25.6%, 55.3%), and 55.6% for L5S1 (95% CI = 43.3%, 67.2%); two level FJA was not observed. The prevalence was significantly greater at the L5S1 level for symptomatic FJA than asymptomatic FJOA (p < 0.001); the rates were not significantly different at level L45 (p = 0.49). Comparisons were not performed at other levels since FJA/FJOA was not observed in both groups.

Conclusions: CLBP due to FJA most commonly occurs at L5S1. Morphologic abnormalities indicative of FJOA in asymptomatic adults occur most commonly at L45. These findings suggest that arthritic changes alone are not the sole etiologic factor mitigating FJA related CLBP.

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LAMIN A DEREGRULATION IN HUMAN MESENCHYMAL STEM CELLS PROMOTES AN IMPAIRMENT IN THEIR CHONDROGENIC POTENTIAL AND IMBALANCE IN THEIR RESPONSE TO OXIDATIVE STRESS

J. Mateos 1, A. De la Fuente 1, I.A. Lesende-Rodríguez 1, M. Arufe 2,3, F.J. Blanco 1,2, 3 Rheumatology Div., Proteomics Unit-ProtoRed/ISIBIC INIBIC-CHUAC, A Coruña, Spain; 2 Dept. of Med., Area of Anatomy and Human Embryology, University of A Coruña, Spain; 3 Rheumatology Div., Cellular Therapy Unit, CIBER-BBN/ISIBIC, INIBIC-CHUAC, A Coruña, Spain

Purpose: Previous work by our group and others indicated that an accumulation of lamin A (LMNA) was associated with the osteoarthritis (OA) chondrocyte phenotype. Mutations of this protein are linked to laminopathies and specifically to Hutchinson-Guilford Progeria Syndrome (HGPS), an accelerated aging disease. Some authors have proposed that a deregulation of LMNA affects the differentiation potential of stem cells. In the present study, we examined the effect of the over-expression of LMNA, or its mutant form progerin (PG), on the mesoderm differentiation potential of MSCs.

Methods: Mesenchymal stem cells (MSCs) from human umbilical cord (UC) stroma have previously been isolated, expanded and differentiated towards mesodermal lineages. For efficient gene delivery of wt LMNA, PG and GFP (Green Fluorescence Protein), we used a lentiviral expression system. GFP-transduced MSCs were used as control for the differentiation study since they present a differentiation capacity similar to that of untransduced MSCs. Osteogenic potential was studied by using alizarin red staining to assess calcium deposits as well as Real-Time PCR of ALP, OC and Runtx2 to assess early and late osteogenic differentiation. Adipogenic potential was studied with Oil Red staining for lipid droplets and Real-Time PCR of PNL, FABP and ADIPQ, for early and late adipogenic differentiation. Chondrogenesis and hypertrophic differentiation were studied using immunohistochemistry and Real-Time PCR of Aggrecan, MMP-13, Type II Collagen, Type I Collagen and Type I Collagen.

Results: We found that over-expression of LMNA or PG by lentiviral gene delivery leads to defects in differentiation potential. PG-transduced MSCs present defects in adipogenic and osteogenic potential. The chondrogenic potential is defective in PG-MSCs, which present a decrease in COL2 and Aggrecan as revealed by both immunohistochemistry and Real-Time PCR. LMNA and PG-transduced MSCs have an increase in hypertrophy markers (MMP-13 and Type X Collagen) during chondrogenic differentiation, as well as a decrease in manganese superoxide dismutase (MnSODM) and an increase of mitochondrial MnSODM-dependent reactive oxygen species (ROS). ROS synthesis was partially (51%) and totally reverted by N-Acetyl Cystine, ROS scavenger, (NAC) at 20 and 40 μg/mL respectively for 1 hour in culture. In addition, defects in chondrogenesis detected by immunohistochemistry and Real-Time-PCR are partially reversed by incubations with NAC at 40 μg/mL for 1 hour.

Conclusions: Our results suggest that OA process could be enhanced by defects in stem cell differentiation, partially due to imbalance in oxidative stress.

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SYNGENEIC, MINOR MISMATCHED, AND MAJOR MISMATCHED TRANSPANTATION OF MESENCHYMAL STEM CELLS DERIVED FROM SYNOVIA IN A RAT MASSIVE MENISCAL DEFECT MODEL

M. Okuno 1,2, I. Sekiya 3, S. Yoshiya 2, T. Muneta 1, Orthopaedic Surgery, Tokyo Med. ana Dental Univ., Tokyo, Japan; 2 Orthopaedic Surgery, Hyogo Coll. of Med., Hyogo, Japan; 3 Cartilage Regeneration, Tokyo Med. ana Dental Univ., Tokyo, Japan

Purpose: We previously reported that intraarticular injection of mesenchymal stem cells (MSCs) derived from synovium promoted meniscal regeneration in a rat massive meniscal defect model. Great number of reports described that bone marrow MSCs had immunoprivileged effects as well as immunosuppressive effects and that allogeneic transplantation of bone marrow MSCs has been successful in injury models. However, this is still controversial, and the opposite results were also reported. As far as synovial MSCs, the influence of allogeneic transplantation have not been investigated at all.

In this study, we performed syngeneic transplantation and allogeneic transplantation of synovial MSCs into the knee joints whose medial menisci were removed. The area of regenerated menisci were compared among the allogeneic transplantation groups, the major antigen mismatch group and the minor antigen mismatch group.

Methods: Cell isolation and culture. This study was approved by institutional animal use committee. Synovium was harvested from the knee joint of 3 strains, F344, Lewis, and ACI rats. After collagenase digestion, nucleated cells derived from synovium were expanded and colony forming cells were collected for transplantation.

Meniscectomy: As recipients, only F344 rats at 10-12 weeks of age were used. Under anesthesia, a straight incision was made on the anterior side of the right knee, the anteromedial side of the joint capsule was cut, and the anterior horn of the medial meniscus was dislocated anteriorly with a forceps. The meniscus was then cut vertically at the level of medial collateral ligament, and anterior half of medial meniscus was removed.

Transplantation: Immediately after the skin incision was closed, 5x10^6 synovial MSCs in 50 μL PBS were injected into the right knee joint of F344 rats. Transplantation of synovial MSCs derived from F344 rat is a syngeneic model. Transplantation of synovial MSCs derived from Lewis rat is a minor antigen mismatch model, in which histocompatibility antigens differ partly. Transplantation of synovial MSCs derived from ACI rat is a major antigen mismatch model, in which histocompatibility antigens differ greatly. Macroscopic and microscopic features for menisci were examined, and the area of regenerated menisci were measured 4 weeks after the surgery.

Results: In the case of synovial MSCs derived from F344 and Lewis rat were transplanted, into the knee of F344 rat, the area to F344 rat was significantly larger in the transplantation side than in the contralateral side (p = 0.034). Contrarily, in the case of synovial MSCs derived from ACI rat were transplanted, into the knee of F344 rat, there was no significant difference in the area of regenerated menisci to the transplantation side and the contralateral side. The area of regenerated meniscal in the ACI transplantation group was significantly smaller than in the F344 and ACI groups (p < 0.003).

Conclusion: For regeneration of removed menisci, similar results were obtained between the syngeneic transplantation and the minor mismatch transplantation model. In the major mismatch model, the results were inferior to the other models.

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CHONDROGENIC DIFFERENTIATION POTENTIAL OF CD56+ SATELLITE CELL AND PDGFRα+ MESENCHYMAL STEM CELL DERIVED FROM HUMAN SKELETAL MUSCLE


Purpose: We recently identified that murine-derived platelet derived growth factor-a positive mesenchymal stem cells (PDGFRα+ cells) have the capacity for adipogenic and osteogenic differentiation in vitro and vivo (Nature Cell, 2010). We also previously reported that two distinct stem cells, CD56+ satellite cells and PDGFRα+ cells were identified in human skeletal muscle and the human PDGFRα+ cells showed successful engraftment and bone-like tissue formation in vivo. However, chondrogenic differentiation potential of human skeletal muscle-derived stem cell is still unknown. In this study, we performed syngeneic transplantation and allogeneic transplantation of human synovial MSCs derived from human skeletal muscle were evaluated for their capacity for chondrogenic differentiation in vitro.

Methods: In this study, we used the human muscular tissue of patients with osteoarthritis of hip, after informed consent were obtained at the time of hip surgery. After enzymatic digestion of human skeletal muscle,
CD56+ cells and PDGFRα+ cells were isolated by using flow cytometry and the cells at fourth passage with 80% confluence were collected and re-suspended in a 15ml polypropylene tube containing 0.5 ml the chondrogenic differentiation medium at a density of 5×10³/ml, centrifuged at 200g for 5min into cell mass. The cell mass were cultured at 37°C in a humidified atmosphere containing 5% carbon dioxide for 21 days with the medium changes twice a week. After the morphological analysis of the cell mass were performed for 21 day, the cell mass was fixed with 4% paraformaldehyde at room temperature, frozen and sectioned (6μm-thick). The section was stained with toluidine blue.

**Results:** Cartilage-like tissue was not morphologically obtained by the cell culture with CD56+ cells, while successful formation of cartilage-like tissue was achieved by the cell culture with PDGFRα+ cells. Moreover, patholo-gical examination demonstrated that toluidine blue was positive at the marginal area of the section with PDGFRα+ cells, while toluidine blue-positive cells of the pathological section with CD56+ cells were not apparently observed in the section with PDGFRα+ cells.

**Conclusions:** PDGFRα+ cells seem to be suitable for cartilage formation in vitro; however, it seems that CD56+ cells cannot adapt to chondrogenic environment in vitro. In future, we would like to pursue analysis of in vivo chondrogenic differentiation of PDGFRα+ cells in detail.

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**FUNCTIONALLY DISTINCT MESENCHYMAL STEM CELL SUBSETS FROM THE HUMAN SYNOVIIUM**

F. Guillo, Jr., C. De Bari, Sr. Univ. of Aberdeen, Aberdeen, United Kingdom; Francesca Guillo and Cosimo De Bari Regenerative Medicine Group, Musculoskeletal Research Programme Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK

**Introduction:** Osteoarthritis is the commonest joint condition character-ized by a progressive breakdown of articular cartilage and other joint tissues. Regenerative medicine offers the opportunity to promote joint repair through biological tissue regeneration. Synovial mesenchymal stem cells (MSCs) may represent an optimal source of chondrogenic cells for joint resurfacing protocols However, until now, the definition of MSCs has relied merely on the retrospective analysis of in vitro culture-expanded cell populations. Moreover, MSCs undergo phenotypic rearrangements during culture expansion becoming heterogeneous with spontaneous differentiation and senescence. The resulting variability may affect the outcome of clinical applications. The aim of this study is the identification of marker sets for consistent enrichment/purification of MSCs from the human synovium.

**Methods:** Synovial cells were isolated enzymatically from human synovium specimens obtained after informed consent from patients with osteoarthritis undergoing joint replacement. Flow cytometric phenotypic analysis on freshly isolated and culture-expanded synovial cells was performed using multiple combinations of conventional MSC markers (CD106, CD271, CD73, CD90, CD13, CD81, CD105 and CD39) in association with CD45, a pan-hematopoietic cell marker not expressed by MSCs. Sorting was carried out using the FACS DIVA high speed digital cell sorter. Sorted cell populations were assessed for clonogenicity, kinetics of growth and chondro-osteogenic differentiation in vitro.

**Results:** Analysis of the phenotype of culture-expanded synovial cells showed that they were largely negative for CD45, as expected, and positive in varying proportions for the MSC markers tested. After removal from their natural environment, MSCs underwent phenotypic rearrangements during in vitro culture, losing the expression of LNGFR and CD106 and acquiring other markers such as CD166 and CD146. After culture expansion, two functionally distinct cell subsets revealed peculiar abilities after sorting. The CD73posCD39neg fraction was significantly more clonogenic and proliferative but less chondrogenic and osteogenic than the CD73posCD39pos fraction. However, no significant differences in terms of kinetics of growth, clonogenicity and chondro-osteogenic potential were observed when the CD73posCD39neg and the CD73posCD39pos cell subsets were purified directly from freshly isolated synovial cell populations.

**Conclusions:** Our findings indicate that, in keeping with the literature, culture conditions can profoundly influence MSC phenotype. MSCs undergo phenotypic and functional change due to the removal from their niches and the use of chemical and physical growth conditions. Notably, we provide evidence of existence of MSC subsets within culture-expanded synovial cell populations, which display distinct chondro-osteogenic potency. The purification of MSCs with predictable biological activity will allow the development of consistent MSC-based joint resurfacing protocols for osteoarthritis.

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**TYPE 1 COLLAGEN AND HEPARAN SULFATE SCAFFOLDS SUPPORT HUMAN CHONDROGENESIS FOR CARTILAGE TISSUE ENGINEERING**

S. Díaz-Prado1,2, E. Muñoz-López1, T. Hermida-Gómez1, I. Fuentes-Boquete1,2, P. Esbrit1, J. Buján1, F.J. De Toro1,2, F.J. Blanco1, INIBIC-CHUC, A Coruña, Spain; 2INIBIC-Univ. of A Coruña, A Coruña, Spain; 1Fundación Jiménez Díaz (Capio Group), Madrid, Spain; 4Dept. of Med. Specialties. Univ. of Alcalá de Henares, Madrid, Spain

**Purpose:** Evaluate the proliferation and chondrogenic potential of human bone marrow mesenchymal stem cells (hBM-MSCs) grown on type 1 collagen and different concentrations of heparan sulfate (HS) scaffolds.

**Methods:** hBM-MSCs and chondrocytes were cultured (150,000 cells/cm²) on the scaffolds for 16 and 30 days. We performed 12 groups of study (Table 1). hBM-MSCs were cultured in chondrogenic medium or DMEM with 20% FBS plus 100 nM PTHrP. Chondrocytes were grown in DMEM with 10% FBS plus 100 nM PTHrP. Chondrogenic differentiation was confirmed by histochemical and immunohistochemical analysis. Transfec-tion (TEM) and scanning (SEM) electronic microscopy were performed to study the cell interior and the morphology.

**Results:** hBM-MSCs and chondrocytes were able to proliferate on type 1 collagen and various concentrations of HS scaffolds, since cells showed high percentages of positivity for PCNA proliferation marker (Fig. 1A). Increased cell proliferation has been associated with a high rate of scaffold degradation. The results indicated that hBM-MSCs proliferated better in chondrogenic medium than in the usual growth medium (DMEM 20%). The study groups with hBM-MSCs grown in chondrogenic medium +100 nM PTHrP, regardless the HS concentration, showed high percentage of cells regarding the scaffold area (more than 80% after 16 days in culture and more than 90% after 30 days in culture). Moreover, they also showed high percentages of positivity for safranin O and type II collagen (Figure 1B and C). Hematoxylin-eosin (H-E) (Fig. 1D) and Mason’s trichrome stainings showed that cells formed aggregates and produced extracellular matrix. By scanning electron microscopy (SEM) we studied the cell morphology in culture over the biomaterials (Fig. 1E). Transmission electron microscopy (TEM) showed that cells had many ribosomes, large number of lipid vacuoles and the accumulation of glycogen, characteristic of the cells with a high activity (Fig. 1F).

**Conclusions:** Our data demonstrated that type I collagen and heparan sulfate scaffolds were optimal for hBM-MSCs and chondrocyte growth and that hBM-MSCs cultured over these scaffolds on chondrogenic medium were able to differentiate to chondrocyte-like cells.

**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Cells</th>
<th>Scaffolds</th>
<th>Culture Medium</th>
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<tbody>
<tr>
<td>I</td>
<td>hBM-MSCs</td>
<td>Col I</td>
<td>Chondrogenic +100 nM PTHrP</td>
</tr>
<tr>
<td>II</td>
<td>hBM-MSCs</td>
<td>Col I + 1% HS</td>
<td>Chondrogenic +100 nM PTHrP</td>
</tr>
<tr>
<td>III</td>
<td>hBM-MSCs</td>
<td>Col I + 2% HS</td>
<td>Chondrogenic +100 nM PTHrP</td>
</tr>
<tr>
<td>IV</td>
<td>hBM-MSCs</td>
<td>Col I + 3% HS</td>
<td>Chondrogenic +100 nM PTHrP</td>
</tr>
<tr>
<td>V</td>
<td>hBM-MSCs</td>
<td>Col I</td>
<td>DMEM + 20% FBS +100 nM PTHrP</td>
</tr>
<tr>
<td>VI</td>
<td>hBM-MSCs</td>
<td>Col I + 1% HS</td>
<td>DMEM + 20% FBS +100 nM PTHrP</td>
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<td>hBM-MSCs</td>
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<td>IX</td>
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