HYDROXYAPATITE-CONTAINING ORGANIC/INORGANIC COMPOSITE WEB FABRICATED VIA ELECTROSPINNING METHOD

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1. Introduction

Natural bone is a highly organized tissue which consists of about 70% hydroxyapatite (HAp) and 30% extracellular matrix (ECM) protein mainly taking up collagen type 1, and it is able to repair itself the fractured regions. However, when bone has the large defect, the natural healing ability of bone cannot be brought out and it is limited. Therefore, many tissue engineering scaffolds of bioactive polymers or inorganic materials have been used for improving regeneration of damaged bone defects. Because the scaffold mimicking the structure of ECM has an advantage in cell activity, the submicron or nanofibers prepared by electrospinning method can be suitable for the fibrous structure of 2-D or 3-D scaffold whose feature resembles collagen fibers in ECM. Most of all, inorganic HAp, with the chemical formula \((\text{Ca}_{10}\text{(OH)}_{2}\text{(PO}_4\text{)}_6)\), is receiving great attention as a bone substitute due to its high biocompatibility, osteoconductivity, and bioactivity. But HAp itself is too brittle and hard to be molded, so it is necessary to make a hybrid composite, which is composed of polymer matrix and HAp. Many natural and synthetic biopolymers, such as collagen, gelatin, fibroin, poly(lactic-co-glycolic acid) (PLGA) and poly(ε-caprolactone) (PCL) and etc, have been used for the organic matrix. And the hybridization of these biocompatible polymers and HAp can be performed through two methods in general. First, the simplest method is the HAp deposition onto the surface of the polymer matrix which is strongly dependent on the biomineralization conditions (surface coating method). An accurate process of biomineralization in vivo is unknown yet, but organic material of composite has apparently large effect on the formation of inorganic crystal. Especially, simulated body fluid (SBF) soaking method counts as a simple and useful way to form the HAp crystal onto the surface of polymer. But the study for controlling the detailed degree of biomineralization and ensuring the deposition uniformity of HAp on polymer matrix has not fulfilled thoroughly yet. The second general method to hybridize is a HAp deposition inside the polymer matrix simply by blending the HAp particles with polymer melt or solution. In this case, there is a strong tendency for the HAp particles to be aggregated in the matrix, resulting in a low dispersibility and stability in the mixture solution. Eventually, the polymer/HAp hybrid composite has not been able to ensure the uniformity of HAp particles distribution. Recently, D. Yang et al have reported the modification of HAp nanoparticle with hyaluronic acid (HA) – dopamine (DA) conjugate for enhancing the dispersibility of HAp nanoparticle on the electric repulsion of HA, and they used the modified HAp particles for preparation of monodispersed HAp layer. In this study, HAp containing silk nanofiber composite web is fabricated by electrospinning through the two ways; HAp deposition onto the surface of the electrospun nanofiber by soaking in SBF solution and deposition of well-dispersed HAp particles inside the micro or nanofiber using HA–DA conjugate. Structural characterization and evaluation of the SF/HAp composite nanofiber webs are performed for confirming the possible use as a tissue engineering scaffold for bone regeneration.

2. Experimental

2.1 Preparation of a concentrated SF solution
B. mori silk cocoons were completely dried and degummed with sodium olate and Na₂CO₃. Then the degummed SF was dissolved in 9.3 M LiBr solution at 60 °C for 30 min and dialyzed in water for 3 days using molecular porous membrane tubing (MWCO 3500). The SF solution was filtered to remove impurities and moved in polyethylene oxide (PEO) solution through the dialysis membrane to obtain a concentrated SF solution (SF aqueous solution of about 12 wt%).

2.2 Modification of HAp particles using HA-DA conjugate

HA was dissolved in DI-water to a concentration of 2mg/ml and then, 0.95mg/ml of DA and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) as coupling agent are added to the solution. The reaction mixture was stirred for 2 h at room temperature, maintaining pH 4.7 with 0.1 N HCl. After the HA-DA conjugate was precipitated in ethanol, it was dissolved in DI-water, and then purified by dialysis in 100 mM NaCl solution and DI-water. HAp particles (purchased from Sigma Aldrich) with average diameter of 100 nm were dispersed in DI-water at a concentration of 5mg/ml, and HA-DA conjugates were added to this solution. After ultrasonication, the reaction was performed with gentle stirring at 60 °C for 3 h. Finally, the solution was centrifuged at 3000 g for 10 min and rinsed with DI-water.

2.3 Fabrication of electrospun SF nanofiber composite containing well-dispersed HAp particle inside

The HA-DA conjugate modified HAp nanoparticles with various concentrations were added into a concentrated SF aqueous solution, and the SF/HAp mixed solution was treated with ultrasonication. Then, the PEO solution dissolved in DI-water for at least 5 days was poured into the SF/HAp solution. The mixed SF/PEO/modified HAp solution was moved into syringe and loaded with a syringe pump for electrospinning. The distance between the spinneret and collector was about 20 cm and applied voltage was 8~12 kV. After electrospinning, SF/HAp composite nanofiber web was treated with methanol for 1 day to crystallize and with DI-water to remove PEO.

2.4 HAp coating onto surface of SF nanofiber using a concentrated SBF solution

The concentrated SBF solution (5X or 10X) was prepared by dissolving in order of NaCl, KCl, CaCl₂·2H₂O, MgCl₂·6H₂O, Na₂HPO₄ and NaHCO₃ in DI-water at 37 °C. Then, electrospun SF nanofiber web was soaked in the concentrated SBF solution and treated with vacuum for 3 min to remove air into the composite web. The soaking time was varied from 10min to 90min. After soaking process, the SF nanofiber composite web coated with HAp was freeze-dried.

2.5 Structure and property measurement of SF/HAp nanofiber composite web

Morphology of SF/HAp nanofiber composites was examined by field emission scanning electron microscope (FE-SEM) and transmission electron microscope (TEM). Mechanical properties were measured using Minimat. The formation of HAp was confirmed by fourier transform infrared spectroscopy (FT-IR) and energy dispersive X-ray spectroscopy (EDS). Zeta potential and size distribution of HA-DA-HAp particles was evaluated by electrophoresis light scattering (ELS). Thermal behavior of SF/HAp composite was examined by differential scanning calorimetry (DSC).

3. Results and Discussion

3.1 SF composite nanofiber web containing well-dispersed HAp particles inside

In general, pure HAp nanoparticles have a low surface charge and therefore, they have a great tendency to aggregate in an aqueous solution. However, it was confirmed that when the HAp nanoparticles were modified with HA-DA conjugates, the modification enhanced the dispersity and stability of the HAp nanoparticles in aqueous solution. As a result of zeta potential measurement, pure HAp particles showed a 1.67 mV of zeta potential, indicating that it is in an almost electroneutral state. On the other hand, HAp nanoparticles modified with HA-DA conjugate exhibited a negative charge of surface (upto -22 kV). It was also found that a diameter of HAp particle was decreased from about 2.16 µm to 370 nm after surface modification with HA-DA. Here, HA
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(hyaluronic acid) plays a major role for enhancing the dispersity and stability of HAp particles due to relatively high negative charge and steric hindrance of bulky HA structure. DA (dopamine) can also play a role of link between HA and HAp particle, based on its adhesive property. Therefore, the HA-DA conjugate system is very useful for obtaining stable dispersion of HAp particles in a solution during fabrication processing. It was observed that most of unmodified HAp particles were precipitated in DI-water within 10 minutes while the modified HAp nanoparticles with the HA-DA conjugate maintained very stable colloidal state in DI-water for more than 3 days without any precipitation. As shown in Figure 1, TEM images confirmed that the HAp nanoparticles were much better dispersed inside the electrospun SF nanofiber when the modified HAp nanoparticles with the HA-DA conjugate were used. In the unmodified case, the HAp nanoparticles were heavily aggregated inside the SF nanofiber, and consequently, the nanofibrous structure cannot be maintained in SF/HAp composite nanofiber web. It is even very difficult to fabricate the composite nanofiber by electrospinning due to the precipitation of HAp nanoparticles in a dope solution. Figure 2 shows FE-SEM images of pure SF nanofiber web and SF/HAp composite nanofiber web, in which the modified HAp nanoparticles with HA-DA conjugate were used in electrospinning. Here, aqueous SF solution was used for the electrospinning. Both nanofiber webs have about 500 nm of average fiber diameter. HAp deposition inside the SF nanofiber did not cause the increase of fiber size but the rugged surface of SF nanofiber formed in composite web due to the dispersion of HAp nanoparticles inside. The FT-IR and EDS analysis were carried out for confirming the chemical structure of SF/HAp composite nanofiber. Figure 3 shows the FT-IR spectra of pure HAp nanoparticles, pure SF nanofiber and SF/HAp composite nanofiber web. Typical absorption peaks of amide I and amide II in silk fibroin and that of PO$_4^{3-}$ in HAp were observed respectively.

3.2. SF/HAp composite nanofiber web prepared by biomineralization of HAp using a concentrated SBF solution

The concentrated SBF solution (5X or 10X) was used for the deposition of HAp crystals on the surface of SF nanofibers. Figure 4 shows FE-SEM images of SF nanofibers surface-coated with HAp particle by soaking in a SBF solution for various times. It confirmed that the biomineralization processing conditions were adequate to produce the SF/HAp composite nanofiber. When the concentrated SBF solution was used for coating the HAp particle onto SF nanofiber web, the amount of HAp formed and the shape of HAp coated were different, depending on the SBF concentration and treatment time. And as a result of FT-IR and EDS analysis, it was confirmed that the material coated onto surface of electrospun nanofiber was calcium phosphate which is the chemical structure of HAp.

4. Conclusion

The SF/HAp composite nanofiber web was successfully fabricated by electrospinning in two different ways; HAp coating onto the surface of the electrospun SF nanofiber by soaking in a concentrated SBF solution and well-dispersed HAp deposition inside the SF nanofiber by modifying the HAp nanoparticles with HA-DA conjugate. Uniform distribution of HAp nanoparticles inside and outside the polymer matrix is very important for HAp containing organic/inorganic composite scaffold. In this study, the hybridization methods of HAp nanoparticle and SF nanofiber were introduced for fabricating the composite scaffold in the use of bone regeneration. Several analytical methods confirmed the structural characteristics of these SF/HAp composite nanofiber webs.

Fig. 1. TEM images of SF/HAp composite nanofiber containing HA-DA conjugate modified HAp particles (left) and unmodified HAp particles (right).
Fig. 2. FE-SEM images of pure SF nanofiber web (left) and SF/HAp composite nanofiber web containing HA-DA conjugate modified HAp nanoparticles (right).

Fig. 3. FT-IR spectra of HAp nanoparticles (nHAp), electrospun SF nanofiber (SF), and SF/HAp composite nanofiber (SF/nHAp).

Fig. 4. FE-SEM images of SF nanofiber web surface-coated with HAp crystals by soaking in a concentrated SBF solution with different times.

Fig. 5. EDS spectrum of SF/HAp composite nanofiber by soaking in a concentrated SBF solution.

5. References


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