Fabrication and Characterization of Collagen/Apatite Scaffolds with Intrafibrillar Mineralized Collagen Fibers

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Statement of Purpose: Natural bone is an outstanding organic-inorganic nanocomposites consisting primarily of collagen fibrils and hydroxyapatite (HA) crystals, which provides skeletal and metabolic functions of the natural bone. Many researchers have attempted to develop bone-like collagen-HA composites via conventional methods consisting of nucleation and growth of HA onto collagen matrices, which does not duplicate the specific organic/inorganic hierarchical nanostructure present in natural bone. [1-2] It is evident that intrafibrillar mineralization is a dominant contributor to the elasticity and hardness of the hard tissues, including both bone and dentin. [3] Thus, synthesis of mineralized collagen fibrils with intrafibrillar mineralization is a key factor for mimicking natural bone.

In this abstract, a biomimetic approach is used to prepare intrafibrillar mineralized collagen fibers. A combination of polycarboxylic acid (polyacrylic acid, PAA) and sodium tripolyphosphate (TPP) was used, where the former acted as a sequestration analog and the latter served asa templating analog. This polymer-induced liquid-precursor mineralization process leads to the intrafibrillar mineralization of collagen fibers, which is identical to the nanostructure of the mineralized collagen fibers and is the basic building block of the hierarchical biomineralized hard tissues.

Methods: Based on the protocol by Rajan et al., [4] type I collagen was extracted from rat tails, purified and freeze-dried. This collagen was then dissolved in 0.02 M acetic acid at 4 ℃ with a concentration of 4.5 mg/mL. Polyacrylic acid (Mw 5000 Da, PAA) with a concentration of 0.8 mg/mL was added to the m-SBF to create metastable calcium phosphate (ACP) nanoprecursors. Meanwhile, the m-SBF was prepared as reported previously. [5] Collagen solution mixed with ACP nanoprecursor at a volume ratio of 1:1. Then Sodium tripolyphosphate (TPP) was dissolved in the solution to reach a final concentration of 1.2 wt%. The pH of the solution was adjusted to 7.2 by addition of NaOH.

To form the scaffold, acollagen/HA hydrogel with intrafibrillar mineralization (Col-Intra HA) was first prepared using a two-temperature process. [6] Then this collagen/HA hydrogel was uni-directionally freeze-dried to create lamellar structure as reported previously. [6]

Results: The Col-Intra HA scaffold with highly hierarchical nano-, micro- and macro-structures was prepared by unidirectionally freeze casting of Col-Intra HA hydrogel (Figure 1). At macro-structure level, Col-Intra HA scaffold consists of unidirectional macropores (Figure 1A). At micro-structure level, each lamella is comprised of aligned micro-lamellae (Figure 1B).

At nano-structure level, the intrafibrillar HA minerals are shown in Figure 2. Figure 2A shows uniform D-banding in the collagen fibers, indicating the formation of intrafibrillar mineralization. Figure 2B shows both the intrafibrillar and extrafibrillar mineralization of the collagen fibers.

The Col-Intra HA scaffold exhibits a high similarity to the complex hierarchical structure to natural bone. These results collectively suggest that the Col-Intra HA scaffold can be successfully prepared using the biomimetic approach with both intrafibrillar and extrafibrillar mineralizations.

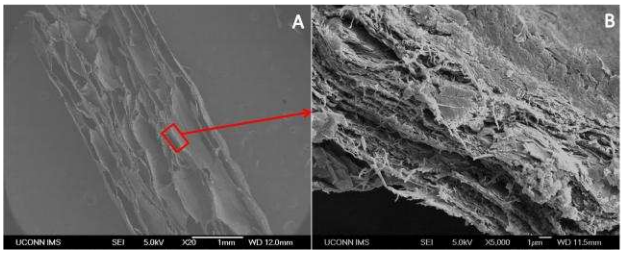

Figure 1. Field emission scanning electron microscope (FESEM) images of collagen/HA scaffold with lamella structure.

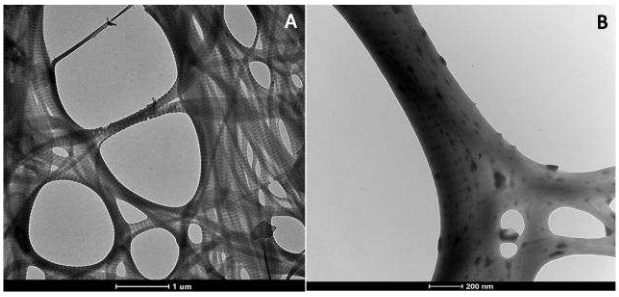

Figure 2. Transmission electron microscope (TEM) images of the unstained intra- and extra-fibrillar mineralized collagen fibers.

Conclusions: A novel collagen-HA scaffold with intrafibrillar mineralization and hierarchical structures has been developed for bone repair. A simple biomimetic approach has been employed to fabricate collagen-HA scaffolds with intrafibrilar and extrafibrillar mineralization of collagen while exhibiting hierarchical nano-, micro- and macro-structure was successfully developed. Structure at the nano-, micro- and macro-level could be easily tailored. Moreover, the scaffolds can be combined with drugs/proteins/cells to produce optimum therapy for different tissue regeneration.

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