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Core–shell structured electrospun biomimetic composite nanofibers of calcium lactate/nylon-6 for tissue engineering

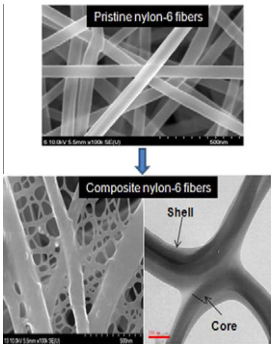
Hem Raj Pant [a,b,*], Prabodh Risal [c,d], Chan Hee Park [a,*], Leonard D. Tijing [e], Yeon Jun Jeong [d], Cheol Sang Kim [a,e,*]

[a] Department of Bio-nano System Engineering, Chonbuk National University, Jeonju 561-756, Republic of Korea
[b] Department of Engineering Science and Humanities, Pulchowk Campus, Tribhuvan University, Kathmandu, Nepal
[c] Department of Biochemistry, School of Medicine, Kathmandu University, Dhulikhel, Kavre, Nepal
[d] Department of Surgery, Chonbuk National University Medical School, Jeonju 516-712, Republic of Korea
[e] Division of Mechanical Design Engineering, Chonbuk National University, Jeonju 561-756, Republic of Korea

HIGHLIGHTS

- Core–shell nanofibers of nylon-6/lactic acid are fabricated by single-spinneret.
- Surface lactic acid is easily converted into calcium lactate by neutralization.
- Calcium lactate coated nylon-6 fiber offers nucleation site for HAp deposition.
- Calcium lactate coated nylon-6 composite fibers has good cell compatibility.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, core-shell structured nylon-6/lactic acid (LA) nanofibers have been produced via single-spinneret electrospinning from the simple blending of LA and nylon-6 solution. The low evaporation rate and plasticizer property of LA was found to be responsible for the formation of point-bonded morphology whereas solvent degradation of nylon-6 with complex phase separation mechanism could give spider-wave-like architecture of the mat and core–shell structure of the composite fibers. The core–shell nanostructure and existence of LA on the shell layer of the fiber were confirmed by TEM and XPS analysis. These fibers were further treated with calcium base to convert surface LA into calcium lactate (CL) which could increase the biocompatibility of composite mat. The SBF incubation test not only confirmed the nucleation capacity of CL to accelerate the deposition of calcium compound on the surface of nanofibers but also facileated the formation of hollow calcium phosphate particles at biomimetic conditions. The adhesion, viability and proliferation properties of osteoblast cells on the composite nanofibers were analyzed by in vitro cell compatibility test. This study demonstrated the non-cytotoxic behavior and good proliferation capacity of calcium lactate/nylon-6 (CL/nylon-6) composite mat. Therefore, the novel nanofibrous composite mat may become a potential candidate for bone tissue engineering.

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* Corresponding authors at: Department of Bio-nano System Engineering, Chonbuk National University, Jeonju 561-756, Republic of Korea. Tel.: +82 63 270 4284; fax: +82 63 270 2460.

E-mail addresses: hempant@jbnu.ac.kr (H.R. Pant), biochan@nate.com (C.H. Park), chskim@jbnu.ac.kr (C.S. Kim).

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1. Introduction

Material research continues to focus on new methods of producing nanomaterials, including an increased understanding of material/process modification techniques, which are inspired from nature (called biomimetic) [1,2]. Recently, electrospinning has been identified as a versatile technique for fabrication of nanofibers-based biomaterial scaffolds [3]. These nanofibers have great potentiality in tissue engineering applications since they mimic the nanoscale properties of native extracellular matrix (ECM) structure in vivo [4]. A wide variety of materials that are desirable for tissue scaffold can be processed via the electrospinning technique. Furthermore, different materials such as monomer, oligomer, drugs, genes, and inorganic nanoparticles are easily incorporated to the electrospun fibers for improving desirable properties of materials [5–8]. Mechanical strength, hydrophilicity, bioactive component, softness, crystallinity, and stable chain conformation of electrospun mats are main concerned for tissue scaffold engineering [9–12]. Different studies have resulted in process improvements that have improved the biocompatibility, and mechanical properties of the resulting nanofibrous mat as well as increased the speed of electrospinning for mass production, and may lead to better control the different features (fiber diameter, fiber orientation, fiber interconnection, and porosity) of electrospun mat for future applications [1,13,14]. Tissue scaffold is a support that mimics the ECM and serves as a temporary skeleton for cell growth, migration, and finally reproduction to allow tissue regeneration. These matrices should be three-dimensional (3-D) interconnected porous networks with large void volumes and high surface-to volume ratios that allows for nutrient supply/transport while providing adequate space for cell migration and attachment within the structure.

Different synthetic as well as natural polymers such as, PCL, PVA, PVAc, PEO, PEG, PLGA, PLCL, PLLA, chitosan, cellulose, gelatin, and alginate are widely used for making tissue scaffold [16,17]. But there are very few reports about the using of nylon-6 electrospun mats as a tissue scaffold even though it is degradable and biocompatible [18–20]. Polymorphic nature, proper mechanical strength, good physical properties, and excellent spinability of nylon-6 can be considered for making its electrospun mat as an effective material for bone tissue engineering [20,21]. However, high crystallinity, low hydrophilicity, slow degradation, and brittleness hinder its applications in biomedical engineering [5]. Therefore, the appropriate modification by suitable materials is essential to address above mentioned drawback of nylon-6 for tissue engineering. In our previous study, we reported a single nozzle electrospinning process to fabricate point-bonded nylon-6/lactic acid composite fibers with sufficient amount of true nanofibers (diameter <50 nm) in the form of spider-wave-like structure and demonstrated that the existence of such structure can improve the mechanical properties and hydrophilicity of nylon-6 mat [5]. Although these improved properties can increase the biocompatibility of nylon-6 mat for tissue engineering, a challenge still remains to further improve the cell viability for tissue engineering needs because our in vitro cytotoxicity result showed that the modified mat has less cell compatibility than pristine nylon-6 mat (described in Section 3). The increased pH, due to the large amounts of LA present in nylon-6 mat, might be the possible cause of its less cell compatibility than pristine nylon-6. Therefore, investigation about the distribution of LA on nylon-6 fibers and its neutralization by calcium base to increase its biocompatibility is potential in this field. Our result showed that core-shell fiber was formed from single electrospinning set up in which LA monomer was shell and nylon-6 was core of the fibers.

There are many reports focused on the core-shell structure of electrospinning nanofibers. Most of them are obtained using co-axile electrospinning set up and very few are obtained from the mixture of polymers from single nozzle electrospinning set up [22-24]. So far, no core-shell structured nanofibers containing organic acid monomer as a sheet of polymer fibers and its successive post-electrospinning neutralization to incorporate organic calcium compound as a nucleation site for the biomimetic mineralization of CaP compounds on their surface have been reported. In the present study, we investigate the mechanism of formation of core-shell structure and effect of acid-base neutralization on the surface of fibers to engineer the new surface functionality (calcium lactate) on nylon-6 fibers. The neutralization of LA by calcium base (formation of calcium lactate) maintains not only neutral pH but also provide Ca^{++} ions to the fibers for nucleation of calcium phosphate (CaP) compounds during bone regeneration. We evaluate the effect of calcium lactate (CL) deposition on the biocompatibility performance (biomimetic mineralization and cell toxicity) of modified nylon-6/CL composite mat. Furthermore, we also study the effect of different functionalities (nylon-6 and CL) on the nucleation, growth, and shape and size of CaP NPs deposited during biomimetic mineralization. Comparison of the pattern and morphology of biomimetic mineralized CaP NPs on the surface of different mats demonstrated the effect of calcium lactate chemistry in controlling the morphology of deposited CaP NPs. Our result showed that the formation of CL on the surface of nylon-6 fibers could enhance the biocompatibility in terms of cell cytotoxicity and nucleation of CaP NPs. These improved properties of nylon-6 mat caused by CL could make it a potential candidate in bone tissue engineering.

2. Experimental

2.1. Materials

In this study, Nylon-6 (Mw ≈ 35000, Kolon, Korea) and lactic acid monomer (Showa, Japan) were used. Formic acid and acetic acid (analytical grade, Showa, Japan) in 4:1 ratio by weight were used as solvents. Calcium hydroxide (0.1 M) solution is prepared using $Ca(OH)_2$ (Junsei, Japan). All the materials were used as obtained.

2.2. Electrospinning

Different nylon-6 mats were electrospun from the 22 wt% nylon-6 solution (4:1 ratio by weight of $HCOOH/CH_3COOH$) containing 0, 3.2, 6.25, 11.7, and 21 wt% LA with respect to nylon-6 solution. Polymer solution was fed through the plastic micro-tip of 5 mL syringe with a diameter and length of 0.3 and 10 mm, respectively. The syringe was clamped at about 20° angle relative to the horizontal axis for self-flowing of solution without syringe pump. Electrospinning process was carried out at 18 KV electric voltage and 18 cm working distance at room temperature where the humidity was 37%. After vacuum dried for 24 h, the fiber mats were used for further analysis. The electrospun mats were named M1, M2, M3, M4, and M5 to indicate the 0, 3.2, 6.25, 11.7, and 21 wt% LA containing nylon-6 mats, respectively.

2.3. Lactic acid neutralization and calcium lactate formation

Nylon-6 composite mat obtained from 11.7 wt% LA containing nylon-6 solution (M4 mat) 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 obtained mat was named CL/nylon-6. After vacuum dried for 24 h at 30 °C, the fiber mat was used for further analysis. Fourier transform infrared (FT-IR) spectra of Nylon-6/LA and CL/nylon-6 composite mats were directly recorded using an ABB Bomen MB100 Spectrometer