not observed in the wound but disappeared by the paracrine effect. This means that injected cells can help cartilage regeneration instead of engrafting and regenerating directly into the wound.
Conclusions: Spheroids, which tend to be similar to pellet cultures, do not exhibit differentiation characteristics, but they could be seen as a result of expression of related genes such as Bax, Bcl-XL and Alcian blue staining. Compared to conventional 2D culture single cells, spheroids cultured in 3D culture dish have high potential of cartilage regeneration as a result of in vitro and in vivo experiment. Spheroids tend to have low potential of cell death rather than proliferation as a result of a significant increase in Bax, a gene associated with apoptosis, and reduction in the proliferation marker, Bcl-XL. In previous paper, we confirmed that spheroids remained longer than single in the intra-articular (IA) cavity of rats. By 6 weeks, the amount of spheroids remained 80% and more than doubled compared to single. Moa (Mode of action) of immunohistochemistry conducted with HN (human nuclear antigen) staining, and injected hASCs were not observed really in surgical site. It means injected hASCs perished from joint after probably exerting paracrine effects. So, we conclude the fact that instead of hASCs going directly to the surgical site to regenerate cartilage, they can help catrilage regeneration.

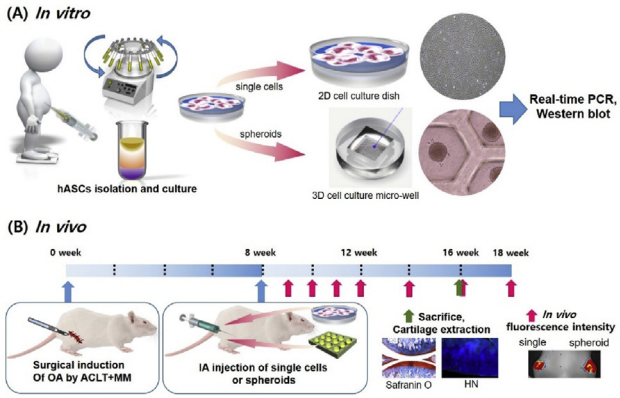

Figure 1. General scheme of *in vitro* and *in vivo* experiments using spheroid. (A) *in vitro* experimental procedures to identify single and spheroid. (B) *in vivo* experimental procedures in surgically-induced OA model to identify the cartilage regeneration efficiency of spheroid.

777
HYALURONIC ACID-CD44 SIGNAL AXIS PLAYS IMPORTANT ROLES IN THE FORMATION OF MESENCHYMAL STEM CELL (MSC) ANTIGEN-POSITIVE CELLS DURING THE TWO-DIMENSIONAL CULTURE

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Purpose: Cultured fibroblastic cells isolated from supra-patella synovial membrane in the knee joint are reported to have characteristics of mesenchymal stem cells (MSCs), since they have high proliferative activity, express surface antigens for MSCs, such as CD73, CD90, CD105, and have capacities to differentiate into chondrocyte-, osteoblast-, and adipocyte-like cells in vitro. Since autologous synovial MSCs can be relatively easily obtained from patients suffering from osteoarthritis by synovial biopsy and in vitro two-dimensional culture, it is expected to apply these cells for cell transplantation therapy to regenerate articular cartilage and meniscus. To establish reproducible and effective tissue regeneration therapy, we consider that the quality control of in vitro expanded MSCs are quite important. However, the molecular and cellular mechanisms how fibroblastic cells with MSC characteristics are dominantly expanded from synovial cells during two-dimensional cultures are still largely unknown. In this study, we performed time course MSC-related cell surface antigen expression analyses during in vitro culture to trace stem cell lineage residing in the synovial tissue.
Methods: This study was approved by the Ethics Committee of Tokyo Medical and Dental University. All patients included in this study gave their full written, informed consent for participation. Primary human synovial cells were isolated from supra-patellar synovial membrane obtained from patients who underwent total knee arthroplasty (TKA) by the collagenase digestion (n=6). One million of nucleated cells were seeded onto 15 cm-diameter culture dishes and maintained in normal growth medium (MEM alpha, 10% Fetal Bovine Serum, and antibiotics) at 37C in a humidified CO2 incubator. At day 0, 1, 4, 7, and 14, cells were detached from the dishes and subjected for cell surface antigen expression analyses. Positive cell fractions for MSC-related surface antigen (CD73, CD90, and CD105) were analyzed using a FACS Verse flow cytometer (BD Biosciences).
Results: There were large individual differences in the expression patterns of the surface antigens before the cell culture. At this time point, average almost 30% of total nucleated cells were positive for CD73 and/or CD90, however, CD105-positive cells were rarely detected. As a result, we found that less than 1% of the nucleated cells isolated from synovial membrane were satisfied the criteria for MSC determined by ISCT (International Society for Cell Transplantation: CD45(-)CD73(+) CD90(+)CD105(+)). Further flow cytometric analyses indicated that the cells positive for both CD73 and CD90 were developed from the cells positive for CD44. Interestingly, there was the bimodality in CD44 expression in synovial fibroblasts (CD44(low) and CD44(high)). At day 1 in culture, almost 70% of the fibroblastic cells were positive for CD44 and all of these cells were CD44(low). CD44 positive cells increased with time and almost all of the cells became positive for CD44 by day 4. Expression levels of CD44 also increased with time and most of the cells became CD44(high) cells in the later stage in culture. All CD44(high) cells were positive for CD73 and CD90 and the cells positive for CD105 were developed only from CD44(high)CD73(+)CD90(+) cells. These results suggest that synovial fibroblasts have plasticity to develop the characteristics of MSCs and there is a hierarchy in MSC-related surface antigen expression during two-dimensional culture. Since CD44 is a functional cell surface receptor for hyaluronic acid (HA), these data suggest that intra-cellular signals activated by HA may play important roles in the plasticity of synovial fibroblasts.
Conclusions: We showed that synovial fibroblasts have high plasticity and form MSCs during the two-dimensional culture. There is a hierarchy in MSC-related surface antigen expression and intra-cellular signals activated by CD44 may play important roles to initiate MSC-antigen expression in synovial fibroblasts.

778
PROGENITOR CELLS FROM HEALTHY AND OSTEOARTHRITIC HUMAN CARTILAGE SHOW POTENTIAL FOR ONE-STAGE REPAIR TREATMENT

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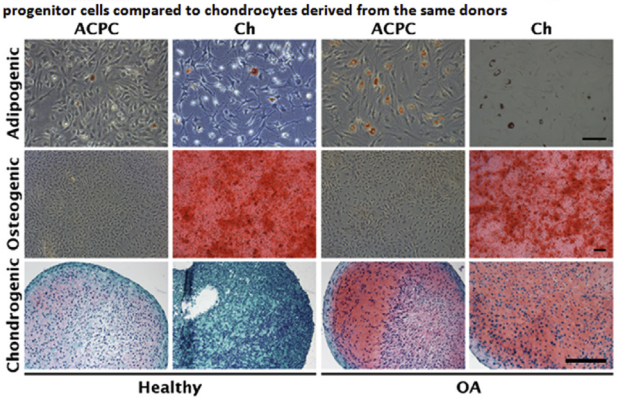
Purpose: This study aims to isolate and characterize articular cartilage-derived progenitor cells (ACPCs) from human cartilage. ACPCs from healthy and osteoarthritic cartilage were compared. In addition, the potential to use these ACPCs in addition to primary chondrocytes for one-stage procedures to treat cartilage defects is assessed.
Methods: Cells were isolated from full-thickness healthy (n=6, age 46-49, mean age 48) and osteoarthritic (n=6, age 41-82, mean age 62) human cartilage. Subsequently, ACPCs were isolated from the total cell population by clonal growth after a differential fibronectin adhesion assay. Mesenchymal stromal cells (MSCs) were isolated from human bone marrow. Healthy and osteoarthritic ACPCs were characterized and compared to MSCs by multilineage differentiation and flow cytometry analysis to assess cell surface marker expression. Full-depth chondrocytes of the same donors were assessed as well. Next, ACPCs were cocultured with osteoarthritic chondrocytes in 3D pellet cultures and compared to cocultures of MSCs and chondrocytes. Pellets were harvested after 28 days and assessed for cartilage-like matrix production using quantitative biochemical analyses for glycosaminoglycans and collagen. (Immuno)histochemistry was performed to visualize proteoglycan and collagen production.
Results: Healthy and osteoarthritic ACPCs were successfully isolated and differentiated into the adipogenic and chondrogenic lineage (Figure 1), but failed to produce calcified matrix when exposed to osteogenic induction media. Full-depth chondrocytes derived from the same donors were able to produce calcified matrix upon induction of osteogenic differentiation. Both ACPC populations met the criteria for cell surface marker expression to identify mesenchymal stromal cells (MSCs) as determined by flow cytometry. Cartilage-like matrix production was observed in ACPC pellet cultures, as well as pellets consisting of a coculture of ACPCs and chondrocytes.

Conclusions: In conclusion, this study provides further insight into a progenitor cell population which is present in both healthy and osteoarthritic human articular cartilage. These populations show similarities to MSCs. Furthermore, ACPCs show potential for use complementary to osteoarthritic chondrocytes in one-stage cartilage repair treatments, as a potential alternative to MSCs.

Figure 1: Trilineage differentiation of healthy and osteoarthritic articular cartilage-derived progenitor cells compared to chondrocytes derived from the same donors



779
COMPARISON OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS, ARTICULAR CARTILAGE DERIVED CHONDROPROGENITORS AND CHONDROCYTES TO ASSESS CELL SUPERIORITY FOR CARTILAGE REGENERATION

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Purpose: Spontaneous cartilage regeneration following damage caused by traumatic injury or degeneration (osteoarthritis) is poor, necessitating cell-based therapy. The goal is to identify a progenitor population within the pool of chondrocytes, which can be expanded in culture; as well as retain the property to differentiate into hyaline cartilage in vivo. Such a population, when isolated from articular cartilage especially from the superficial layers using fibronectin adhesion assay, has been found. Although this cell isolate shows superiority in terms of chondrogenic potential, reduced tendency towards hypertrophy and is also classified as a mesenchymal stem cell (MSC), conflicting reports when comparing these cells with cultured chondrocytes on the basis of cell surface markers and gene expression necessitate isolation of purer clones based on characteristic differentiating markers. This study aimed to assess cell superiority for cartilage repair by comparing bone marrow MSC, chondroprogenitors and chondrocytes expanded in culture.
Methods: Articular cartilage and bone marrow (BM) aspirate were obtained from three human osteoarthritic knee joints (Age: 53.3 ± 10.21 years). Three arms including a) BM-MSCs, b) chondroprogenitors using Fibronectin adhesion assay and c) cultured chondrocytes were taken up to passage 0 and characterized for growth kinetics (cumulative population doubling-CPD: up to passage 2 and galactosidase senescence assay), CD marker expression (MSC markers- positive: CD105, CD73 and CD90; negative: CD34, CD45 and HLA-DR and markers of enhanced chondrogenic potential: CD29, CD49e, CD49b, CD146 and CD166), RT-PCR (markers of chondrogenesis: SOX9, Aggrecan and Collagen II, marker of hypertrophic chondrocyte: Collagen I, and markers of hypertrophy: RUNX2 and MMP13) and trilineage differentiation. Data was compared using one-way ANOVA with post hoc Bonferroni correction. A P value of < 0.05 was considered significant.
Results: CPD of chondroprogenitors and BM-MSCs was seen to be comparable but significantly higher than chondrocytes at passage 1 (**P=0.001**) and 2 (**P=0.028**). When surface marker expression was compared, CD105, CD73 (vs CP), CD49e and CD29 values were seen to be significantly lower in BM-MSCs as compared to chondroprogenitors and chondrocytes (**P<0.05**). Also, CD146 (**P≤0.001**) expression was significantly lower while CD49b (**P<0.01**) was notably higher in chondroprogenitors than BM-MSCs and chondrocytes. Comparison of gene expression showed high expression of Aggrecan and low expression of RUNX2 and MMP13 in all groups. BM-MSCs showed lower expression of SOX9 as compared to chondroprogenitors and chondrocytes (**P=0.007**). Trilineage potential was seen to be comparable in all groups although less uptake of alizarin red was seen in chondroprogenitors as compared to BM-MSCs and chondrocytes.
Conclusions: Our Results indicate that since chondroprogenitors display properties of inherent stemness and chondrogenesis as evident by surface marker and gene expression, they may be a potential candidate for cell-based therapy. This is further validated by promising results seen in trilineage differentiation. Our study is the first of its kind to report a distinguishing marker in the form of CD49b and CD146, to differentiate between chondroprogenitors and chondrocytes, although further evaluation based on sorting, expansion and re-assessment of cell characteristics will provide conclusive data to re-affirm the same.

780
A COMPARISON OF CHONDROGENESIS ORIENTED DIFFERENTIATING PROTOCOLS FOR INDUCED PLURIPOTENT STEM CELLS

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Purpose: Induced pluripotent stem cells (iPSCs) technology has been proposed as an excellent method for engineering of cartilage and bone. Currently, iPSCs are being extensively studied in regenerative medicine strategies, disease modelling and drug screening in OA. These studies, however, still show a large variability and, more importantly, expansion of the cells following differentiation is shown to rapidly decrease quality of deposited cartilage extracellular matrix (ECM). We set out to compare molecular and histological differences between two approaches, aiming at establishing a controllable protocol ensuring deposition of high-quality cartilage.
Methods: A step-wise method was applied to differentiate iPSCs towards chondroprogenitor cells (CPCs) directly followed by chondrocytes depositing cartilage ECM. Alternatively iPSC were differentiated first towards mesenchymal stromal cells (iMSCs) followed by chondrogenesis. Cells were characterized by flow cytometry for mesenchymal stromal cell (MSC) associated markers (e.g. CD90, CD73, CD31, CD45, CD105). Chondrogenic differentiations were performed during 3 and 5 weeks in a 3D pellet culture system and compared to cartilage generated from human MSCs and from human primary chondrocytes. Subsequently, pellets were analysed by histology (Alcian Blue, collagen type 2), and by RT-qPCR.
Results: Flow cytometry analyses confirmed similarity of the iMSCs with bone marrow derived MSCs while chondrocytes generated by the two protocols significantly differed in their characteristics. Specifically some iMSCs could only differentiate into chondrocytes while others did not deposit any ECM. However, when applying the step-wise direct protocol, the chondrogenic differentiation appeared more consistent while simultaneously showing deposition of high-quality cartilage ECM (Figure 1). Gene expression analyses show a significant difference between the matrix genes *COL2A1, COL1A1* and *COL10A1* in cartilage deposited by chondrocytes generated with the two methods. *COL2A1* is higher expressed in chondrocytes generated by CPCs, while the iMSCs-derived chondrocytes have a higher expression of *COL1A1* and *COL10A1,* suggesting a more hypertrophic cartilage. The generated iMSCs and CPCs show similar expression levels in these genes compared to MSCs and autologous neo-cartilage respectively.

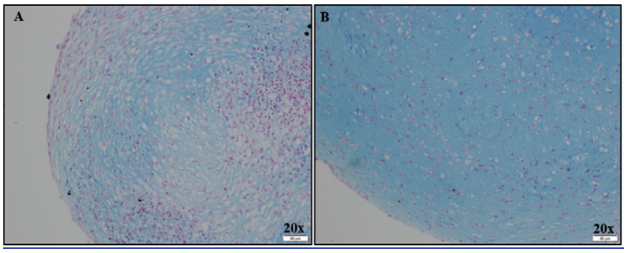

Figure 1. Representative example of glycosaminoglycans detected with Alcian Blue staining of 3D chondrocyte pellets generated from iMSCs (A) and from CPCs (B) measured at 20x at week 5 following chondrogenesis.