20.6

Tissue engineering of osteochondral constructs in vitro using hBMSCs, a hybrid matrix and a bioreactor

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Purpose: The aim of this study was to establish an in vitro system using effects of initial compression forces followed by different stress patterns on human bone marrow stromal cells (BMSC) in a 3-D osteochondral matrix in a bioreactor.
Methods and Materials: Human BMSC were harvested (donors age 24-53 y) and after density centrifugation cells were proliferated for three passages in culture media supplemented 3 ng/ml FGF-2. Cells were seeded into the osteochondral hybrid scaffold (CaReS (Ars Arthro, Esslingen) and Tutobone (Tutogen Medical GmbH, Neunkirchen)) with a concentration of $1x10^6$ cells per ml. Pressure and vacuum forces were applied in a specially developed glass kit prior exposure to stress in a bioreactor for 1, 7, 14, 21 and 28 days. Next to standard staining GAG and DNA were quantified. Biomechanical tests were conducted.
Results: Penetration and cell distribution was demonstrated homogenous and vital over time (88%±8,9%). GAG and DNA quantification showed no significant differences over time and different stress patterns. Mechanical tests showed a significant enhancement of matrix stiffness after primary compression (first 24 h) and over 28d under mechanical stimulation a 12x enhancement vs. non stimulated static compressed control ($p=0.033$).
Conclusions: The integration of mechanical stimulation in the tissue engineering process may lead to a progress in the structural and biomechanical properties of these tissues. Sufficient initial scaffold stability remains a key issue in a successful strategy. Further studies need to clarify influence of compression, different mechanical and hydrostatic stress patterns over different periods.

20.7

CD44 isoform expression in human articular chondrocytes during de-differentiation process

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Purpose: CD44, the principal hyaluronan receptor, is essential for matrix homeostasis. Beside the most common standard form (CD44s) several transcript variants exist. These are traced back to two different splicing sites, exons 6-15 and 19-20. Expression of exon 19 (CD44-st) instead of 20 (CD44-lt) leads to a very short cytoplasmic domain lacking the ability of cytoskeletal interaction. Although the functions of most CD44 transcript variants are not known, it was shown that the cytoplasmic domain is required for pericellular matrix assembly. In this study we analysed the expression of CD44 isoforms in human articular cartilage and chondrocytes during the de-differentiation process in monolayer culture.
Methods and Materials: CD44 isoforms, hyaluronan synthases, UDPGD, Sox9, Col2, Col1 and aggrecan expression was determined by realtime RT-PCR und RT-PCR. Flow cytometry and immunofluorescence were used for analysis at protein level.
Results: CD44s was slightly increased in monolayer culture. During de-differentiation we found a dramatic increase (up to 50 fold in contrast to native cartilage) in CD44-st expression. In RT-PCR no variable exons (6-15) were detected. HAS1 was detected neither in native cartilage nor in monolayer culture. No change occurred in HAS2 and HAS3 expression. UDPGD was increased mainly in the first days of culture. Sox9 expression correlated with course of Col2 expression.
Conclusions: Cell-matrix interactions are crucial for cartilage homeostasis and matrix remodelling. The increase of CD44-st expression causes losing of cell-matrix interactions via the cytoskeleton and decreases matrix assembly. Our data indicate that CD44-st may be responsible for the reduced potential of matrix assembly in cultured chondrocytes.

21.3

In vivo maturation of engineered articular cartilage on hydroxyapatite

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Purpose: The Alginate-Recovered Chondrocyte (ARC) method is a tissue engineering method that generates cartilaginous tissues without the use of a scaffold. Hydroxyapatite has been clinically used as a bone substitute. The purpose of this study was to test whether a biphasic osteo-chondral tissue, fabricated in vitro by using the ARC method and hydroxyapatite, could undergo maturation in vivo.
Methods and Materials: Rabbit chondrocytes were cultured in alginate beads in the presence of 20% fetal bovine serum (FBS) for 2 weeks. The beads were dissolved and the cells with their cell-associated matrix were recovered and seeded onto a porous hydroxyapatite block, partially filled with sodium hyaluronate. After 2 weeks of culture, the osteochondral composite grafts were transplanted into subcutaneous pockets in 12 nude mice and evaluated histologically, immunohistochemically and biochemically after 4 and 8 weeks.
Results: The overall size of the cartilaginous tissue on the osteochondral composite graft gradually decreased, while the surface became more stiff and opaque. The proteoglycan content increased significantly in the initial 4-week time interval, but decreased thereafter [0-wk: 3.62±0.6; 4-wk: 23.3±6.24 ($p<0.01$); 8-wk: 13.1±0.72 ($p<0.05$) µg/µg DNA]. The collagen content significantly increased in a time-dependent manner [0-wk: 44.8±11; 4-wk: 120±21.5; 8-wk: 199±66.2 ($p<0.05$) µg/µg DNA]. The histological and immunohistochemical examinations of the cartilaginous tissue revealed that it contained a cartilage-like matrix.
Conclusions: Biphasic osteo-chondral constructs could be formed using the ARC method and hydroxyapatite. The results indicate that this de novo technique producing an osteochondral composite with potential for the surgical repair of an osteochondral defect.

21.4

One-year follow up of osteo-chondral regeneration in rabbit by scaffold free mesenchymal stem cell implantation

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Purpose: We've previously reported the development of scaffold free cell delivery system, and showed good healing of rabbit osteochondral defect by implantation of mesenchymal stem cells (MSCs) until 12 weeks. Deterioration of the repaired cartilage with time, however, is always concerned since reports on the long term studies showed extensive fibrillation of repaired articular surface. The purpose of this study is to evaluate the efficacy of the osteochondral regeneration until one year after implantation.
Methods and Materials: MSCs were obtained from Japanese white rabbit. The MSC were molded into cylinder like shape as previously reported. Osteo-chondral defect was created in the same rabbit knee and the plug was transplanted without patch. The knees were immobilized for one week. The rabbits were euthanized at 24, and 52 weeks after operation. The specimens were scanned with micro CT (MCT), decalcified, and stained with Safranin O, anti-collagen type II.
Results: At 24, and 52 weeks, all surface area of the defect was covered with hyalin cartilage. Stiffness and smoothness of the repaired area were almost the same as normal area. Histologically, both GAGs and type II collagen were detected in repaired cartilage. MCT showed ossification was detected only in the subchondral bone level; yet, new bone formation was not completed.
Conclusions: In this study, our regenerated cartilages were maintained smooth surface and hyaline cartilage phenotype until 52weeks after implantation. In conclusion, our data showed little deterioration of the regenerated cartilage until 52 weeks, and our system may useful for clinically osteochondral regeneration and good tool for research.