Pluronic-based Hydrogel in Chondrogenesis of Adipose-derived Stem Cells

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Statement of Purpose

Stem cell-based tissue engineering represents a promising solution for the repair of cartilage defects. Among the multiple sources of stem cells, adipose-derived stem cells (ASCs) are useful in cartilage tissue engineering, due to their relatively easy accessibility and abundance in quantity. To induce chondrogenic differentiation of ASC, an appropriate 3D environment is critical. Due to the injectable property, Hydrogels possess a great potential in tissue engineering. Among them, Pluronic hydrogels are thermosensitive and would change their structure and physical property in response to the surrounding environment, temperature.[1] They are nontoxic poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) triblock copolymers (F127) that can undergo a sol-gel transition at proper concentration. Given the capability of transforming growth factor (TGF)-β1 to direct chondrogenic differentiation of stem cells, we developed TGF-β-incorporated Pluronic system and postulated that it might provide ASCs with a suitable environment toward chondrogenesis. In this study, Pluronic-based hydrogel was synthesized and evaluated for its chondrogenic potential *in vivo*.

Materials and Methods

A Pluronic derivative, F127-glycolide-4-methacryloxyethyl trimellitic anhydride (META) (F127-G5-META, FGM), was synthesized. Once heparin was grafted onto it, heparin-grafted Pluronic (FGM-Hep) was subjected to the incorporation of TGF-β1 to produce FGM-Hep-TGF-β1. The chemical structure of the resultant F127 derivative block copolymers was analyzed by FTIR, GPC, and ^{1}H and ^{13}C NMR. Sol-gel transition and particle size of the copolymers were characterized by tube tilting method and light scattering, respectively. For the quantitative assay of heparin, toluidine blue method was used. TGF-β1-incorporated hydrogel was then tested for chondrogenic differentiation of ASC. Human ASCs were isolated from human adipose tissue obtained by suction-assisted lipectomy (SAL) and cultured in Dulbecco's Modified Eagle Medium (DMEM), supplemented with ITS^{+}, ascorbic acid, dexamethasone, and TGF-β1. To improve the long-term physical stability of F127, hyaluronic acid (HA) was crosslinked and used with F127. This HA/F127 hydrogel was mixed with ASCs and subjected to gelation with the increased temperature. The cell-encapsulated hydrogels such as HA/F127, HA/F127/FGM-hep, and HA/F127/FGM-Hep-TGF-β1, respectively, were then implanted into nude mouse for 3 weeks and evaluated for cell viability, cell number, histology, and gene expression of chondrogenic markers.

Results and Discussion

The thermosensitive FGM-Hep- TGF-β1 triblock copolymer was successfully synthesized. The pattern of sol-gel transitions was not significantly different among the F127 derivative copolymers (Fig. 1). The gelation was noticed at the polymer concentration of 16% at 37°C. The release profile of the growth factor showed that the incorporated TGF-β1 was gradually released up to 30 days from the hydrogel system (data not shown). From histological analysis, a sign of chondrogenic differentiation *in vivo* was identified with Safranin O staining (Fig. 2). Retrieved at 3 weeks, the growth factor-containing hydrogel (FGM-Hep-TGF-β1) appeared to be better in the induction of chondrogenesis of ASCs than the F127/HA control. This study suggested that F127-based hydrogel might be useful as an injectable tissue-engineered scaffold for *in vivo* chondrogenesis of ASC.

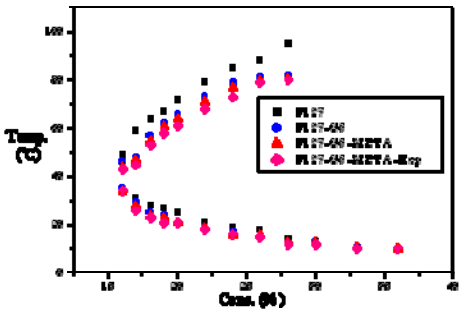

Fig. 1. Sol-gel transition of F127 derivative copolymers.

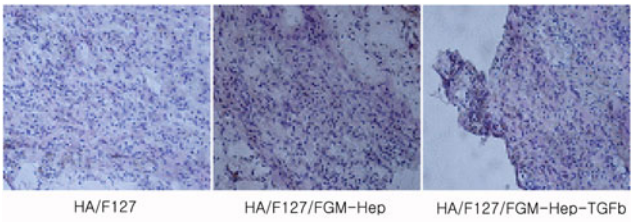

Fig. 2. Safranin O staining of the implanted hydrogels for 3 weeks.

Acknowledgment

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Reference

1. K. Y. Lee and D. J. Mooney, "Hydrogel for Tissue Engineering", *Chem. Rev.*, 101, 1869-1879 (2001).