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Synthesis, characterization, and mineralization of polyamide-6/calcium lactate composite nanofibers for bone tissue engineering

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ABSTRACT

Polyamide-6 nanofibers containing calcium lactate (CL) on their surface were prepared by neutralization of lactic acid (LA) in core–shell structured polyamide-6/LA electrospun fibers. First, simple blending of LA with polyamide-6 solution was used for electrospinning which interestingly formed a thin LA layer around polyamide-6 nanofibers (core–shell structure) and then subsequent conversion of this LA into calcium lactate via neutralization using calcium base. FE-SEM and TEM images revealed that plasticizer capacity of LA led the formation of point-bonded structure due to the formation of shell layer of LA and core of polyamide-6. The bone formation ability of polyamide-6/calcium lactate composite fibers was evaluated by incubating in biomimetic simulated body fluid (SBF). The SBF incubation test confirmed the faster deposition of large amount of calcium phosphate around the composite polyamide-6/calcium lactate fibers compared to pristine polyamide-6. This study demonstrated a simple post electrospinning calcium compound coating technique of polymeric nanofibers for enhancing the bone biocompatibility of polyamide-6 fibers.

1. Introduction

Bone tissue scaffold is designed to serve as the matrices of tissue formation capability which fulfill a few basic requirements, such as high surface area, high porosity, required surface properties permitting cell adhesion, cell differentiation and proliferation, good mechanical integrity, non-cytotoxicity, and osteoconductivity [1–3]. Structural bone substitutes made from polymer fibers containing calcium compound have been popular in bone tissue engineering. In the past few years, several different electrospun nanocomposite fibers had been devised and explored for potential bone regeneration applications [4–9]. All these studies showed that the presence of calcium compound on polymer matrix serves as nucleation sites for the formation of crystal around the fibers. However, electrospinning of blends made by mixing inorganic materials with viscous spinning solutions prior to electrospinning causes the nanoparticle agglomeration, decreased spinnability of polymer, decreased mechanical properties of fibers, and reduced calcium compound loading capacity. Therefore, post electrospinning loading of calcium compound on the surface of nanofibers can solve the above noted problems in this field. However, proper post electrospinning deposition of calcium compound on the surface of fibers is not easy task because of lacking of specific interactions between polymeric and inorganic molecules.

In this work, our strategy is to design and fabricate biomimetic and bioactive bone tissue scaffolds that resemble the native extracellular matrix (ECM) as closely as possible to nucleate the calcium phosphate during implantation. In this context, our current endeavor is to use the synthetic polymer polyamide-6 as a matrix to develop biomimetic fast mineralization on its surface. Polyamide-6 shows structural and molecular similarity to the natural collagen of human bone and has good mechanical properties, nontoxicity, and degradability [10–14]. However, low hydrophilicity, brittleness, and low degradation rate of polyamide-6 fibers hinder its application in tissue engineering [15]. Therefore, modification of polyamide-6 fibers using plasticizer bioactive component containing calcium ions, without distributing the internal structure of fiber, is essential for this application. Here, our hypothesis is to fabricate core–shell structured polyamide-6 composite fibers in which bioactive acidic organic material such as lactic acid forms shell of the fibers which can be subsequently converted into calcium lactate by neutralization with calcium base. This simple technique not only

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provides a local reservoir of calcium ions on the surface of fibers for the stable nucleation sites to rapid crystal growth but also prevents from the disturbing of internal structure and chain orientation of polymer fibers.

We have already reported that LA modified polyamide-6 could alter some unique properties of the mat required for biocompatibility (such as increased hydrophilicity, softness and mechanical strength, stable chain conformation, and decreased crystallinity) [15]. Here, our concern is to investigate detailed morphological structure of composite mat and to convert surface LA layer to calcium lactate without disturbing the internal structure of polymer fibers for increasing biocompatibility for bone tissue engineering. The neutralization of surface LA on the core–shell structured polyamide-6/LA hybrid fiber using calcium base ($Ca(OH)_2$) could homogeneously deposit calcium compound around the fiber that can act as nucleation of bone like materials. So far, no bone tissue regeneration scaffold using post electrospinning acid base neutralization technique to deposit organic calcium compounds on the surface of fibers has been reported. These improved properties of polyamide-6 mat caused by calcium lactate could make it a potential candidate in bone tissue engineering.

2. Experimental

2.1. Materials

Polyamide-6 (medium molecular weight, Kolon, Korea) and lactic acid monomer (Showa, Japan) were used in this work for the fabrication hybrid electrospun mat. Formic acid and acetic acid (analytical grade, Showa, Japan) in 4:1 ratio by weight were used as solvents. Calcium hydroxide (0.1 M) is prepared using $Ca(OH)_2$ (Junsei, Japan). All the materials were used as received.

2.2. Electrospinning

Pristine and composite mats of polyamide-6 were electrospun from the 22 wt% solution containing different amounts of LA (0 and 5 g) in 30 g of polyamide-6 solution as reported in our previous work [15]. A viscous solution was placed into a 5 ml plastic syringe equipped with a polystyrene micro-tip (0.3 mm inner diameter and 10 mm length). A high voltage power supply (CPS-60 K02V1, Chungpa EMT, Korea) of 18 kV to the syringe micro-tip was supplied to electrospun the nanofibers. The working distance between the tip of the syringe and rotating collector was adjusted at 16 cm. After vacuum dried for 24 h, the fiber mats were used for further analysis.

2.3. Calcium lactate formation by neutralization

Hybrid polyamide-6 mat obtained from 5 g LA containing polyamide-6 solution was used for further modification. The composite mat (5 cm × 5 cm) was kept into the beaker containing 100 ml of 0.1 M $Ca(OH)_2$ solution for 5 min, and the mat was named polyamide-6/CL. After vacuum dried for 24 h at 30 °C, the fibrous mats were used for further analysis.

2.4. Biomimetic mineralization with simulated body fluid (SBF)

For SBF incubation test, pristine and polyamide-6/CL mats were kept into 24 well plate and SBF solution is added over the mats and incubation was carried out up to 10 days. The SBF incubation test was carried out at 37 °C in 5% CO_2 atmosphere with SBF solutions renewed every 3 days. The SBF solution used in the present study was commercially available Hank's balanced salt solution (Aldrich, H2387-1L). A bottle of Hank's balanced salt with 0.097 g of $MgSO_4$, 0.350 g of $NaHCO_3$ and 0.185 g of $CaCl_2$ was taken into the 1 l volumetric flask and then the volume was made 1 l by adding distilled water.

2.5. Quantification of mineralization using Alizarin Red S

The mineralization of pristine polyamide-6 and polyamide-6/CL scaffolds was quantified using Alizarin Red S (ARS). Here, ARS was dissolved in deionized (DI) water to make 40 mM solution whose pH was adjusted to 4 using NaOH solution [16]. The solution was then filtered through a 0.8 μm mesh to remove undissolved particulates and stored in the dark. Ten days incubated scaffolds were rinsed with DI water and then transferred into a new 24-well plate and fixed in 3.7% buffered formaldehyde for 30 min. The scaffolds were stained with an excess of ARS solution for 30 min on an orbital shaker. The scaffolds were rinsed repeatedly in deionized water to wash off all the unbound dye until clear wash solution was obtained. Then the scaffolds were transferred to a new plate and treated with 50% acetic acid solution for 30 min on an orbital shaker to extract bound dye. The solubilized dye was pipetted out, diluted in water and the absorbance measured at 550 nm in a 96-well plate using spectrophotometer.

2.6. Characterization

Fiber morphology of the as-spun pristine polyamide-6 and polyamide-6/LA composite nanofibers was observed using field-emission scanning electron microscopy (FE-SEM, Hitachi S-7400, Hitachi, Japan). Samples were cut from electrospun mats collected on polyethylene sheet. Dried electrospun scaffolds were mounted on aluminum stubs and sputter-coated by an ultrathin layer of Pt. The fiber diameter distribution was determined using Image J (NIH, USA) software. For each electrospun mat, about 100 fibers were considered from three different images to calculate the average diameter. The core–shell structure of the prepared composite nanofibers was characterized by transmission electron microscopy (TEM, JEM-2010, JEOL, Japan) operating at 200 kV. For TEM observation, fibers were directly deposited onto a TEM grid. The TEM grid was also submerged in water for one week, dried and observed again by TEM to discriminate the components in the shell and core region of the fiber.

Fourier transform infrared (FT-IR) spectra of calcium lactate, pristine polyamide-6, polyamide-6/LA, and polyamide-6/CL mats were directly recorded using an ABB Bomen MB100 Spectrometer (Bomen, Canada). The mineralization of pristine polyamide-6 and polyamide-6/CL mats was observed by using scanning electron microscopy (SEM) (GMS 5900, JEOL, Japan). The SEM images of scaffolds were obtained after 10 days incubation in SBF to monitor the mineralization of calcium compound on the nanofiber surface. The amount of calcium and phosphate deposited on the surface of fibers was analyzed by using energy dispersive X-ray (EDX) spectrometer attached to emission scanning electron microscope (SEM).

3. Result and discussion

Fig. 1 shows typical FE-SEM images of as-spun pristine polyamide-6 and polyamide-6/LA nanofibrous composite mats. It is clearly shown in Fig. 1a that the pristine polyamide-6 mat has non-bonded fibers whereas the addition of LA led the transformation from the non-bonded to the point-bonded fibrous structure, and large amounts of thin nanofibers (true nanofibers) having fiber diameter <50 nm (spider-wave-like structure) were also formed (Fig. 1b). The plasticizer effect of LA might be the cause of formation of point-bonded structure which was also explained in our previous report [15]. Since solvent evaporation rate is an important factor for determining the fiber morphology of electrospun