were expressed at low levels, while Col1a1, VEGF and alpha11 integrin were highly expressed together with TIMP-1, TIMP-2 and MMP-13. Upon osteogenic and chondrogenic differentiation these genes were differentially regulated. Cytokine and growth factor profile was assessed at protein level using an antibody array. Here, we observed secretion of TIMP-1, MCP-1, VEGFα-165, while CINC-2 and β-NGF were detected intracellularly. This expression profile was dependent on differentiation state of MSCs while other factors embedded into the array could not be detected irrespective of culture conditions.

Conclusions: Our gene expression results provide a foundation for a more reproducible and reliable quality control of rat bone marrow derived MSCs used for osteo-chondrogenic differentiation studies. Additionally, we demonstrate secretion and production of cytokines and growth factors from a heterogenous BM cell population which may be crucial for MSCs fate determination and sustenance.

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CHONDROCYTE VIABILITY IN HUMAN TIBIAL PLATEAUS CRYOPRESERVED WITH THE NATURAL GLYCOSYLATED HYDROQUINONE, ARBUTIN

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Purpose: Chondrocytes are essential to maintain the integrity of the articular cartilage matrix. Freezing of intact articular cartilage, even in the presence of cryoprotective agents, like glycerol or dimethyl sulfoxide, seriously compromises the viability of chondrocytes and, thus, the integrity and durability of cryopreserved osteochondral allografts used to repair several types of cartilage defects. This study aims at evaluating the ability of the natural glycosylated hydroquinone, Arbutin, to protect articular chondrocytes from the damaging effects of freezing/thawing, using human tibial plateaus as a model of osteochondral allografts.

Methods: Tibial plateaus were harvested from cadaver tissue donors within 24 hours of death, in strictly aseptic conditions according to the standards of the Tissue and Bone Bank of the University Hospital of Coimbra, Portugal, and the Portuguese Transplantation Organization. Donor age ranged from 17 to 53 years old. Articular cartilage from all donors appeared grossly normal without signs of fibrillation or other lesions. The lateral and medial tibial plateau tissue samples from each cadaver tissue donor were incubated in Ham F-12 Nutrient Mixture with or without arbutin (50 or 100 mM) for 30 minutes to 1 hour at 37°C and then frozen at -20°C/24h. After the initial cooling, the osteochondral pieces were transferred to a biofreezer at -80°C and kept in these conditions for a maximum of 2 months. Thawing was achieved by immersion in Ham F-12 Nutrient Mixture at 37°C for 1h. Chondrocyte viability was assessed in situ by a modification of the MTT reduction assay and by staining cryostat sections with Calcein AM and Propidium Iodide. Where applicable, statistical analysis was performed using the Student’s t test. Results were considered significant for p<0.05.

Results: Chondrocyte viability in the osteochondral pieces immediately before freezing, was identical in all the conditions tested. After freeze/thawing, chondrocyte viability, expressed as the percent MTT reduction relatively to the viability of chondrocytes in fresh cartilage, was significantly higher in tibial plateaus treated with arbutin than in those treated with the nutrient mixture alone (Control), as shown in table 1. However, fluorescence microscopy showed that living chondrocytes were restricted to the superficial cartilage layers.

Results are the mean ± SE of 4 different experiments, each performed in duplicate.

Table 1. Chondrocyte viability in freeze/thawed cartilage relatively to the fresh tissue

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Arbutin, 50mM, 30 min</th>
<th>Arbutin, 50mM, 1h</th>
<th>Arbutin, 100mM, 30 min</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>32.0 ± 10.8</td>
<td>34.2 ± 2.9</td>
<td>28.5 ± 6.2</td>
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Conclusions: The results presented indicate that Arbutin is a low toxicity cryoprotective agent that effectively preserves chondrocyte viability, at least, in the superficial cartilage layers. Nevertheless, further studies are required to identify conditions of cryopreservation with this natural compound that allow the protection of chondrocytes in the entire depth of the articular cartilage, which is essential to maintain cartilage integrity in cryopreserved osteochondral allografts.

P386
GENE EXPRESSION PROFILING OF HUMAN CARTILAGE AND BONE GENERATED BY TISSUE ENGINEERING

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Purpose: Osteoarthritis (OA) as the most prevalent disorder of the musculoskeletal system is a consequence of mechanical and biological events destabilizing tissue homeostasis in articular joint tissues. Thus, it involves the disturbance in the normal balance of degradation and repair in articular cartilage, synovial membrane and subchondral bone. Current means and surgical procedures for an efficient treatment are restricted to symptomatic measures or are quite unsatisfactory in the long-term evaluation. Tissue Engineering (TE) provides new sustainable therapy strategies based on the transplantation of cells as suspensions or in combination with scaffolds to the defect side. Mesenchymal progenitor cells like mesenchymal stem cells and periosteal progenitor cells meet the demands of regenerating complex defects like articular knee cartilage and the underlying bone occurring due OA progression.

Since several years we investigate the regenerative properties of periosteal progenitor cells. In a new approach we developed three-dimensional (3D) cell culture techniques comprising the use of periosteal progenitor cells mixed with fibrinogen and embedded in PGLA biomaterials to allow the formation of osseous and cartilaginous tissue in vivo and in animal models. The aim of this study was to characterize human cartilage and bone tissue engineered on the molecular level.

Methods: With regard to a possible translation into the clinic, we performed the experiments in a clinically applicable setting: Human periosteal cells from mastoid (n=3) were isolated and cultured in complete DME-medium containing human serum. Passage 3 cells were mixed with human fibrinogen, embedded in PGLA fleeces and induced according to osteogenic or chondrogenic standard protocols.

Results: After one, two and four weeks of osteogenic induction the formation of mineralized bone matrix was shown by von Kossa staining. Chondrogenic differentiation was demonstrated by collagen type II immunohistochemistry and by alcian blue staining after two, four and six weeks of induction. Cell viability was investigated by using PI/FDA staining. RNA from culture expanded periosteal progenitor cells, 3D bone grafts (timepoint: 7, 14 and 28 days), native bone, 3D cartilage graft (timepoint: 14, 28 and 42 days) and native cartilage were used for genomewide gene expression profiling analysis using the Affymetrix HG U133 plus 2.0 oligonucleotide microarray. The analysis by pattern discovery displayed the regulation of distinct genes already described as relevant in osteogenesis and chondrogenesis, like genes involved in matrix formation, and also of possible new marker genes.
Conclusions: 3D tissue culture of expanded human mastoid-derived periosteal progenitor cells in resorbable PGLA fleeces initiates bone and cartilage formation on the cellular and molecular level. The generation of different mesenchymal tissue makes 3D tissue cultured periosteal progenitor cells promising candidates for the treatment of OA. Further investigations with periosteal progenitor cells from donors with OA were necessary for an autologous therapy strategy.

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EFFECT OF BLOOD ON THE MORPHOLOGICAL, BIOCHEMICAL, AND BIOMECHANICAL PROPERTIES OF NEO-CARTILAGE, SYNTHESIZED BY ISOLATED CHONDROCYTES PRE-SEEDED ONTO A BIOLOGICAL SCAFFOLD

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Purpose: The use of autologous chondrocytes seeded onto a biological scaffold represent a current valid tool for cartilage repair. However, the effect of the contact of the blood to the engineered construct is unknown. The aim of this work was to investigate the effect of blood contact on the morphological, biochemical and biomechanical properties of engineered cartilage.

Methods: Articular chondrocytes were enzymatically isolated from swine joints, expanded in monolayer culture and seeded onto collagen membranes for two weeks. Peripheral blood was obtained from animals of the same species. The cell-seeded collagen membranes were placed in contact with the blood, diluted with medium, for three days ("blood" group). As controls ("control" group), some samples were left in medium, with no blood contact. Some samples were retrieved from cultures after the blood contact, some others were left in standard culture conditions for 3 more weeks. Samples were analysed grossly, histologically, biochemically (MTT analysis), and by biomechanical analysis under unconfined geometry.

Results: Upon retrieval, samples from both groups showed increasing dimensions and weights overtime, with higher mean values recorded for control group. Biochemical evaluation demonstrated a transient reduction of the mitochondrial activity due to blood contact. Histological evaluation demonstrated evident cartilage-like matrix production for both groups. Biomechanical data showed a reduction of the values in the early culture time, followed by a stabilization regardless the presence of the blood.

Conclusions: The results obtained from this study demonstrate that the contact to blood of the samples determine a transient reduction of the mitochondrial activity. However, the morphological parameters seem to show a continue production of the cartilage matrix components overtime. This tissue engineered cartilage structure is easily reproducible and it could represent a valuable model for studying the behaviour of different variables on the newly formed cartilage.

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NEW TISSUE GROWTH AFTER COLLAGEN MENISCUS IMPLANT (CMI) PLACEMENT INCREASES ACTIVITY LEVELS AFTER TWO YEARS

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Purpose: Absence of meniscus tissue leads to decreased clinical function and activity levels. In a previous study, we reported significant correlation between actual measured amounts of meniscus tissue removed at meniscectomy and symptoms, function and activity 2 years after surgery. In particular, it was noteworthy that patients with >50% remaining meniscus tissue were significantly better in all categories measured than patients with <50% total meniscus tissue remaining. The purpose of the present study was to determine, prospectively, changes in Tegner activity levels from preoperative to 2 years postoperative in patients who received Collagen Meniscus Implants (CMI) and were documented to have >50% total meniscus tissue at 1-year relook arthroscopy.

Methods: In a randomized controlled investigational device clinical trial (Level of Evidence I), 138 patients 18 to 60 years old underwent partial medial meniscectomy and placement of a CMI to fill the meniscus defect. There were 64 acute (no prior meniscus surgery) and 74 chronic (1 to 3 prior partial meniscectomies on the involved meniscus) patients. At index surgery, meniscus defect size was measured with specially designed instruments, and the percent of meniscus loss was calculated based on these actual measurements. Relook arthroscopy was performed at 1 year on 124 patients (93% surgical follow-up), and percent total meniscus tissue (remnant + new tissue) was determined by making these same measurements and calculations. Patients were followed clinically for a minimum of 2 years after CMI placement. At each follow-up, all patients completed questionnaires, including a Tegner score to assess activity. We then determined changes in Tegner score from the index surgery to 2 years status post CMI in these patients.

Results: Of 124 relooks, 111 patients (90%) had >50% total meniscus tissue. In these patients, average Tegner activity scores improved by two levels from 3 to 5 from preoperative to 2 years status post CMI. This increased change in activity levels significantly correlated with total meniscus tissue >50% (r=0.21, p=0.02). These findings mirrored those we previously reported for partial meniscectomy patients in which >50% of the meniscus was maintained.

Conclusions: There is a significant correlation between change (increase) in Tegner activity levels over 2 years and percent total meniscus tissue in patients who receive the CMI as treatment for meniscus loss and have >50% total meniscus tissue. This study confirms the importance of preserving as much meniscus tissue as possible at the time of repair or meniscectomy. It clearly supports the potential positive benefits of regrowing or regenerating lost meniscus tissue to assist patients in regaining their activity.

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CHONDROGENIC PROGENITOR CELLS DERIVED FROM LATE STAGES OF HUMAN OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is such a widespread complication of old age that it is expected to become the fourth leading cause of disability by the year 2020. As a hallmark of the pathogenesis of late stages of the disease, in addition to the diseased chondrocytes (type 1 cells), type 2 cells emerge. While