Fabrication of mineralized zein/hydroxyapatite nanofibrous membranes

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Introduction

Biomineralization of hydroxyapatite (HAp), the key ingredient for strength and stability in the human skeletal system, has attracted much attention in the past decades. Mimicking the biomineralization process has showed promise in the development of bone tissue engineering scaffolds and void fillers. Organic polymers have been studied as templates for the bio-inspired approach, including collagen and gelatin (Zhang W. Chem. Mater. 2003; 15: 3221.), peptide-amphiphile (Hartgerink JD. Science; 2001; 294: 1684.), poly (lactic acid), poly (lactide-co-glycolide) and some surfactants. Zein, a prolamin fraction of corn protein, has attracted interests for promising applications in the areas of biomaterials and drug delivery. The present study is focused on the benefits of zein protein as an organic template in biomineralization process. Zein nanofibrous membranes were electrospun and used as a scaffold for mineralization of synthetic HAp. The potential of mineralized zein nanofibrous scaffold for bone tissue engineering was explored by combing the unique structural features generated by electrospinning with precipitation of HAp regulated by the organic molecular system.

Methods

Purified zein was purchased from Acros Organics (New Jersey, USA). Formic acid solutions with zein concentrations ranging from 30% to 60% (w/v, g/mL) were electrospun to form fibrous membranes. The fibrous membrane was subjected to a treatment cycle by incubating in 0.5 M freshly made aqueous solution of $CaCl_2$ and 0.3 M Na_2HPO_4 solution at pH 9 for a period of 30 min, respectively. An alternate incubating was carried out for three cycles. The mineralized zein fibrous membrane was then characterized by scanning electron microscopy (SEM), energy-dispersive spectroscopy (EDS), and wide-angle X-ray diffraction (WAXD). *In vitro* cytotoxicity of the zein/HAp nanofibrous scaffolds were evaluated by MTT assay.

Results

Randomly oriented apatite crystals were observed on mineralized zein nanofibers (Fig. 1). As determined by EDS, the Ca/P ratio of the apatite formed on zein nanofibers was approximately 1.56, close to the value of 1.67, expected for the chemical formula $Ca_{10}(PO_4)_6(OH)_2$. Comparing the XRD patterns with the hydroxyapatite standard, mineralized zein nanofibers have similar diffraction reflections 002, 211, 300, 130, 222 and 213 centered at 2θ of 25.6°, 32.0°, 34.5°, 39.4°, 46.4° and 49.9° (Fig. 2). As shown in Fig. 3, MTT results obtained for the mineralized nanofibrous membranes electrospun from 35%, 45% and 55% (w/v) zein solutions are not significantly different from those of the pristine zein membranes. It should also be noted that there was no measurable difference in cytotoxicity of zein membranes after incubation for 1, 3 and 5 d.

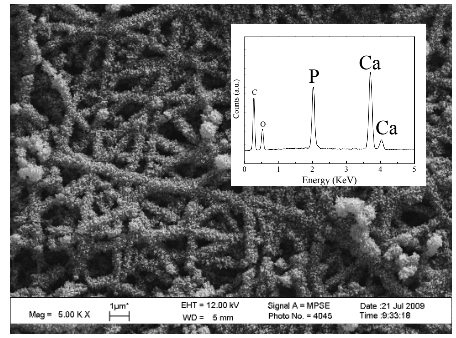

Figure 1. SEM image of mineralized zein nanofibrous membranes electrospun from 45% (w/v) formic acid solution. Magnification: ×5000.

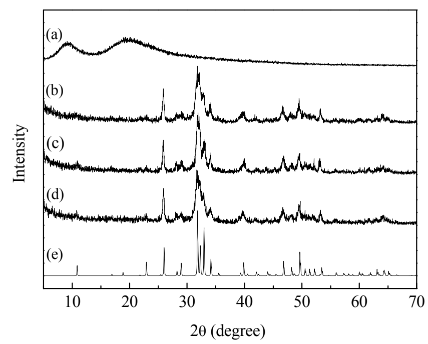

Figure 2. X-ray diffraction patterns of (a) pristine zein nanofibrous membrane, mineralized zein nanofibrous membranes electrospun from (b) 35%, (c) 45%, (d) 55% (w/v) formic acid solutions, (e) hydroxyapatite standard.

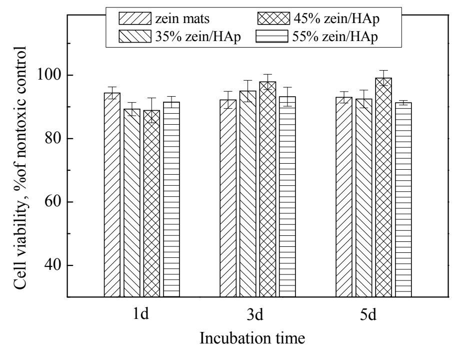

Figure 3. Cytotoxicity of mineralized zein nanofibrous membranes after incubation for 1, 3 and 5 d.

Conclusions

Zein/HAp nanofibrous membranes were fabricated by electrospinning and precipitation of minerals regulated by the organic molecular system. Morphological and structural details of the mineralized membranes were confirmed by SEM, EDS, and WAXD. MTT results indicate that the mineralized nanofibrous membranes did not induce cytotoxic effects, which is promising for biomedical applications.

Abstract #862