A Biomimetic Collagen-apatite Scaffold with Unique Multi-level Lamellar Structure for Bone Tissue Engineering

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Statement of Purpose: Inspired by the natural bone, collagen-apatite (Col-Ap) scaffold has been widely studied for bone tissue engineering. The biomimetic structure of Col-Ap composite which includes the organization of the collagen fibers and crystal phase of apatite plays an important role in new bone formation. An ideal scaffold should also possess a 3-D structure with interconnected pores to facilitate cellular activities such as vascularization and transport of nutrients and metabolic waste while maintaining sufficient mechanical strength to support cell adhesion and physiological loading.

In the current study, we fabricated mineralized collagen hydrogel by a novel biomimetic technique using modified simulated body fluid (m-SBF) containing collagen. This is the first paper works on a 3-D macro porous scaffold while maintaining the high fibrillar density and biomimetic property in the construct. Other unique property of the scaffold includes the control of the pore size and pore orientation over a wide range from nano to macro dimensions.

Methods: Biomimetic Col-Ap hydrogel was synthesized using a collagen containing modified simulated body fluid (m-SBF). Col-Ap hydrogel was frozen uni-directionally in a custom-made mold under different cooling rates. The freeze-dried scaffolds were subsequently cross-linked, rinsed and re-lyphilized. Thus obtained scaffolds were characterized using field emission scanning electron microscopy. The rheology measurement was conducted using in situ time sweep on a ARG2 rheometer with a coquette. The mechanical test was performed using Dynamic Mechanical Analyzer.

Results: Gelation of collagen in m-SBF was studied using a rheometer (Figure 1). Initially, neutralization of the pH and increase of temperature triggered the assembling of triple-helical tropocollagen molecules into collagen fibrils and in situ precipitation of apatite onto the collagen matrix. The kinetic of collagen assembling and apatite crystal growth was well controlled by different processing conditions to form a mechanical stable hydrogel as shown in Figure 2 a1&a2.

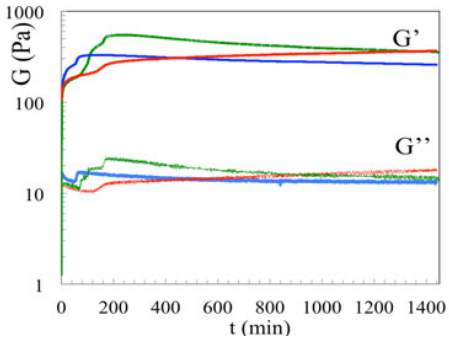

Figure 1. Gelation behavior of Col-Ap monitor by measuring the time dependence of the elastic and viscous modulus of Col-Ap solution prepared at three different processing conditions.

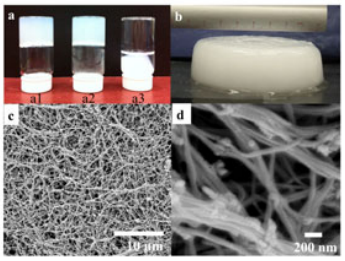

Figure 2. Photograph of hydrogel prepared at three different processing conditions (a), Photograph of hydrogel prepared for freeze casting (b), FESEM image of hydrogel dehydrated by ethanol solution and then super critical point dried (c&d).

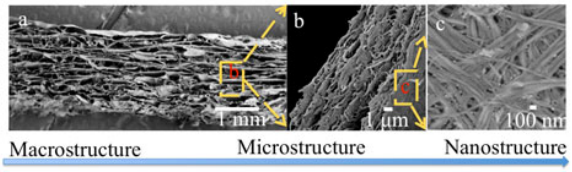

Figure 3. The freeze-dried hydrogel possesses a multi-level lamellar structure consisting of aligned macropores (a), aligned nanolayers in each lamellae (b) and biomimetic surface morphology (c).

Collagen density (g/L)	Compression modulus-X (kPa)	Compression modulus-Z (kPa)
2.5	222.6 ± 69.1	59.5 ± 10.3
3.9	2912.5± 802.0	149.9 ± 25.6

Table 1. Mechanical properties of freeze dried Col-Ap hydrogel

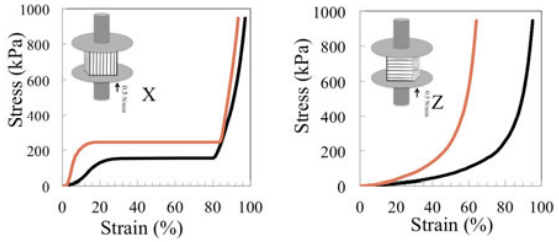

Figure 4. Representative unconfined compressive stress-strain curves of Col-Ap scaffolds.

Conclusions:

A novel biomimetic scaffold with multi-level lamellar structure was developed for bone tissue regeneration. Mineralized collagen hydrogel was prepared by a simple biomineralization process using collagen containing m-SBF. Then Col-Ap hydrogel was frozen at different temperatures at unidirectional freezing direction. As a result, scaffold with controllable macro and micro uniaxial pore structure was successfully prepared. With its hierarchical lamellar pore structure and excellent biomimetic properties, this novel scaffold has great potential to meet the needs of different bone tissue engineering applications.