Outcome of Pre-Differentiated Mesenchymal Stem Cell-Loaded Hydrogel Implants in Chronic Osteochondral Defects in Sheep Model

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Introduction
The use of mesenchymal stem cells (MSCs) to treat osteochondral defects caused by sports injuries is of particular interest. However, there is a lack of large animal models studying the benefits of chondrogenic pre-differentiation in vitro for repair of osteochondral defects. We hypothesized that chondrogenic in vitro pre-differentiation of MSCs embedded in a collagen-I-hydrogel currently in clinical use for matrix-associated autologous chondrocyte transplantation (MACT) would facilitate the regeneration of a chronic osteochondral defect in ovine stifles.

Materials and Methods
An osteochondral incision (Ø 7 mm) was made in an initial operation on the sheep knee and bone marrow aspirate was obtained from the iliac crest of 18 merino sheep. The isolated and expanded MSCs were cultured with 0.5x10⁷ cells/ml in a collagen-I-hydrogel. One part of the MSC gels was pre-differentiated with chondrogenic medium with 10 ng/ml TGF-β3, whilst the other part of the gels was not pre-differentiated. The implantation of the grafts then followed in the chronificated defect 6 weeks after defect creation. The knee joints were explanted and investigated macro- and histologically according O’Driscoll and the ICRS-VHAS after 6 and 12 months. For detecting differences (p<0.05) non-parametric Mann-Whitney-U Test was used.

Results
Gene expression, immunohistology and measurements of s-GAG enabled the successful demonstration of the chondrogenic in vitro pre-differentiation. After 6 months in vivo the pre-differentiated MSC hydrogel implants showed the best results and yielded an O’Driscoll Score of 17.38 and an ICRS VHAS of 14.25 (Fig. 1) by displaying a defect repair with morphologic characteristics of hyaline cartilage such as columnarization, presence of collagen II and marginal expression of collagen I (Fig. 2). No differences were found in histological scores between 6 and 12 months concerning both groups.

Discussion and Conclusions
The collagen-I-hydrogel implants based on pre-differentiated autologous MSCs lead to a partial hyaline type structure of the regeneration matrix after 6 and 12 months and show significant better results compared to undifferentiated MSC-loaded grafts. MACT with pre-differentiated MSCs may be a promising approach for repair of focal, chronic osteochondral defects.

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Fig. 1. O’Driscoll and the ICRS-VHAS histological scoring after 6 months (p<0.05).

Fig. 2. Representative toluidine blue stained sections of the repaired osteochondral defects 6 months after treatment.