Efficient Re-differentiation of De-differentiated Chondrocytes in Heparin-Based Hydrogel : *In-vitro* and *In-vivo* Study

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Statement of Purpose: Tissue engineering approach is one of the promising ways for regeneration of articular cartilage. Even though chondrocyte expansion by monolayer culture *in vitro* is essential to get sufficient cells for cartilage repair, expanded chondrocytes easily lose their chondrogenic phenotype leading to de-differentiation. Previously, we found that a heparin-based hydrogel is a promising matrix for 3-D culture of primary chondrocytes.[1] In this study, we characterized and optimized the efficiency of the heparin-based hydrogel for inducing the re-differentiation of de-differentiated chondrocytes. Then, we evaluated the cartilage tissue formation of the heparin-based hydrogel containing de-differentiated chondrocytes in *in vivo* by subcutaneous implantation in nude mice and by implantation to partial defect site in rabbits.

Methods: Heparin-based hydrogels were prepared by a Michael-type addition reaction between thiolated heparin and diacrylated poly (ethylene glycol)[2]. Isolated chondrocytes, from knee cartilage of New Zealand white rabbits, were sufficiently expanded in monolayer culture, leading to de-differentiation, and then were cultured in the heparin-based hydrogels under a normal cell culture condition (DMEM with 10 % FBS only) without any chondrogenic factors. We characterized the effect of initial precursor concentration of these hydrogels on the re-differentiation of chondrocytes and the GAG production to find the optimized condition for chondrocyte culture *in vitro*. In addition, we also characterized the proper *in-vivo* chondrogenesis by subcutaneous implantation of the cell/hydrogel construct in mice and by applying to a partial defect model in young rabbit knee joint cartilage.

Results: Results showed highly efficient re-differentiation of the chondrocytes for all polymer concentrations. Among them, 10 wt % hydrogel was the most optimal concentration for re-differentiation *in vitro*; showing significantly faster and higher expression of Type II collagen and aggrecan in real time PCR **(Figure 1)** and immunostaining experiments as well as an enhanced deposition of glycosaminoglycan (GAG). The *in-vivo* culture of de-differentiated chondrocytes in the 10 wt % heparin-based hydrogel also showed the proper chondrogenesis of the implant, and the accelerated healing of cartilage defect **(Figure 2).**

Conclusions: Completely de-differentiated chondrocytes were effectively re-differentiated and produced GAGs and ECMs *in vitro* within a week without addition of any growth factors or chondrogenic components in the culture medium when cultured in heparin-based hydrogels. Effective cartilage regeneration by the heparin-based hydrogel containing de-differenriated chondrocytes was also confirmed *in vivo*.

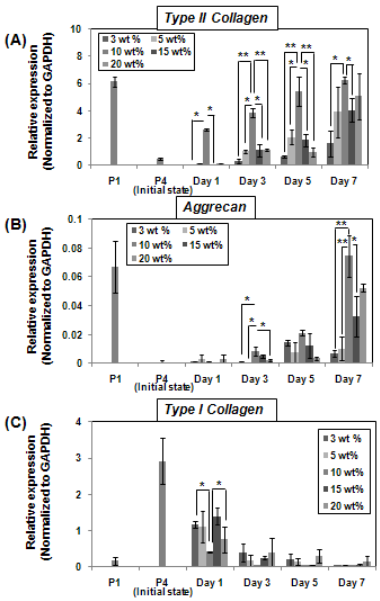

Figure 1. Real-time RT-PCR analyses *in vitro*. *p<0.05, and **p<0.001

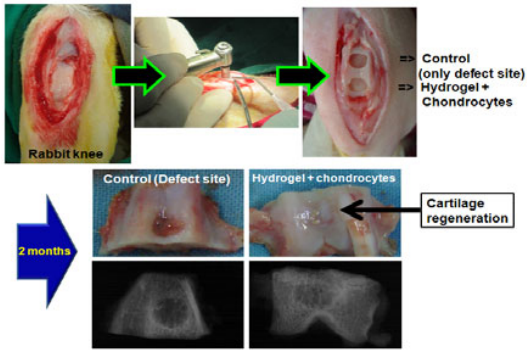

Figure 2. Implantation of cell/heparin-based hydrogel construct to partial defect site in rabbit knee and extraction of construct after 2 months.

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References:

1 M,Kim. et al, Tissue Engineering C 2010; 16:1-10
2. Tae, G. et al, Biomacromolecules 2007;8:1979-1986