Enhanced Chondrogenesis of Mesenchymal Stem Cells in Collagen Mimetic Peptide-Mediated Microenvironment

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Statement of Purpose: Collagen is the most abundant extracellular matrix protein in our body and is known to have important roles in delivery of various growth factors and cartilage repair. Thus, collagen gel has been used as a scaffold for mesenchymal stem cell encapsulation and differentiation into chondrocytes. Collagen alone, however, has several problems: weak mechanical strength and large porosity due to its loose network structure, shrinkage upon implantation, and immune response in the host body (1). Previously, as an alternative, a PEODA scaffold conjugating collagen mimetic peptide has been developed and shown to retain native collagen in a hydrogel (Fig.1). Collagen mimetic peptides, -(Pro-Hyp-Gly)$_x$-, have the unique triple helical conformation of collagen and are able to physically interact with natural collagen (2,3). In this research, CMP-mediated microenvironment is suggested as an approach to provide an efficient bio-mimetic scaffold for differentiation of MSCs.

Methods:

Photoencapsulation of MSCs in hydrogel MSCs were isolated from bone marrow as previously described (4) and expanded in mesenchymal stem cell growth medium (Cambrex Bioscience). CMP, Pro-Hyp-Gly)$_7$-Tyr, and acryloly(ACRL)-PEG-CMP were synthesized as described previously (2). Control PEODA macromer solution was prepared by mixing 0.05% (w/v) photoreactive initiator (Igracune 2959; Ciba Speciality Chemicals) and 10% (w/v) poly (ethylene glycol) diacrylate (PEODA: Nektar, molecular weight: 3400 g/mol) in PBS. Macromer solution of CMP-conjugated PEODA (CMP/PEODA) hydrogel composed of 2% (w/v) ACRL-PEG-CMP and 8% (w/v) PEODA. Harvested MSCs were resuspended in the macromer solution (100 μL) at a concentration of 20×10^6 cells per mL. The solution was photopolymerized by UV exposure (EXFO Acticure 4000; wavelength: 365 nm; intensity: ~5 mW/cm^2). The hydrogel construct was cultured in chondrogenic medium with 10 ng/mL TGFβ-1 (RDI) for three weeks.

Histology and immunostaining Hydrogels were fixed overnight in 4% paraformaldehyde and prepared using standard histology techniques. Sections of a construct were stained for Safranin O, type I, II and X collagens.

Biochemical Analysis Constructs (n=3) were lyophilized and papain digested. DNA content was determined with 33258 Hoechst dye. Glycosaminoglycan (GAG) content was determined by DMMB dye, and total collagen content, by hydroxyproline assay.

Results/Discussion: Cell productions of type II collagen as well as GAG, indicators of MSC chondrogenesis, were significantly increased in CMP-conjugated PEODA construct than those in control PEODA construct (Fig.2 and 3). Moreover, decrease in type X collagen, a hypertrophy marker, in CMP/PEODA hydrogel as well as minimal expression of type I collagen, a major type of collagen in bone, suggest the efficient chondrogenic differentiation of MSCs in CMP/PEODA gel (Fig.2).

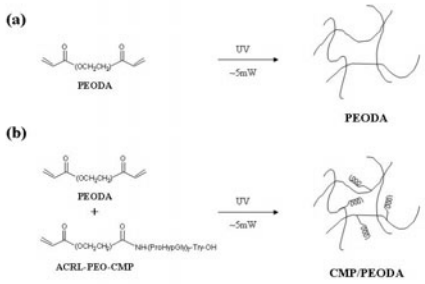

Figure 1. (a) PEODA control (b) CMP/PEODA hydrogel.

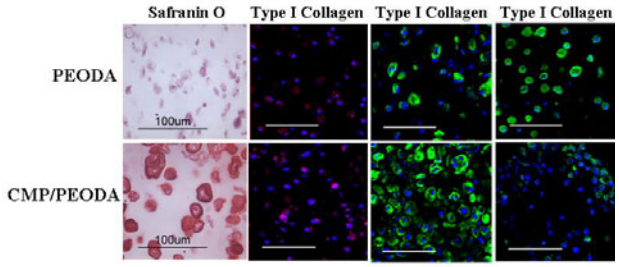

Figure 2. Histology and Immunostaining.

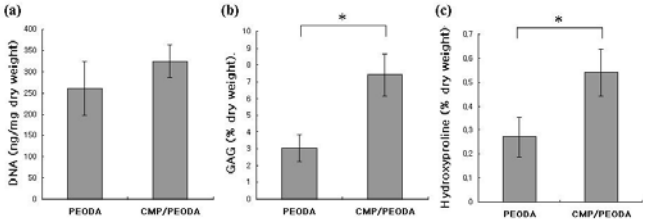

Figure 3. Biochemical Assays. (a) DNA (b) GAG (c) Hydroxyproline contents.

Conclusions: MSCs were encapsulated in CMP-conjugated PEODA hydrogel, and after three-week culture, chondrogenesis of MSCs were evaluated. Our results indicate that CMP-mediate microenvironment enhanced efficient chondrogenesis of MSCs. The CMP-conjugated PEODA scaffold using CMP collagen affinity is promising not only for cartilage tissue engineering application, but also for other tissue engineering applications that require native bio-active scaffolds.

References: (1) Aigner, T., and Stove, J. Adv Drug Deliv Rev 2003;55:1569-1593. (2) Lee, H.J., et al. Biomaterials 2006;27:5268-5276. (3) Wang, A.Y., et al. J Am Chem Soc 2005;127:4130-4131. (4) Williams, C.G.,et al. Tissue Eng 2003;9:679-688.