Uniaxial BCP/Collagen Bi-layered Scaffold for Osteochondral Regeneration by Stem Cells Migration

Yun-Jeong Seong, Seol-Ha Jeong, Hyoun-Ee Kim.
Department of Materials Science and Engineering, Seoul National University, Seoul, Republic of Korea

Statement of Purpose: Articular cartilage has a limitation on self-repair capability due to avascularity [1]. Among various approaches to regenerate the osteochondral defects, cell migration has been spotlighted since it reduces immune reaction [2]. In this study, bi-layered scaffolds with uniaxial channels were fabricated to induce mesenchymal stem cells (MSCs) migration with higher accessibility from bone marrow, as compared with the random porous scaffolds. In addition, *in vivo* study was conducted with respect to the effect of channel diameter on cell migration and osteochondral regeneration.

Methods: Uniaxially aligned biphasic calcium phosphate (BCP) scaffold was fabricated by sequential co-extrusion using camphene. A scaffold with random pores, the control group, was created by dynamic freeze casting. Collagen layer on BCP scaffold was unidirectionally freeze-dried and crosslinked with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). The overall structure and surface morphology was observed by μ-CT and SEM, respectively. *In vivo* analysis was performed on New Zealand white rabbits. On patellofemoral groove, cylindrical osteochondral defect was created and 4 types of scaffolds were implanted; random pores with the size of 270 μm (R-270), uniaxial channels with the diameter of 620, 270 and 140 μm (U-620, U-270, and U-140). After 6 and 12 weeks, cartilage and subchondral bone regeneration were evaluated by histological and immunohistochemical staining and ICRS scoring system.

Results/Discussion: BCP/Collagen bi-layered structure was fabricated uniformly (**Fig. 1A**). Collagen showed aligned structures with 100 μm pores (**Fig. 1B**) and it was attached well on the channels of BCP scaffold (**Fig. 1C**). BCP scaffold maintained its uniaxial channels (**Fig. 1D**) and all the channels were arranged at same intervals (**Fig. 1E-F**). Under high magnification, rough surface with longitudinal grains was observed (**Fig. 1G**) and micropores with the size of 3 μm were presented on the whole surface (**Fig. 1H**). From *in vivo* results, cartilage was hardly formed in R-270, whereas, osteochondral tissues were regenerated conspicuously in U-270 (**Fig. 2A-B, G-H**). Chondrocytes were arranged in their original morphology in U-270. Immunohistochemically stained images with antibody of type I collagen showed that R-270 revealed symptom of ossification in contrast with U-270 (**Fig. 2C, I**). Differentiation in U-620 was not proceeded well, showing only ossifying fibrous tissues, since stem cells and other vascular supplements excessively reached to the defects in short time (**Fig. 2D-F**). U-140 showed better regeneration than U-620, however subchondral bone was not connected with the channels. (**Fig. 2J-L**) Since the channels were blocked before regeneration. Therefore, chondrocyte distribution and density were not promising, as compared with U-270.

Conclusions: Uniaxial BCP/collagen scaffolds were successfully fabricated by sequential co-extrusion and unidirectional freezing. The uniaxial channels accelerated MSCs migration, resulting in superior osteochondral regeneration compared to random porous structure. In addition, diameter of channels affected cell migration and the 270 μm showed the most outstanding regeneration.

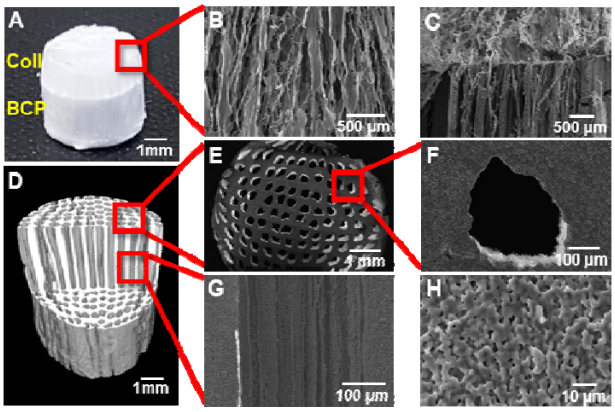

Fig. 1. U-270 scaffold. (A) Optic and (B-C) SEM images about (B) collagen (C) interface between collagen/BCP. (D) μ-CT and (E-H) SEM images about (E-F) cross section and (G) vertical section of channels and (H) micropores.

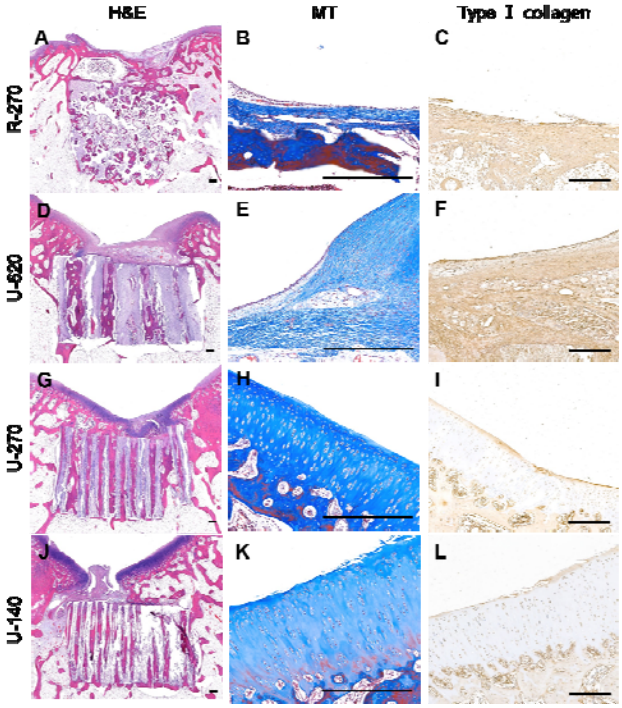

Fig. 2. Histologically and immunohistochemically stained tissues of (A-C) R-270, (D-F) U-620, (G-I) U-270 and (J-L) U-140 scaffolds with H&E, MT and type I collagen after 12 weeks. Scale bars = 300 μm.

References:

[1] Coburn et al. PNAS 25 (2012) 10012–10017
[2] P. Chen et al. Biomaterials 39 (2015) 114-123