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In situ chondrogenic differentiation of bone marrow stromal cells in bioactive self-assembled peptide gels

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Articular cartilage is a specific tissue that lacks nerves and blood vessels and has limited self-repair abilities. Accordingly, it is necessary to develop new technology for the regeneration of cartilage to overcome therapeutic limitations. Recently, there have been several studies investigating the use of peptide hydrogel scaffolds, which are biocompatible and have low immunogenicity, for cartilage tissue engineering. In this study, we used self-assembled peptide hydrogels with repeating peptide sequences and bioactive motifs at the end of repeating sequences, which are collagen mimetic peptides (CMPs). CMPs that have a unique collagen-like triple helical conformation have been shown to associate with collagen molecules and fibers via a strand invasion process. In order to confirm the biological activities of the modified bioactive peptide hydrogels, the role of functional motifs in *in situ* chondrogenic differentiation of rabbit bone marrow stromal cells (rBMSCs) was examined. To compensate for the weaker mechanical properties of peptide hydrogels, we used poly (L-lactide-co-caprolactone) (PLCL) scaffolds, which were loaded with the self-assembled peptides into which the bioactive motifs had been incorporated. Then, we performed *in vitro* and *in vivo* analyses with the rBMSC/PLCL-peptide hydrogel complexes. The results indicated that the secretion of a cartilage-specific extracellular matrix and gene expression concerned with chondrogenic differentiation were increased by CMP motifs. In conclusion, it was confirmed that CMP-modified self-assembled peptide hydrogels could effectively enhance chondrogenic differentiation *in situ*, and, consequently, they could be a good biomaterial for cartilage tissue engineering.

[**Key words:** Collagen mimetic peptide; Self-assembled peptide; *In situ* chondrogenic differentiation; Bioactive; Bone marrow stromal cells]

The gradual increase in the aging population in modern society has resulted in a growing number of osteoarthritic patients. Articular cartilage is a specific tissue that lacks nerves and blood vessels and has limited self-repair abilities (1–3). For treatment of cartilage defects, current therapies such as medication, cartilage substitutes, and cell therapy are complicated by the formation of fibrous cartilage, which has lower mechanical strength and limited regenerative capabilities (4–6). Therefore, it is necessary to develop new technology for the regeneration of cartilage to overcome these therapeutic limitations (7–9). In cell therapy, mesenchymal stem cells (MSCs) have been utilized for tissue repair and the regeneration of cartilage in recent years, and the potential of MSCs for cartilage repair has been well documented (10,11). For the successful engraftment of injected MSCs and regeneration of tissues, cells need to be surrounded by other cells as well as many extracellular ligands and other matrix proteins in a three-dimensional (3-D) structure. These environments not only enhance interactions between cells and the extracellular matrix (ECM), but also interchange oxygen, hormones, and nutrients, and remove waste products (7,12–14).

Recently, there have been many studies on peptide hydrogels, which are biocompatible and have low immunogenicity, for potential therapeutic applications (15–21). Self-assembled peptides (SAP) are versatile biomaterials typically composed of two distinctive sides, hydrophobic and hydrophilic, allowing their self-assembly into ordered nanostructures for 3-D environments, which occur either spontaneously or in response to an exogenous stimulus (22–24). Since self-assembled peptides are composed of synthetic amino acids, they can be biodegraded by various proteases in the body and exhibit superior biocompatibility with tissues compared with other natural hydrogels derived from animals (15). Also, they do not induce an immune response and are easy to incorporate with numerous functional cellular motifs to achieve their desired function. Such properties of SAP hydrogels allow them to be utilized as biologically compatible scaffolds. These peptide hydrogels were assembled into nanofibers, which were approximately 5–10 nm in size and had 3-D nanofiber structures similar to the natural ECMs. Kisiday et al. (16) studied peptide scaffolds composed of n-KLDLKLDLKLDL-c as a model for cartilage repair. They developed a method to encapsulate chondrocytes within the scaffold and confirmed the increase of cartilage-like, ECM-rich proteoglycans and type II collagen, which are indicative of a stable chondrocyte phenotype (16). Furthermore, self-assembled peptides can be easily designed and modified in a variety of ways. A collagen mimetic

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TABLE 1. List of primers used in real-time PCR analysis of rBMSC complex-constructs.

Primer name	Forward sequence	Reverse sequence	Product size (bp)
Aggrecan	TCGAGGACAGCGAGGCC	TCGAGGGTGTAGCGTGTAGAGA	85
Type II collagen	GGCAATAGCAGGTTCACGTACA	CGATAACAGTCTTGCCCCACTT	70
Type X collagen	CAAGGCACCATCTCCAGGAA	AAAGGGTATTTGTGGCAGCATATT	70
SOX-9	TACGACTACACCGACCACCA	CTCCTCAAGGTCGAGTAGGC	217

TABLE 2. List of primers used in RT-PCR analysis of rBMSC complex-constructs.

Primer name	Forward sequence	Reverse sequence	Product size (bp)
Type II collagen	TTTCCCAGGTCAAGATGGTC	CTTCAGCACCTGTCTCACCA	358
Type I collagen	CATCTCCCCTTCGTTTTTGA	CTGTGGAGGAGGGTTTCAGA	594
Type X collagen	TGGAGTGGGAAAAAGAGGTG	GTCCTCCAACTCCAGGATCA	600
hActin	GCCCCTCCATCGTCCACCGC	GGGCACGAAGGCTCATCATT	493

peptide (CMP) composed of a specific amino acid sequence, -(Pro-Hyp-Gly)$_n$-, acts on a collagen-specific molecular hitchhiker. CMP forms a triple-helix conformation similar to the native protein structure of natural collagens. Lee et al. (25) reported that CMP could bind to natural collagen via a triple-helix strand association process, resulting in CMP-collagen interactions that help the scaffold to retain cell-secreted collagen and promote the fast accumulation of ECM products. Collagens are present in the ECM as fibrillar proteins and are related to cell behaviors such as cell proliferation, cell differentiation, and cell—cell or cell-ECM communication. In addition, Nöth et al. (26) reported that collagen hydrogels have been shown to promote chondrogenesis. However, synthetic collagen has potential limitations in immunogenicity and exhibits batch-to-batch differences (27). Therefore, rather than using synthetic collagen, we chose CMP as a natural collagen material for our experiments. In our previous works, we developed highly elastic PLCL scaffolds for mechano-active tissue engineering, and we showed that the elastic mechanical properties of the PLCL scaffolds enhanced the expression of chondrogenic-specific genes and the secretion of cartilage-specific ECMs (28,29). In this study, we

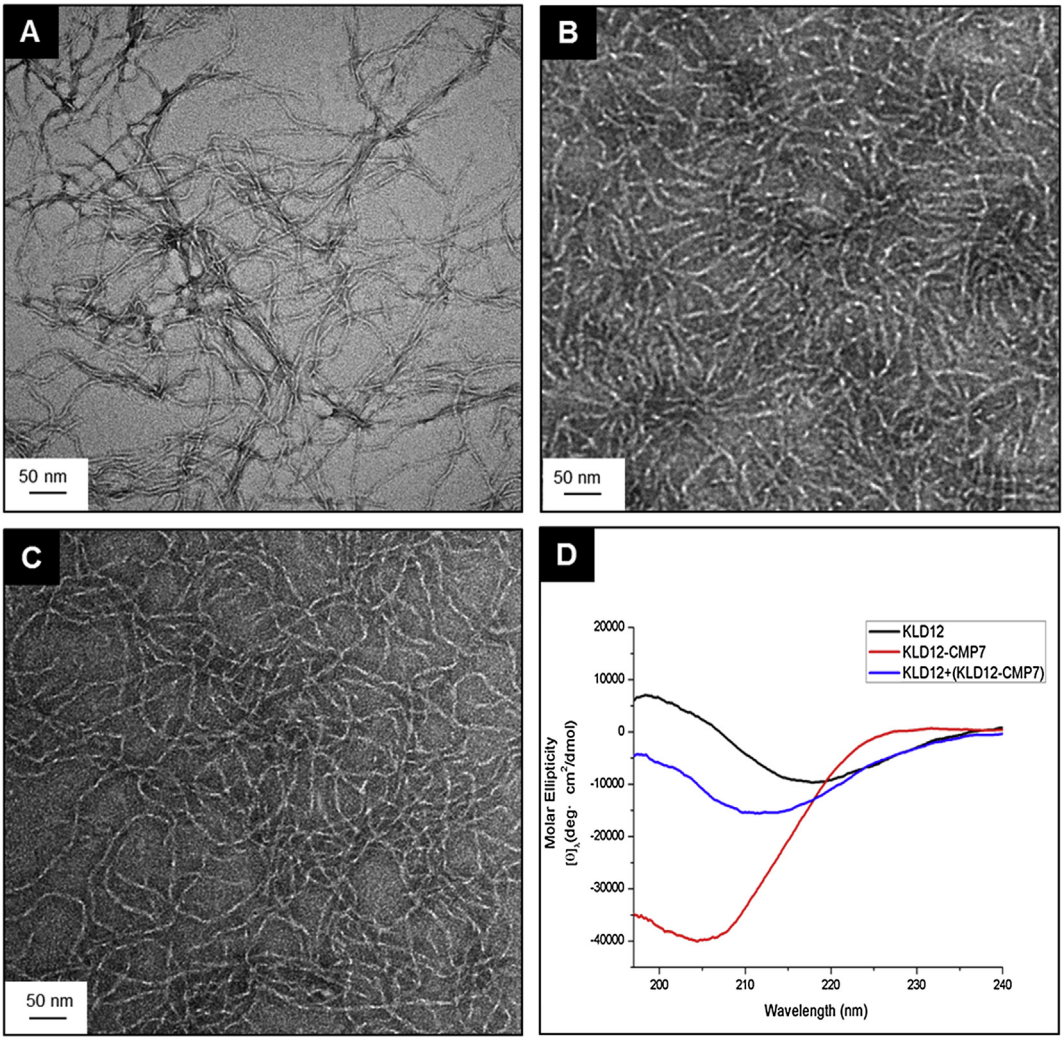

FIG. 1. Structural analysis of bioactive peptides: transmission electron microscopy (TEM) images of (A) KLD12, (B) KLD12-CMP7 and (C) KLD12 and (KLD-CMP7). (D) Circular dichroism (CD) spectra of each peptide.