms of OA. Previous studies have shown that synovial activation is involved in mediating cartilage destruction during experimental OA. Recently it has been shown that Adipose-derived Stroma/Stem Cells (ASCs) express immunosuppressive characteristics. The aim of our study was to explore the effect of intra-articular injection of ASCs on synovial activation and cartilage destruction during experimental OA.
Methods: ASCs were isolated from inguinal fat surrounding the popliteal lymph nodes and cultured for two weeks according to standard procedures. ASC were characterized with FACS analysis on a set of specific cell surface markers. Experimental OA was induced by injection of collagenase into murine knee joints, which causes instability and cartilage destruction. Collagenase-induced OA is characterized by thickening and activation of the synovial lining layer. ASCs were injected

into knee joints at various time-points after induction of OA. OA phenotypes were measured within 8 weeks after induction. Total knee joints were isolated and processed for histology. Synovial activation was measured using an arbitrary scale (0 to 3) and cartilage destruction was measured in 4 different layers of the knee joint (medial and lateral tibia and femur) according to the scoring method of Pritzker et al.. Moreover, damage to the cruciate ligaments was scored using an arbitrary scale (0 to 5).

Results: After culture FACS analysis showed that the adherent fraction expressed characteristic markers for stem cells (positive staining for Sca-1, CD-44 and CD-105 and negative staining for CD-11b and cKIT). Using RT-PCR, we found that ASCs expressed high mRNA levels of TIMP 1, 2 and 3 but not TIMP 4. A single dose of ASCs (20×10^3in mouse serum) was injected into the knee joint of mice, 7 days after induction of osteoarthritis. Synovial activation was significantly inhibited at day 14 (9%) and day 42 (35%) when compared to serum treated joints. Destruction of cartilage was also significantly inhibited at day 14 (54%) and at day 42 (35%). Inhibition of cartilage destruction was particularly found in the medial tibia. Interestingly, ASC-treatment had a protective effect on the cruciate ligaments. At day 42, damage to the ligaments was reduced by nearly 50% in the ASC treated joints when compared to controls. In line with that, 87.5% of the control animals showed a dislocation of the knee joint, whereas only 25% of the ASC treated animals. In contrast to early treatment, injection of the same dose of ASCs, 14 days after induction of OA only showed a small inhibiting effect (11%) on synovial activation when measured at day 42. Although cartilage destruction diminished with 28%, these values did not reach significance at that time-point.

Conclusions: Our study indicates that a single injection of ASCs into the knee joints of mice with collagenase-induced osteoarthritis gives protection of synovial activation and cartilage destruction when given shortly (day 7) after induction of experimental OA, possibly by inhibiting MMP activity and protection of damage to cruciate ligaments.

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BETA-XYLOSIDES INHIBITION OF CHONDROITIN SULPHATE SUBSTITUTION ON MATRIX PROTEOGLYCANS PERTURBS THE DIFFERENTIATION OF BONE MARROW STEM CELLS INTO A CHONDROGENIC LINEAGE

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Purpose: In a previous study (Hayes et al. J Histochem Cytochem. 2007, 56: 125–138) we reported that novel chondroitin sulphate (CS) sulphation motifs on cell-associated proteoglycans (PGs) may be putative biomarkers of progenitor/stem cell sub-populations (Dowthwaite et al. J Cell Sci. 2005, 117: 889–897). Recent studies indicate that unique CS sulphation motifs are localized in putative stem/progenitor cell niches at sites of incipient articular cartilage & other musculoskeletal tissues, which indicates their potential importance in cell differentiation during development. In this study, we investigated the importance of CS chains in the differentiation of bone marrow stem cells to the chondrogenic phenotype in vitro using p-nitrophenyl xyloside (PNPX) as a competitive inhibitor of CS substitution on matrix PGs.

Methods: Bovine bone marrow stem cells (BMSCs) were isolated from 7-day-old cow legs and cultured as monolayer for 4 weeks with chondrogenic medium ± 0.25mM PNPX. At each week, samples were analysed for sulphated glycosaminoglycans (sGAG), real-time PCR, Western Blotting & immunohistochemistry (IHC) using monoclonal antibodies recognising native and enzyme-generated epitopes & neoepitopes. The expression and distribution of structural chondroitin sulphation proteoglycans (CS-PGs) were analysed by immunofluorescent staining combined with confocal microscopy scanning.

Results: BMSCs cultured in chondrogenic medium start to aggregate and form mini-cell beads in 3 days and these mini cell beads clustered together to form a large cartilaginous cell bead in 2–4 weeks. There was no structural CS-PGs expression including aggrecan, biglycan & decorin when cells remained as monolayer. However, these CS-PGs were observed as long as the cells form mini-cell beads, suggesting an initiation of chondrogenic process. BMSCs cultured with 0.25mM PNPX still form the mini-cell beads and the single cartilaginous cell bead although it is 3–5 days later when compared with the control. This indicated that PNPX delayed the cell clustering and bead formation, a key milestone during the chondrogenic differentiation process. Real-time PCR and Western blotting results indicated that PNPX significantly inhibited or delayed the expression of chondrogenic markers such as aggrecan, SOX-9 & type II collagen gene and/or protein expression, suggesting the delay or inhibition of chondrogenic process. Interestingly, using monoclonal antibodies 7D4 and 6C3 identifying different epitopes along CS chains, we found that PNPX did not alter 7D4 staining but completely deplete the expression of 6C3 epitopes at the early stage of culture (1 week). Confocal microscopy analysis indicated that there was a colocalisation between 7D4 and aggrecan as well as biglycan core protein in the extracellular matrix of mini-cell bead after 2 weeks culture with or without PNPX. Differently, the colocalisation between 6C3 and biglycan was disrupted by PNPX, and there was no apparent colocalisation between 6C3 and aggrecan core protein. After 4 weeks of culture, 7D4 staining was observed across the cartilaginous-like bead and there was no difference between control and PNPX group. 6C3 staining was also evident across the whole bead in the control group. However, it was absent on the surface of the bead cultured with PNPX. Taken together, this highlight that different composition of CS chains may play distinct roles in the chondrogenic differentiation, and 6C3 epitopes are more important than 7D4 during bMSCs chondrogenesis.

Conclusion: These results indicated that CS sulphation motifs may play an important role in BMSCs differentiation into chondrogenic lineages. Its precise mechanism is not clear yet, but CS sulphation motifs may be involved in the cell aggregating, the initiation of chondrogenic process and extracellular matrix-cell interaction during the whole chondrogenesis.

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DOES ACOUSTIC HOMOGENEITY CORRELATE WITH TISSUE QUALITY IN ENGINEERED CARTILAGE?

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Purpose: Osteoarthritis related cartilage defects affect a large and growing fraction of the population. As cartilage does not self-repair, these defects present a long term problem; the end-point is usually total joint replacement. Patching these defects with living tissue-engineered (TE) tissue, particularly based on mesenchymal stem cells (MSCs), is being considered as an approach to avoid or delay arthroplasty. In this approach, biocompatible scaffolds are seeded at high density with MSCs and then exposed to a chondroinductive medium *in vitro*. Despite uniform initial conditions, the engineered cartilage develops in a centripetal fashion. After several weeks in culture, there is a wide range of possible outcomes. The best case results in solid cartilage specimens being generated, the worst in a cartilage shell of varying thickness surrounding an immature soft center. From the outside, without resorting to histological evaluation, these extremes appear visually identical. This study is a proof-of-concept of the use of an inexpensive, non-imaging, ultrasound system to assess the internal homogeneity of TE cartilage samples by non-destructive testing.

Methods: Deidentified hMSCs were obtained from three healthy volunteer donors under the terms of an IRB approved protocol. The cells were culture-expanded until the end of 1st first passage and were then seeded at 8×10^8 cells/ml onto 6 mm diameter × 3 mm thick macroporous collagen-chondroitin sulfate scaffolds with different formulations. The cell-seeded constructs were then grown for up to 3 weeks in a perfusion bioreactor. In parallel, calf stifle joints were obtained from a local abattoir; the cartilage was shaved off and 6 mm diameter disks were then punched out using a biopsy punch. The normal cartilage disks or the engineered tissue were immersed in buffered saline. A Panametrics V116-RM ultrasound transducer was mounted in a custom jig and positioned at 90° to the surface of the test sample. A Panametrics 5072PR pulser-receiver and a Picoscope 3206 oscilloscope were used to capture the ultrasound data, after which the test samples were processed for histology.

Results: In normal bovine cartilage, clear acoustic reflections were obtained from the front and rear faces of the biopsy, and very few internal reflections were found. This correlates well with the homogeneous histological aspect of the native cartilage plugs. In the engineered tissue, in addition to the front and rear faces of the sample, numerous internal reflections of comparable magnitude could be found. Histological examination of the engineered tissue samples showed an irregular differentiated shell surrounding an undifferentiated cellular core. Thus,