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Autologous nasal chondrocytes and a cellulose-based hydrogel for the repair of articular cartilage defects

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Purpose: Articular cartilage has low capacities for spontaneous repair. To promote the repair of this tissue, amplification and transfer of autologous chondrocytes using a three-dimensional matrix appear promising. In this attempt, we developed a self-setting and three-dimensional matrix consisting in a silanized cellulose derivative (Si-HPMC). In previous works, we showed that our hydrogel enabled the proliferation of human and rabbit chondrocytes, the production of sulfated GAG and the expression of the main chondrocytic markers during a three-dimensional culture in vitro. In parallel, the capacity of our hydrogel to allow the formation of a cartilaginous tissue in vivo was determined by transplantation of human nasal chondrocytes and Si-HPMC in subcutaneous pockets in nude mice. Therefore, we sought to evaluate the preclinical interest of autologous chondrocyte transplantation with Si-HPMC in rabbit articular cartilage defects.
Methods and Materials: After harvesting and amplification during 4 weeks, nasal autologous rabbit chondrocytes were transplanted with Si-HPMC in critical-size defects created in rabbit articular cartilage. Implants were histologically characterized for the presence of sulfated GAG (Alcian blue staining) and collagen (Masson's trichrome staining). The presence of type II collagen was investigated by immunostaining.
Results: After a 6-week implantation, histological analysis of implants revealed the formation of a repair tissue exhibiting a histological organization similar to that of healthy articular cartilage with a positive staining for sulfated GAG and total collagen. Immunohistological analysis of type II collagen showed that the repair tissue was hyaline-like cartilage.
Conclusions: Our results indicate that Si-HPMC hydrogel appears a potential scaffold for the cellular therapy of articular cartilage.

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Effect of cell number on the quality of the repair tissue in Autologous Chondrocyte Transplantation (ACT) in an in-vivo model

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Purpose: Until now it is still unclear if the number of implanted cells in autologous chondrocyte implantation (ACT) has an effect on the quality of the repair tissue. To address this issue we performed ACT with different cell numbers in an in-vivo model of human articular defects.
Methods and Materials: Human full-thickness (2-4mm) articular cartilage discs, 10mm in diameter, attached approximately 3mm of subchondral bone, were obtained from human femoral heads upon joint replacement. Chondral defects (2 and 4 mm in diameter) were set without violating subchondral bone. Human chondrocytes derived from femoral heads were isolated and cultivated for up to 3 passages. The defect was completely filled with a cell suspension of either 10^6 or 10^7 cells/ml and cells were allowed to adhere to the cartilage inner surface. Defects were covered with human periosteum fixed with a drop of fibrin sealant. Discs were implanted subcutaneously in the back of nude mice for 5 and 8 weeks. The repair tissue was evaluated by histology with analysis of cell number, cell morphology, spatial orientation and production of extracellular matrix.
Results: The concentration of 10^7 cells resulted in significant increase in the quality of repair tissue over time with differentiated chondrocytes in chondron-like structures and formation of proteoglycane-rich extracellular matrix. In comparison the samples with lower cell-numbers revealed fibrous or fibro-hyaline tissue with less differentiated chondrocytes and extracellular matrix.
Conclusions: The quality of the repair tissue after ACT in respect of cell differentiation and formation of proteoglycane-rich extracellular matrix is significantly dependent on the number of implanted cells.

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Cell-free scaffold implantation in the horse

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Purpose: The principle of matrix associated autologous chondrocyte transplantation (MACT) is to deliver chondrocytes associated with a biomaterial to the cartilage defect. Up to now it is still a question to which extent cells from the surrounding tissues participate to the final repair tissue. In order to investigate the amount of cells invading the transplants, horses were treated with cell-free MACT-scaffolds.
Methods and Materials: Cell-free biomaterials as a collagen type I gel (CaReS®), a collagen type I/III membrane (Biogide®), and a hyaluronan scaffold (Hyalograft®C) were implanted into the lateral facette of the femoropatellar joint of two horses. The regenerative tissue was taken 1.5 years after implantation and analysed by histochemical, immunhistochemical, ultrastructural methods and element analysis (EDAX).
Results: In all defects fibrous tissue was formed. The matrix showed a certain degree of cartilage-like organisation such as the vertical orientation of collagen fibres and a superficial layer of lubricin-like granular material. However, the tissue was negative for collagen type II and poor in proteoglycans. The matrix was well infiltrated by cells which sometimes showed signs of differentiation such as spherical cell morphology and a pericellular matrix. Both, regular and uneven cell-distribution were observed. Signs of cell invasion were found at the border to the native cartilage as well as at the transplant surface.
Conclusions: The results of the cell-free scaffold implantation showed that invading cells strongly participate to tissue formation. The formed tissue is not hyaline-like and non-uniform. The implantation of cell-free scaffolds cannot be used for cartilage repair in the horse model.

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Validation of a transport system for tissue engineered products

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Purpose: The aim was to develop an optimal transport system for tissue engineered constructs.
Methods and Materials: Chondrocytes were isolated, culture expanded and seeded in Hyaff-11 scaffolds. After 14 days of culture the constructs were packed in a transport system. The primary packing consisted of a 2-container system that assures the viability and sterility. The secondary packaging was a plastic cylinder approved for transport of biohazards material. The outer packaging consisted of a polystyrene box and gel packs. The boxes were then placed in ambient temperature on a shaking plate for 48h. During the 48 hours the box was also turned up side down three times to mimic a real transport situation After the simulated 48 hour transport the constructs were tested for handling properties, viability and its histoarchitecture.
Results: No difference in handling properties of the constructs was seen between before and after the 48 hours simulated transport. All constructs were stable and showed no signs of disruption. The viability was controlled by staining the constructs with a Live-Dead staining. Only viable cells (green) was seen and no signs of dead cells. There was no difference before or after the simulated transport. The Histology showed cells through the whole membrane and matrix production within the membrane and no disruption of the construct. No difference was seen before and after the simulated transport.
Conclusions: We show that we have a transport system that ensures a good transport of the tissue engineered construct between the GMP laboratory and the hospital.