THE EFFECTS OF ARTICULAR CARTILAGE FRAGMENTS AND SYNOVIAL MEMBRANES ON CHONDROGENESIS OF BONE MARROW-DERIVED MENCESHMYAL STEM CELLS


Purpose: There are many approaches have been developed to manage and treat articular cartilage injuries. For example, bone marrow stimulating techniques, mosaicplasty and autologous chondrocyte implantation. However, current clinical therapies still have some limitations regarding to the formation of fibrocartilage. On the other hand, tissue engineering of cartilage has been recognized as a new treatment for cartilage damage, but it still has some issues need to be verified. In this study, we wanted to create an articular mimetic environment for better hyaline cartilage formation. Therefore, the components of the articular such as cartilage fragments and synovial membranes were added and the effects on chondrogenesis of bone marrow-derived mesenchymal stem cells (BM-MSCs) which were used for cartilage tissue engineering were investigated (schemed as figure 1).

Methods: Cartilage fragments, synovial membranes and BM-MSCs were tested alone or in different combinations (as table 1) for chondrogenesis. The components of different groups were wrapped by type I collagen gel and treated with induction medium. The matrix were analyzed at 0, 14, and 28 days after induction. Scanning electron microscopy and histological staining by H&E and alcian blue were employed. Glycosaminoglycans (GAGs) content were measured and gene expression of Col I, Col II, Col X, Sox9, and aggrecan of matrix were analyzed as well.

Results: Type II collagen gene expression of groups containing cartilage fragments were increased. When BM-MSCs were co-cultured with cartilage fragments, histological and GAGs analysis showed that GAGs secretion from BM-MSCs was increased and the proliferation of BM-MSCs which closed to cartilage fragments was promoted. Finally, aggrecan and type II collagen expression of BM-MSCs were also increased while chondrocyte hypertrophy was inhibited after incubation for 2 weeks.

Conclusions: We demonstrated that the articular cartilage fragments and synovial membranes in combination could promote chondrogenic differentiation of BM-MSCs. We hope these preliminary results could help develop an optimal condition for hyaline cartilage repair and be able to be applied along with bone marrow stimulating techniques in clinical treatment in the future.

Experiment groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Cartilage fragments</th>
<th>Synovial membranes</th>
<th>BM-MSCs</th>
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<tbody>
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ROLE OF POLYAMINES IN CHONDROGENESIS OF ADIPOSE DERIVED STEM CELLS

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Purpose: Adipose derived stem cells (ASC) are an attractive cell source for regenerative purposes in orthopedics, because of their accessibility and high differentiation potential, upon delivery of appropriate stimuli which mimic the organogenetic natural microenvironment. Polyamines are naturally occurring, positively charged polycations able to interact with negatively charged compounds and structures within the living cell thus controlling several cellular processes including cell differentiation and already implicated in bone growth and development. Hence, we investigated the effects of exogenously added spermine in chondrogenesis of ASC recapitated in 3D micromass cultures, to tease out the effects on gene and protein expression of key chondrogenesis regulatory transcription factors, markers and effectors.

Methods: Adipose tissue was obtained from the surgical subcutaneous area of 20 OA patients (age 29-72) undergoing hip arthroplasty. ASC were obtained from the stromal vascular fraction with conventional procedures and used within p2-p3 passage. To monitor phenotypic homogeneity, flow cytometric analysis of the cells at p1 passage was carried out to evaluate the expression of CD 31, 34, 45, 271, 44, 73, 90 and 105. Micromasses were seeded in control (D-MEM 10% FCS and 50 μg/ml ascorbic acid) or chondrogenic medium, with or without the addition of 5 μM spermine to evaluate its chondrogenic ability across 1, 2 and 3 weeks chondrogenic maturation. Osteogenic medium was used as control. We evaluated the effects of spermine addition on molecular markers of chondrocyte differentiation at the level of gene (real time PCR) and protein (western blot and immunohistochemistry) expression as well as the effects on extracellular matrix deposition and mineralization.

Results: The number of ASC per unit (g) of adipose tissue was inversely correlated with the age of both male and female subjects and positively correlated with the body mass index. ASC samples were highly positive for CD44, CD73 and CD90, being the expression of the latter inversely correlated with the age of both male and female subjects and positively correlated with the body mass index. ASC samples were highly positive for CD44, CD73 and CD90, being the expression of the latter inversely correlated with the age of both male and female subjects and positively correlated with the body mass index.

Conclusions: Polyamine can represent a potential tool to improve the differentiation of ASC for bone tissue engineering via recapitulation of endochondral ossification.

Acknowledgement: This work supported by FIRB (MIUR, Italy) and Fondi cinque per mille (Ministero della Salute, Italy). The authors wish to thank Prof C. Ventura for helpful discussion during the early phase of the work.

IMPLANTATION OF BILAYERED PLLA SCAFFOLDS LOADED WITH MESENCHYMAL STEM CELLS (MSCS) IN A SHEEP MODEL OF OSTEochondRAL LESIONS

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Purpose: Cartilage injuries are poorly repaired after surgery, since cartilage is a tissue with a very limited capacity to regenerate. Apart from other
surgical attempts, autologous chondrocyte implantation (ACI) was the first cell therapy approach developed for these kinds of lesions. Chondrocyte usage nevertheless is limited by the dedifferentiation occurred when expanded in monolayer. Bone marrow-derived Mesenchymal Stem Cells (MSC) emerge as ideal cells to be implanted in the site of cartilage lesions.

**Methods:** Here we use MSC to treat sheep cartilage lesions implanted in the upper cartilage layer of bilayered PLLA scaffolds, which hold a lower layer enriched with Hydroxyapatite to facilitate osteointegration of the scaffold.

**Results:** After evolutions of 1.5, 3 and 6 months, lesions showed a slight but non significant decrease in the mankin score for cartilage found in the lesioned area (in animals treated with unloaded and cell loaded scaffolds) suggesting a progressive degradation of the tissue. Nevertheless, staining with Safranin-O showed the presence of more cartilaginous tissue in animals evolve for 6 months with MSC loaded scaffolds, demonstrating a more regeneration of the tissue when MSC were implanted in cartilage lesions. Unfortunately new formed tissue did not appear in the cartilage layer, but inside the hole performed during surgery, which might suggest some incapacity of the scaffold to support the presence of cells aligned with the surface in order to integrate tissue to the surrounding cartilage. Finally, collagen type II and aggrecan were found in the regenerated tissue.

**Conclusions:** The use of MSCs is a promising alternative for the treatment of cartilage damage, but administration in the core of scaffolds needs to be improved.

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**MESENCHYMAL STEM CELL THERAPY IN A RAT MODEL OF OSTEOARTHRITIS**

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**Purpose:** Mesenchymal stem cells (MSCs) are promising candidates for cartilage regeneration and osteoarthritis (OA) therapies, due to their ability to form cartilage and to secrete immunomodulatory factors. Since OA comprises many different processes, we evaluated the effects of intra-articularly injected cultured MSCs on multiple aspects of OA pathology, including pain, cartilage degeneration and subchondral bone changes, in a rat model in vivo. In order to explore potential options to enhance clinical translatability, we studied rat- and human cultured MSCs, as well as a rat model in vivo. In order to explore potential options to enhance clinical translatability, we studied rat- and human cultured MSCs, as well as a rat model in vivo. In order to explore potential options to enhance clinical translatability, we studied rat- and human cultured MSCs, as well as a rat model in vivo.

**Methods:** Osteoarthritis was induced unilaterally in 32 male wistar rats freshly isolated rat bone marrow mononuclear cells (BMMNCs). Methods: Osteoarthritis was induced unilaterally in 32 male wistar rats (16 weeks old) by intra-articular injection with 300 μg mono-iodoacetate (MIA). Three weeks after OA induction rats were randomly divided into four treatment groups: 1. Control (saline); 2. Rat MSCs; 3. Rat BMMNCs and 4. Human MSCs. Rat MSCs and human MSCs were given at a dose of 1 x 10^6 cells in 50ul saline per joint, rat BMMNCs were given at a dose of 10 x 10^6. Rats where euthanized four weeks after treatment injections. Before and after OA induction, as well as 4 weeks after treatment, hind limb weight distribution between the osteoarthritic and the contralateral healthy joint was used as an index of joint discomfort. Ex vivo uCT analysis was performed to assess changes in the subchondral bone plate and histology was used to evaluate loss of glycosaminoglycans and development of structural damage in the cartilage at the endpoint of the study. Statistical analysis was performed with repeated measures ANOVA and paired t-tests for hind limb weight distribution, with one way ANOVA and unpaired t-tests for μCT data, and with non-parametric Kruskal-Wallis and Mann-Whitney-tests for histology scores. For all tests, p values <0.05 were considered statistically significant.

**Results:** Hind limb weight distribution (Figure 1): After MIA injection, significantly less weight was distributed to the affected leg compared to baseline (P < 0.001), indicating pain sensation. The rats treated with rat MSCs distributed significantly more weight to their affected paw 4 weeks after treatment than before treatment (P = 0.003), whereas none of the other groups showed a significant change in weight distribution. The groups however, did not differ statistically significant at the endpoint of the study.

Subchondral bone changes: MIA injected knees had a significantly more porous (P < 0.001) and thinner (P = 0.005) subchondral plate than control knees. Rats treated with rat MSCs or rat BMMNCs showed the least subchondral bone changes, although no statistically significant differences between treatment groups were observed.

**Conclusions:** The treatment with injection of rat MSCs caused a significant increase in weight distributed to the affected limb, indicating a reduction of joint discomfort. This is the first study that evaluates the effect of cell therapy on pain in a small animal OA model. In addition, the injection of rat MSCs caused a trend towards reduction of cartilage and subchondral bone changes, albeit no significant differences were observed between any of the treatment groups. The absence of statistically significant effects of the treatments on cartilage and bone structure might be related to several factors, including the time of injection, the OA model or the number of injected cells. To improve treatment effect, cell number, cell type and the time point of treatment should be further optimized.

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**CARTILAGE- AND SUBCHONDRAL BONE DERIVED FACTORS REGULATE COLLAGEN PRODUCTION IN MSC**

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**Purpose:** Functional repair of critical size articular cartilage defects is still a considerable challenge in the field of tissue regeneration. To effectively improve the quality and durability of tissue-engineered neocartilage a better understanding of microenvironmental cues provided from surrounding cartilage and subchondral bone tissue and the modulating influence of neighbouring cells and tissue interfaces in vivo on chondrogenic differentiation of MSC are essential. This study is designed to identify microenvironmental conditions which promote, prevent or interfere with chondrogenic differentiation of mesenchymal stem cells (MSC) and to analyse their impact on hyaline cartilage like quality and biomechanical properties of regenerated tissue.