Transplantation of nasal chondrocytes with an innovative self-setting hydrogel for cartilage repair

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INTRODUCTION: Cartilage is a specialized connective tissue that consists of relatively few cells embedded in an extracellular matrix. It is well known that the intrinsic repair capacity of articular cartilage is limited. 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 of a silanized cellulose derivative (Si-HPMC). In previous works, we showed that Si-HPMC enabled the proliferation of articular chondrocytes and the expression of the main chondrocytic markers during a three-dimensional culture *in vitro*(1). The aim of the present work was to determine whether this hydrogel could be a suitable scaffold for nasal chondrocytes-based cartilage engineering. We therefore tested the ability of nasal chondrocytes associated with Si-HPMC to allow the formation of a cartilaginous tissue in nude mice subcutaneous pockets and rabbit articular cartilage.

MATERIALS AND METHODS: The synthetic scaffold consisted of a reticulated hydrogel of silanized hydroxypropylmethyl cellulose (Si-HPMC). Primary chondrocytes isolated from human nasal cartilage (HNC) were investigated for cellular viability (MTS assay) and proliferation assay in contact with the hydrogel. To assess cell viability within hydrogel, cells were stained with cell tracker green (CTG) and observed in confocal microscopy. To assess the influence of Si-HPMC on chondrocytic phenotype, specific hallmarks of chondrocytes (type II Collagen and aggrecan) as well as type I collagen were investigated at the level of mRNA using RT-PCR. The sulfated glycosaminoglycans (GAG) synthesis in three-dimensional culture was investigated by Alcian blue staining. The ability of Si-HPMC to form a cartilaginous tissue was evaluated by *in vivo* transplantation of human nasal chondrocytes associated with Si-HPMC in subcutaneous site of nude mice for three weeks. The preclinical interest of nasal chondrocytes transplantation was thereafter investigated in rabbit. Rabbit nasal autologous chondrocytes (RNAC) were amplified *in vitro* during 4 weeks before transplantation with Si-HPMC in defects created in rabbit articular cartilage for 6 weeks. Implants were histologically characterized for the presence of GAG (Alcian blue staining) and collagen (Masson's trichrome staining). Cell morphology was observed with haematoxylin/eosin staining. The presence of type II collagen was investigated by immunostaining.

RESULTS: Results showed that our hydrogel enabled the proliferation of HNC, the production of sulphated GAG and the expression of the main chondrocytic markers during a three-dimensional culture *in vitro*. Histological analysis of human nasal chondrocytes transplanted in nude mice during three weeks revealed the production of a cartilage-like extracellular matrix containing GAGs and collagen. The presence of chondroid nodules positively stained for type II collagen confirmed the formation of a hyaline cartilaginous tissue

Histological analysis of RNAC transplanted in an articular cartilage defect revealed the formation of a repair tissue exhibiting a histological organization similar to that of healthy articular cartilage. Immunohistological analysis of type II collagen showed that the repair tissue was a hyaline-like cartilage.

DISCUSSION & CONCLUSIONS: Our results demonstrate the *in vitro* bioactivity of Si-HPMC with respect to HNC. Considering the donor site morbidity occurring after harvesting articular cartilage during standard repair procedures, our data suggest that nasal cartilage is a clinically relevant cell source for tissue engineering. This study also demonstrates the potential of hybrid construct associating nasal chondrocytes with Si-HPMC for engineering cartilage. Whether this hybrid constructs may be able to support the formation of a biomechanically competent cartilage remains to be considered.

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