Efficacy of Pluronic/Hyaluronan Composite Hydrogel in Chondrogenesis of Human Adipose-Derived Mesenchymal Stromal Cells

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Introduction: Thermosensitive, injectable hydrogels are of particular interest in clinics, due mainly to their minimally invasive nature for human applications. Hydrogels have a structural similarity to the natural macromolecules and they are generally recognized biocompatible and are useful in drug delivery and in tissue engineering.[1,2] Hydrogels are often physically mixed with growth factors to elicit specific cellular responses, such as differentiation of mesenchymal stem cells (MSCs). In addition, bioactive molecules-loaded microparticles are encapsulated in hydrogels or hydrogel itself is chemically modified to conjugate biomolecules. In this study, Pluronic F127 is chemically modified in order to anchor a chondrogenic growth factor, transforming growth factor-beta 1 (TGF-β1). To support its poor mechanical stability, crosslinked hyaluronan (HA) was physically mixed with F127. We hypothesize that while physical stability of HA/F127 is maintained, chondrogenesis is prompted in the aid of chondrogenic factors,TGF-β1 and dexamethasone (Dex), incorporated in the composite hydrogel.

Materials and Methods: Pluronic F127 (Mw: 12,600) was chemically modified through the grafting of glycolide (G) and 4-META (M), respectively. The obtained F127-G5-META (FGM) was coupled with heparin, producing FGM-hep (referred as F127 hereafter). Once HA (Mw: 1,600K) was crosslinked, two hydrogels were physically mixed in the final concentration of F127 (20%) and crosslinked HA (5%), respectively. Sol-gel transition of the composite hydrogel was tested at various temperatures by tube tilting method. Meanwhile, the heparin-bound F127 hydrogel was immersed in TGF-β1 solution in order to immobilize TGF-β1. For a HA/F127/TGF/Dex hydrogel system, Dex was simply blended with HA/F127/TGF. Release profile of TGF-β1 from the hydrogels was examined at room temperature. Human adipose-derived stem cell (ASC) was harvested from fat tissue and isolated by collagenase digestion and differential centrifugation. Passaged ASCs ($1x10^6$) were mixed with F127 and HA, in a serum-free Dulbecco's modified eagle medium (DMEM) supplemented with ascorbic acid, and ITS^+ (insulin, transferrin, and selenious acid with linoleic acid and BSA). For *in vivo* experiment, hydrogels (250 ul) were injected into nude mouse subcutaneously, with the test groups of HA/F127, HA/F127/TGF, and HA/F127/TGF/Dex, respectively. The injected hydrogels were monitored for 2 and 4 weeks. Distribution of ASCs in the hydrogel was identified using DAPI staining. Secretion of chondrogenic marker, type II collagen was evaluated using immunofluoresence. In addition, the ASC-encapsulated hydrogels were directly injected into the defects of knee articular cartilage of rabbits. After the animals were sacrificed in 4 weeks, the defect areas were harvested, decalcified and then stained using Alcian blue.

Results and Discussion: Pluronic F127 derivatives were synthesized through a series of reactions. Tested with a variety of concentrations of F127, F127/HA composite hydrogel was thermosensitive at body temperature (data not shown). Meanwhile, when the release pattern of TGF-β1 from the F127/HA/TGF hydrogel was examined using ELISA, the growth factor was continuously released for up to 20 days. (Fig. 1). When the retrieved samples from rabbit knee were stained using Alcian blue, the defect area was found significantly eroded, poorly securing the defect sites (Fig. 2a). The hydrogel-filled area appeared to be better in maintaining its original volume but a large number of empty spots were detected in the construct (Fig. 2b). The construct in Fig. 2(c) was rather intact, reserving most of the initial mass in the defect. A sign of chondrogenic differentiation was noticed from the blue stained areas, indicating the formation of cartilage matrix, GAG.

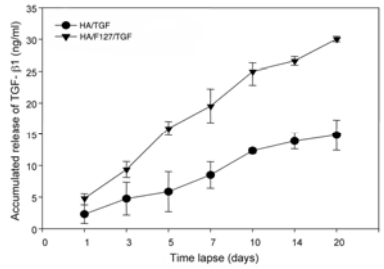

Fig. 1. Release profiles of HA/F127/TGF hydrogel.

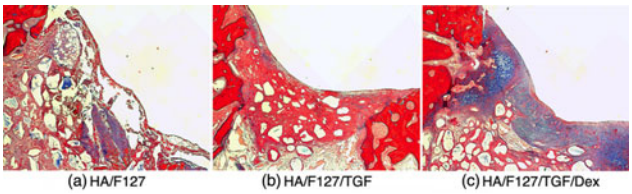

Fig. 2. Alcian blue staining of the hydrogels implanted in rabbit knee articular cartilage defects.

Conclusions: In this study, thermoreversible HA/F127 composite hydrogel was successfully manufactured. Some positive effects in inducing chondrogenic differentiation of ASC were confirmed using the chondrogenic factors-incorporated HA/F127 hydrogel.

References: 1. Na K et al. *Biomaterials*. 2007;28:2631-2637. 2. Yasuda A et al. *Tissue Eng*. 2006;12:1237-1245.

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