results indicate that the amyloid structures could potentially be used for cartilage tissue engineering. The next steps include culturing chondrocytes with amyloid structures in a 3D hydrogel to provide a more realistic surrounding to the cells and to preserve the chondrocyte phenotype. Microscopy image of bovine chondrocytes (dark) in culture with α-synuclein amyloid aggregates (transparent). The dark color of the cells is caused by formazan crystals as a result of the MTT assay. Scale bar represent 100 μm.

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THE CANINE MODEL TO TEST FUNCTIONAL TISSUE-ENGINEERED CARTILAGE

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Purpose: Our laboratory has adopted a chondrocyte-seeded collagen sponge system for tissue-engineering of cartilage. We have recently developed an efficient procedure to amplify human chondrocytes in culture with a cocktail of fibroblast growth factor (FGF)-2 and insulin (FI). This cocktail allows the amplified chondrocytes to build a cartilage matrix in collagen scaffolds, in the presence of bone morphogenetic protein (BMP)-2, insulin and triiodothyronine (BIT). Here, we investigated if the dog model can be used to test in vivo implantation of the cartilage construct for resurfacing articular cartilage defects.
Methods: Osteochondral blocks were harvested from cadavers of skeletally mature male beagle dogs. Lateral and medial femoral condyles (weight bearing sites) and femoral trochlea (non weight bearing site) were processed for immunohistochemical analyses. Several primary antibodies against collagen proteins were screened on tissue sections, with particular attention given to their ability to specifically recognize collagen molecules characteristic of cartilage (type II collagen) or bone and fibrotic tissues (type I collagen). For in vitro reconstruction of tissue-engineered cartilage, isolated canine articular chondrocytes were amplified with the FI cocktail then cultured for 3 weeks in collagen sponges with the BIT cocktail. The neo-synthesized matrix was evaluated by Western-blotting and immunohistochemical analyses.
Results: We selected commercially available human anti-type I and anti-type II collagen antibodies (Novotec, France) that were able to discriminate canine type I and type II collagen proteins, as judged by the mutually exclusive immunohistological stainings of cartilage and bone by the two antibodies. In addition we developed an original antibody capable of detecting the IIB form of type II collagen, the unique cartilage-specific isoform of type II collagen. Regarding cartilage reconstruction in the collagen scaffolds, canine chondrocytes were able to synthesize large amount of cartilage-characteristic proteins (such as type II and type IX collagens). Interestingly, our Western-blotting analysis also indicated that most of the type II collagen produced was type IIB. Very importantly, our immunohistochemical and Western-blotting analyses revealed extremely low amounts of type I collagen production.
Conclusions: The combination of the FI and BIT cocktails during expansion and culture of adult canine chondrocytes in collagen scaffolds led to the formation of cartilage whose biochemical composition closely resembles to native cartilage. Thus, canine and human chondrocytes respond similarly to the FI and BIT cocktails. We have also shown here that the biochemical quality of the cartilage matrix reconstructed by canine chondrocytes can be evaluated by the use of specific antibodies. The next step will be to test the functional properties of the tissue-engineered cartilage after implantation in the dog knee joint. We have selected the adult canine animal model because it offers several advantages: its clinical relevance with respect to anatomy, function, and clinical disorders in veterinary medicine (similar to humans, dogs lack significant intrinsic ability to heal cartilage defects and suffer from cartilage problems such as osteochondritis dissecans and OA); the ability to perform surgical treatments such as arthroplasty, chondrocyte transplantation and osteochondral grafting, as well as postoperative management protocols. These features are important for future validation and comparison of tissue engineering to the accepted clinical treatments.

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CHARACTERIZATION AND USE OF EQUINE BONE MARROW MESENCHYMAL STEM CELLS IN HORSE CARTILAGE ENGINEERING

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Purpose: Articular cartilage, is of great importance for physiological mobility. The structure and the function of this tissue, which is characterized by a poor self-repair capacity, are frequently disrupted or damaged upon physical trauma or in degenerative osteoarthritis (OA). Given that musculoskeletal disorders are the leading cause of poor performance or early retirement of sports and race horses, treating horses for these disorders is relevant. The proposed therapeutic approach, which was first developed for human, has the potential of being considered as a pre-clinical step for human medicine because the horse is recognized as an excellent model for the study of articular cartilage disorders in humans.
This study aims to improve the Autologous Chondrocytes Implantation (ACI) technique by using Mesenchymal Stem cells (MSCs) from bone marrow (easy to collect in horse) as the cell source. Thus, MSCs were, first, characterized before being cultured with chondrogenic conditions in order to find the combination that best enhances and stabilizes the characteristics of the chondrocyte phenotype, in order to perform clinical trials in horse.
Methods: MSCs from equine bone marrow were isolated and expanded in monolayer cultures until 4 passages. These cells were characterized by analyzing their proliferative potential, their pluripotency (with microenvironmental stimuli) and their capacity to express MSCs specific phenotypic markers defining the MSCs (flow cytometry). In parallel, MSCs differentiation in chondrocytes was accomplished via a combinatory approach based on the association of 3D-culture in type I collagen sponges, low oxygen tension with chondrogenic factors (BMP-2, TGF-β1) and RNA interference (siRNA to down-regulate type I collagen and HtrA1 protein expression). Finally, an extensive analysis at gene and protein levels corresponding to differentiated, dedifferentiated and hypertrophic chondrocyte phenotypes was performed.
Results: Our results show a very high proliferation potential of MSCs isolated from equine bone marrow. Furthermore, the isolated cells satisfy the various criteria of stem cells definition (pluripotency, expression of surface markers). In addition, siRNAs targeting equine Col1a1 and Htra1 have been functionally validated. Finally, we show that the BMP-2 and TGF-β1 combination strongly induces the differentiation of MSCs in chondrocytes. Thus, the combined use of specific culture conditions defined within the laboratory, with specific growth factors and siRNAs association leads to the in vitro synthesis of a hyaline type neo-cartilage by chondrocytes which present an optimal phenotypic index, comparable to the one established with induction of specific differentiation of human MSCs in chondrocytes, and close to the one of differentiated/healthy chondrocytes.
Conclusions: These data represent a first step in the development of equine clinical trials, which are planed - to better understand the reaction of cartilage tissue after a few weeks of intra-articular implantation and - to make the proof of concept in a large animal model. This approach will allow us to explore the criteria necessary to begin to consider the development of cell therapy for cartilage repair in humans.