Injectable chitosan-Pluronic® hydrogel for cartilage regeneration

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Statement of Purpose: Recently, cartilage regeneration using scaffolds such as PGA, PLLA, PLGA, and polysaccharide-based hydrogels have been in receipt of much attention in tissue engineering. Especially, chitosan-based hydrogel is a good candidate for cartilage regeneration, since it share some characteristics with a variety of glucosaminoglycan (GAG), which is one of the major macromolecules found in articular cartilage. GAG plays a critical role in regulating expression of the chondrocytic phenotype and in supporting chondrogenisis. Injectable applications using thermosensitive hydrogel have received a considerable attention for cartilage regeneration due to patient's comfort by ease of applications.

In this study, we investigate *in vitro* evaluation of injectable chitosan-Pluronic® (CP) hydrogel using bovine chondrocyte for cartilage regeneration, based on the minimal invasive technique, and study *in vitro* release behavior of bone morphologic protein (BMP).

Methods: CP copolymer was prepared by coupling Pluronic® with chitosan using EDC/NHS chemistry at room temperature for 24hrs. The physico-chemical properties of synthesized CP copolymer were characterized by ^{1}H-NMR, FT-IR, and TGA. The sol-gel transition behavior of aqueous polymer solution was investigated by vial tilting method. Cell viability test of CP hydrogel was performed by MTS assay, including alginate gel as a control group (3D cell culture). In addition, dimethylmethylene blue (DMB) assay, RT-PCR, and live/dead staining assay were performed to confirm amount of GAG contents, revelation of gene, and cell viability, respectively. Furthermore, *in vitro* protein release study was investigated using BMP-7. For *in vitro* BMP-7 release study, the proteins were simply mixed with the CP solution as ambient temperature and the release behaviors of the proteins from the hydrogel matrix were investigated at predetermined time interval.

Results/Discussion: The chemical structure of CP copolymers was characterized by ^{1}H-NMR, FT-IR, and TGA. Its aqueous solution showed the sol-gel transition behavior around body temperature. In the result of MTS assay using bovine chondrocyte, injectable CP hydrogel was more biocompatible than alginate gel as shown in Figure 1. In results of RT-PCR and live/dead staining assay, gene expression and number of cells of CP hydrogel were more increased than those of alginate gel, respectively. Figure 2 shows the results of DMB assay. Amount of GAG contents in hydrogel matrix was increased significantly by contrast with alginate gel. These results demonstrate that chitosan moiety of injectable CP hydrogel could enhance the cell-compatibility. In BMP-7 release test, BMP-7 has shown the sustained release behavior from CP hydrogel.

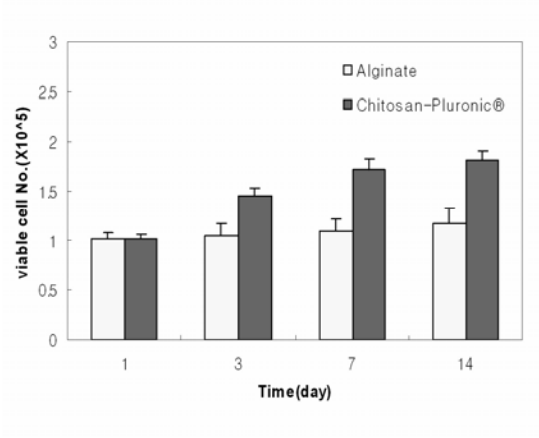

Figure 1. The MTS assay result of CP hydrogel

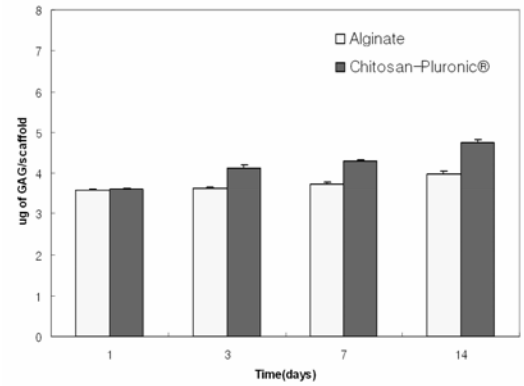

Figure 2. The DMB assay of CP hydrogel

Conclusions: Injectable CP hydrogel as a cell-supported scaffold and growth factor carrier was developed. The obtained results suggest that CP hydrogel has a potential for cartilage regeneration and it can be also useful as a new biomaterial for various biomedical applications.

References:

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