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MENISCAL REPLACEMENT IN CANINE MODEL USING AUTOLOGOUS SYNOVIUM-DERIVED STEM CELLS-SEEDED SCAFFOLD

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Purpose: Meniscus is one of the most frequently injured structures in the knee joint. Loss of meniscal tissue resulting from meniscal tear and meniscectomy leads to cartilage degradation and osteoarthritis, but all the treatment modalities such as meniscectomy, meniscal repair, and even meniscal transplantation have their own weaknesses, and thus tissue engineered meniscal substitutes are being investigated for their possible role in meniscus-injured or deficient knees. We evaluated regeneration of meniscal tissue using poly(ε-caprolactone) (PCL) scaffold in vitro and also in rabbit model [1,2,3]. Synovial cells have been reported to have high proliferation and multi-lineage-differentiation potential. Enhancement of meniscal healing by synovial flap has already been reported. In this study, we have seeded cells on collagen-coated PCL scaffold in vitro and observed cellular proliferation and matrix formation for normal meniscus-approximated tissue formation. We also transplanted meniscal implant seeded with or without autologous synovial stromal cells and evaluated meniscal tissue regeneration in canine model.
Methods: *Engineering Meniscal Prosthesis:* The scaffold was fabricated with poly(ε-caprolactone) (PCL: mw ~80,000) with a pore size between 425 and 500μm. The dog-meniscus shaped prosthesis was produced by melt-molding particulate-leaching method, which was designed after original dog meniscus. Type I collagen was coated on the scaffold to enhance the cell attachment. The porosity and the compression strength was measured. *In Vitro Cell Seeding Study:* Cells were obtained from synovium and meniscus from New Zealand white rabbits. Synovial cells and meniscal cells were seeded onto meniscal scaffolds and incubated at 37°C and 5% CO_2. Cell-seeded scaffolds were harvested for assays on day 1, 3, 7, 14. Assays included cellular proliferation, live cell number, glycosaminoglycan synthesis, microscopic histology, and scanning electron microscopic examinations. *In Vivo Implantation:* Synovium was harvested from the knee joint of a mongrel dog. Stromal cells were isolated and expanded for 4 weeks and passage 2 cells were used for seeding onto the scaffold with the density of 1×10^7cells/ml. At 1 week after seeding, cell-laden scaffold was implanted in the medial meniscal defect created in the right knee joint of the dog. In the left knee joint, cell-free scaffold was implanted in the same defect for comparison. **Results:** The scaffold has the suture strength of 2.4kg for 3× 7mm bloc, and the compression modulus was 400kPa. Both synovial and meniscal cells adhered and proliferated during the 14-day culture period. The number of synovial cells was significantly greater than that of meniscal cells on day 14. GAG production normalized to DNA content was not different between the two groups during the culture period. Both cells generated matrix on PCL scaffold and scaffolds seeded with synovial cells showed more even and abundant distribution of cells and matrices than those with meniscal cells. Scaffolds implanted in dog knee joint showed significant fibrous tissue formation and the finding was more distinct with the synovial stromal cell seeded scaffold. At 4 weeks, regenerated tissue replaced the meniscal defect and formed firm attachment between the implanted material and the peripheral rim of residual meniscus. Scaffold was not yet resorbed and no visible change on the chondral surface was observed.
Conclusions: In vitro study showed that synovial cells proliferated more rapidly than meniscal cells without the difference of capacity in matrix production. We also demonstrated the feasibility of meniscus-shaped PCL scaffold for meniscal regeneration in canine model. Preincubation of the scaffold seeded with synovial cells before implantation may have the advantages compared with the cell-free scaffold.

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ANAPHYLATOXINE RECEPTORS AND COMPLEMENT REGULATORY PROTEINS IN TENDON: INTERRELATION WITH IMMUNOREGULATORY CYTOKINES

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Purpose: Tendon injury induces an inflammatory response, mediated by pro-inflammatory cytokines. The complement cascade might be involved in tendon healing and scar formation. Hence, the aim of this study was to analyze whether key complement components are expressed in tendon as well as in cultured tenocytes and regulated by immunoregulatory cytokines.
Methods: The gene expression of the anaphylatoxine receptors (C3aR, C5aR) and the cytoprotective complement regulatory proteins (CRPs): CD35, CD46, CD55, CD59 was analysed in human tendon, cultured primary tenocytes and to directly estimate the general expression level, also in human blood-derived leukocytes (peripheral blood mononuclear cells: PBMCs and neutrophile granulocytes). Further, their time-dependent regulation by tumor necrosis factor (TNF)α, interleukin (IL)-6, IL-10 alone or TNFα combined with either IL-6 or IL-10 (10 ng/mL, 6, 24, 48 h) was assessed by RTD-PCR in cultured tenocytes. Protein expression of C5aR and CD55 was studied using immunohistochemistry in tendon and cultured tenocytes. Tenocytes were identified by scleraxis and tenomodulin expression.
Results: C3aR, C5aR, CD46, CD55 and CD59 mRNA was expressed in tendon and cultured tenocytes, whereas CD35 expression could only be detected in tendon tissue and leukocytes. Complement expression was generally higher in tendon compared with that in cultured tenocytes. The anaphylatoxine, CD35 and CD55 gene expression level was superior in neutrophiles and PBMCs compared with the expression in tendon. On the contrary, the CD46 and CD59 gene expression in tendon exceeded the levels observed in the studied leukocytic cell types. In response to cytokine stimulation, the anaphylatoxine receptors and CRPs revealed a time-dependent expression profile in cultured tenocytes: The C3aR gene expression was up-regulated by TNFα alone and in combination with IL-6 or IL-10 at all investigated time points. C5aR expression was suppressed by TNFα treatment combined with IL-6 (24 and 48 h) or with IL-10 (48 h) treatment, but not at 6 h. IL-6 alone impaired significantly the CD46 and CD55 expression (6 and 48 h) as well as that of CD59 (24 h). At 6 h, TNFα/IL-6 and TNFα/IL-10 co-treatment suppressed significantly the CD46 and CD55 gene expression. Additionally, C5aR and CD55 protein expression was demonstrated in tenocytes.
Conclusions: Both, the anaphylatoxine receptors which bind the complement cleavage fragments C3a and C5a thus triggering responses to complement activation as well as the cytoprotective CRPs are expressed in tendon and tenocytes. Tendon, a typical bradytrophic connective tissue, displays a unique complement expression profile and tenocytes might contribute to complement homeostasis in tendon. The generally weaker expression of complement in cultured tenocytes compared with the native tissue might be caused by the absence of systemic inductive stimuli during culture as well as by the presence of a certain degree of other cell types in tendon. The time-dependent complement regulation by immunoregulatory cytokines in tenocytes might have relevance in tendon injury, healing and scar formation. Further studies should assess complement activity, the interplay with other systemic regulatory factors and consequences of complement inhibition in tendon.

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MENISCUS CELLS PHENOTYPE: DIFFERENCES FROM YOUNG TO ADULT IN SWINE

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Purpose: The meniscus plays an important role in the biomechanics of the knee joint. This tissue has a poor healing potential, partly due to the absence of vasculature in the inner portion. Due to the presence of both collagen I and II, the meniscus has properties of fibrous and cartilaginous tissue. The term fibrochondrocytes has been introduced to identify the meniscus cells: their phenotype is known to be close to that of chondrocytes in the inner meniscus and fibroblasts in the external area, but a clear distinction between the different phenotypes with particular association to their development is still missing. This work was aimed to study the characteristics of swine meniscus cells by analyzing three different areas of both young and adult meniscus: the inner avascular zone, the intermediate and the external vascular zone.
Methods: Meniscus cells morphology, proliferation and gene expression were evaluated.
Results: The data showed that in the young meniscus the inner region is composed of cells that are similar to chondrocytes in terms of morphology but still immature in terms of gene expression; the intermediate and exter-