Fabrication of Hydroxyapatite/Cotton Nanofiber Scaffolds for Biomedical Application

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Electro spun nature fibers have been investigated as scaffolding materials for bone tissue engineering. Our study was to develop a novel composite cotton nanofiber scaffolds by attaching hydroxyapatite (HA) to cotton nanofibers (CF) using an adhesive material (the mussel-inspired protein polydopamine) as a superglue. Result show that the sheet-like HA was successfully deposited on the CF in short time (30h) and 37°C (human body temperature). The diameter of HA functionalized CF was 1 µm, and the surface become rougher. These results demonstrate this HA/CF scaffold is promising for repair bone defects.

Keywords: Electro spun; Cotton nanofiber (CF); Hydroxyapatite(HA); Polydopamine.

1. Introduction

Tissue engineering is new interdisciplinary field that seeks suitable scaffolds materials for cell growth, proliferation and differentiation to allow tissue regeneration. Bone is composed of hydroxyapatite, highly aligned collagen fibrils and proteins. Some charged proteins can facilitate binding of calcium and thus serve as nucleation sites for HA mineralization [1]. Synthetic bone grafts that promote the natural mineralization would be excellent candidates for the repair bone defects. Electrospinning cellulose-based materials has been attracted much attention for bone tissues [2]. As well known, cotton cellulose is the most abundant polymer in nature and has good properties, such as, chemical stability, biocompatibility, mechanical strength and biodegradability [3]. Some studies have report the biomedical application of cotton cellulose [4, 5]. In our study, HA/CF scaffolds were produced by electrospinning and hydrothermal growth technique. In order to let the HA coated on the CF tightly, we need choose a kind of chemical substances (simulating charged proteins in human body) to accelerate HA nucleation on the substrate. In 2007, inspired by the composition of adhesive proteins in mussels, Lee used dopamine self-polymerization to form thin, surface-adherent polydopamine films onto a wide range of inorganic and organic materials [6]. Besides, Lee found that it can induce hydroxyapatite mineralized on the materials spontaneously [5]. Therefore, the dopamine was used for coated CF.
2. Experimental

2.1. Fabrication of HA/CF scaffold

Cotton linters cellulose (DP=12000) was gotten from Zhejiang Academy of Agricultural Sciences. All chemical reagents purchased from Aladdin Chemistry are analytical grade and used without further purification. The electrospinning process of cotton scaffolds was same as our previous research [7]. The CF scaffolds were immersed in 0.2gL⁻¹ dopamine·HCL in Tris buffer (PH=8.5) for 8h. Then the scaffolds were washed with deionized water and dried with a stream of N2 gas. The mat coated with polydopamine was transferred into 1.5 simulated body fluid (SBF) for 30h and incubated at 37°C. The nucleation of hydroxyapatite was controlled by concentrating Ca²⁺ ions at the CF. The composition of 1.5×SBF was as follows: Na⁺, 213.0; K⁺, 7.5; Mg²⁺,2.25; Ca²⁺, 3.75; Cl⁻, 221.7; HCO₃⁻, 6.3; HPO₄²⁻, 1.5; SO₄²⁻, 0.75mM. Finally, the PH adjusted at 7.4. After the SBF treatment was completed, the samples were washed in distilled water three times and dried with N2 gas for further characterization.

2.2. Characterization methods

The surface morphology of the samples was investigated using Field-emission scanning electron microscope (FESEM, Hitachi S-4800) equipped with an Energy dispersive X-ray spectroscopy (EDS), and a transmission electron microscope (TEM, JEM 2100, 200kV). The phase structures of the HA/CF nanocomposites were studied by powder X-ray diffraction (XRD, Bruker AXS D8-discover).

3. Result and Discussion

The SEM images in Fig.1a reveal that electro spun fibers have uniform and smooth surfaces, with a diameter of 100-300nm. Figure 1b show SEM images of a cotton cellulose fiber after immersed in solution of dopamine for 8h. The surface of fibers become very rough and the diameter become larger. Further, from the TEM (Figure 1D) we can see polydopamine film was formed on the cellulose. It can act as the bridging material at the interface of organic and inorganic phases. Figure 1C show the CF substrate was fully and uniformly covered by sheet-like HA after mineralization for 30h. From Figure 1D (the zoom of Figure 1C), The obtained mineral composite has a macro porous structure. TEM (Figure 1E) images show the diameter of composite structure is about 1μm. The macro porous structure of HA can provide some channels for nutrient transportation.
Figure 1. SEM images of A) electro spun cotton nanofibers, B) nanofiber coated with polydopamine, C) nanofiber coated with HA, D) the zoom of C, E) TEM image of cotton cellulose/polydopamine nanocomposite, F) cotton cellulose/HA nanocomposites.

The EDS indicates that there are only C, O, Ca and P elements in the composite fibers, the Ca/P ratio for the agglomerates is 1.63, which is close to the theoretical ratio of HA (Ca/P was 1.67) (Figure 2). It can proved that the mineral grown on the surface was hydroxyapatite rather than octacalcium phosphate, which is structurally similar to hydroxyapatite.
The XRD pattern of the as-obtained sample in Figure 3 shows that the broad diffraction peaks of the CF at 10-20°, which can be ascribed to amorphous cellulose structure. 25° was correspond to double-sided foam tap. Meanwhile, XRD peaks at 2θ' angers of 25.8, 31.8, 34.0 correspond to HA. Because of the low temperature and short time when preparing HA, the crystalline quality of HA was low. The XRD result further supports that HA are coated on the surface of fiber.

4. Conclusions
A novel HA/CF nanocomposite was successfully synthesized by using polydopamine functioned CF immersed in SBF. Sheet-like HA was coated on the CF
uniformly and the sheet of HA with macro porous structure. The HA with macro porous structure and low crystallinity was beneficial for cell growth, proliferation. Besides, the ability for CF form biologically relevant apatite coatings within a short period of time and body temperature (37°C) may increase the osteoconductivity and osteoinductivity of the cotton cellulose, making them excellent candidates for implantable bone grafts.

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References