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Calcium phosphate deposition rate, structure and osteoconductivity on electrospun poly(L-lactic acid) matrix using electrodeposition or simulated body fluid incubation

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Abstract

Mineralized nanofibrous scaffolds have been proposed as promising scaffolds for bone regeneration due to their ability to mimic both nanoscale architecture and chemical composition of natural bone extracellular matrix (ECM). In this study, a novel electrodeposition method was compared with an extensively explored simulated body fluid (SBF) incubation method in terms of the deposition rate, chemical composition, and morphology of calcium phosphate formed on electrospun fibrous thin matrices with a fiber diameter in the range from about 200 nm to about 1400 nm prepared using 6, 8, 10 and 12 wt% poly(L-lactic acid) (PLLA) solutions in a mixture of dichloromethane and acetone (2:1 in volume). The effects of the surface modification using the two mineralization techniques on osteoblastic cell (MC3T3-E1) proliferation and differentiation were also examined. It was found that electrodeposition was two to three orders of magnitude faster than the SBF method in mineralizing the fibrous matrices, reducing the mineralization time from about two weeks to an hour to achieve the same amounts of mineralization. The mineralization rate also varied with the fiber diameter but in opposite directions between the two mineralization methods. As a general trend, the increase of fiber diameter resulted in a faster mineralization rate for the electrodeposition method but a slower mineralization rate for the SBF incubation method. Using the electrodeposition method, one can control the chemical composition and morphology of the calcium phosphate by varying the electric deposition potential and electrolyte temperature to tune the mixture of dicalcium phosphate dihydrate (DCPD) and hydroxyapatite (HAp). Using the SBF method, one can only obtain a low crystallinity HAp. The mineralized electrospun PLLA fibrous matrices from either method similarly facilitate the

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proliferation and osteogenic differentiation of preosteoblastic MC3T3-E1 cells as compared to neat PLLA matrices. Therefore, the electrodeposition method can be utilized as a fast and versatile technique to fabricate mineralized nanofibrous scaffolds for bone tissue engineering.

Keywords

Electrodeposition; SBF incubation; Calcium phosphate; Nanofiber; Bone tissue engineering

1. Introduction

The repair of damaged or diseased osseous tissue, especially in large defects, remains a major clinical challenge [1, 2]. To overcome the various limitations of conventional therapies, tissue engineering approaches have emerged as a promising new strategy for bone repair, in which osteogenic cells and/or therapeutic molecules (such as growth factors) can be integrated into three-dimensional (3D) porous scaffolds to create an appropriate micro-environment to induce tissue regeneration by mimicking the natural way [3].

In bone tissue engineering, a porous scaffold serves as a temporary extracellular matrix (ECM) for osteogenic cells and a 3D template to guide new bone formation. A scaffold with a good biocompatibility, controllable biodegradability, and sufficient strength is required to regenerate bone of a large size [3, 4]. Moreover, it is desirable for a scaffold to mimic certain chemical composition or/and physical architecture of native bone ECM to enhance its biological function [3, 5-11]. Natural bone ECM is an organic/inorganic nanocomposite material, in which partially carbonated hydroxyapatite (HAp) nanocrystals and collagenous fibers are well organized in a hierarchical architecture [12]. Mineralized scaffolds have been shown to advantageously promote osteogenic cellular activities, mineral deposition, and bone formation [13-19]. Several techniques including electrospinning [20-23], phase separation [24, 25], and self-assembly [26, 27] have been developed to create nanofibrous polymer or polymer-ceramic composite scaffolds. Nanofibrous composite scaffolds fabricated using these techniques have improved the bone-forming capability of cells over their single-component counterparts [14, 28, 29].

Simulated body fluid (SBF) has been used to generate surface-mineralized polymer composite scaffolds [30-33]. The obtained calcium phosphate is a bone-like apatite, similar to the natural bone mineral in composition and structure. However, this is a time-consuming process that takes a few weeks to achieve an appropriately mineralized layer [34, 35], which may result in partial degradation of the polymer materials and alter the release characteristics of any encapsulated therapeutic agents or biological factors. Recently, several techniques have been used to accelerate the mineralization process of electrospun matrices, including surface hydrolysis [36], plasma treatment [37], and surface functionalization via layer-by-layer (LBL) self assembly [38], wherein the mineralization process can be accelerated by activating or introducing functional groups on fiber surface, such as carboxyl, phosphate, and hydroxyl groups [39, 40]. However, the mineralization rate or/and the mineral structure remains not well controlled.

Electrodeposition has been extensively utilized to deposit a bone-like apatite coating on metallic substrates (e.g., stainless steel, titanium and their alloys) to improve their bioactivity and biocompatibility [41-44]. However, to date very little research has been done on electrodeposition of apatite onto a polymer scaffold. We recently demonstrated the feasibility of applying electrodeposition techniques to coat calcium phosphate onto the surface of a nanofibrous scaffold [45]. The purpose of this study was to evaluate the electrodeposition method against the SBF method in depositing calcium phosphate on

electrospun poly(L-lactic acid) (PLLA) nanofibers. These two mineralization methods and resulting matrices were compared in terms of deposition rate, composition and morphology of the formed coating. Moreover, the osteoblastic cell adhesion, proliferation and osteogenic differentiation on the two types of matrices were also evaluated.

2. Materials and methods

2.1. Materials

PLLA with an inherent viscosity of approximately 1.6 was purchased from Boehringer Ingelheim (Ingelheim, Germany) and was used as received. Other chemical reagents were obtained from Fisher Scientific (Pittsburgh, PA). Fetal bovine serum, penicillin-streptomycin, trypsin-EDTA, and -MEM were purchased from Gibco BRL Products, Life Technologies (Grand Island, NY).

2.2. Matrix preparation by electrospinning

PLLA solutions with concentrations of 6 wt%, 8 wt%, 10 wt%, and 12 wt% were prepared by dissolving PLLA pellets into a mixture of dichloromethane and acetone (with a volume ratio of 2:1). A solution was placed in a 10 ml plastic syringe fitted with an 18-gauge needle. The nanofibers were electrospun at 18 kV by using a Gamma high potential supply (Gamma High Potential Research, Inc, Ormond Beach, FL). A stainless steel electrode collector (20 mm × 20 mm × 0.2 mm) or aluminum foil was located at a fixed distance of 15 cm from the needle tip. The solution was fed into the needle using a syringe pump (78-0100I, Fisher Scientific, Pittsburgh, PA) at a flow rate of 3 ml/h. For the electrodeposition process, the nanofibers were collected on the electrode to a thickness of about 200-300 μm. For the SBF process, the nanofibers with the same thickness as that for the electrodeposition process were collected on an aluminum foil. The nanofibers were dried overnight under vacuum at room temperature to remove residual solvents.

2.3. Electrodeposition

A schematic diagram of experimental setup for fabricating mineralized nanofibers using electrospinning and electrodeposition is shown in Figure 1. Electrodeposition was performed under potentiostatic conditions in a two-electrode system in which a platinum plate electrode (20 mm × 20 mm × 0.2 mm) served as the counter electrode and the fiber-covered stainless steel electrode as the working electrode. The distance between the two electrodes was fixed at 2.5 cm. A 250 ml electrochemical beaker was immersed in a water bath to maintain the designated temperature. The electrolyte was a solution of 0.042 mol/l $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and 0.025 mol/l $\text{NH}_4\text{H}_2\text{PO}_4$. Prior to electrodeposition, the fiber-covered electrodes were immersed into alcohol for 1-2 minutes to reduce the hydrogen gas evolution at the deposition electrode. The process parameters such as solution temperature, electrical potential and deposition time were variables and specified in the related texts. Upon the completion of the electrodeposition, the mineralized PLLA mesh was removed from the stainless steel electrode, freeze-dried and stored for structural characterization or cell culture studies.

2.4. SBF method

Electrospun matrices were cut into a square shape with dimensions of 20 mm × 20 mm. The 1.5× SBF was prepared as previously reported [30]. The square matrices were incubated in 40 ml solution of 1.5× SBF maintained at 37°C for mineral deposition. The SBF was renewed every 24 hours. After being incubated for the predetermined time periods, the samples (triplicates for each matrix) were removed from the solution and immersed in 400 ml deionized water overnight to remove the soluble inorganic ions. All the samples were vacuum dried at room temperature for 72 hours before further characterization.

2.5. Characterization

The un-mineralized (control) and mineralized matrices were examined by using a Philips XL30 FEG scanning electron microscope (SEM) operating at 10 kV. The samples were coated with gold using a sputter coater (Desk-II, Denton vacuum Inc., Moorstown, NJ). The coating time was 100 s and 140 s for un-mineralized and mineralized matrices, respectively. The average fiber diameters were determined from over 50 random measurements on a typical SEM image using ImageJ software (National Institutes of Health, USA).

X-ray photoelectron spectroscopy (XPS, Perkin-Elmer, model PHI 5400) was used to determine the film surface composition. All surface spectra were obtained over the range of 0-1000 eV operated at an anode potential of 15 kV and an emission current of 20 mA with the Al K α source. Samples were attached to the aluminum sample platform with a double-sided tape. The take-off angle was 30° with respect to sample plane. The pressure during analysis was maintained at about 10⁻⁹ Torr. Survey spectra and the high-resolution region of the spectra were recorded using 89.45 and 17.90 eV analyzer pass energies. All binding energies were referenced to the peak of aliphatic carbon at 285.0 eV. Quantitative analyses were performed using peak areas and elemental sensitivity factors. The Ca/P atomic ratio was calculated to characterize the chemical composition of the deposited mineral crystals.

To investigate the crystalline phase of the deposits, the mineralized fibrous samples (20 × 20 mm) were analyzed using a Rigaku rotating anode X-ray diffractometer equipped with Cu K α radiation source (40 kV, 100 mA). The diffraction scans were recorded at 2 θ = 10-70° with a scanning rate of 10 °/min.

2.6. Cell culture and seeding

The thawed mouse calvaria-derived preosteoblastic cells (MC3T3-E1) were cultured in a complete medium (α-MEM supplemented with 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin) in a humidified incubator at 37°C with 5% CO₂. The medium was changed every other day.

Three types of matrices, including neat PLLA nanofibrous matrix (neat-PLLA, as control), SBF mineralized PLLA matrix (SBF-PLLA), and electrodeposition mineralized PLLA matrix (ED-PLLA), were used for cell seeding and evaluation. All the matrices for cell culture were prepared from a 10 wt% PLLA solution, and the two kinds of mineralized matrices had similar mineral contents (about 50% in weight). Each matrix was cut into a circular disc and wetted by soaking in 70% ethanol for 30 min, washed three times with PBS for 30 min each, and twice in cell culture medium for 1 h each on an orbital shaker (3520, Lab-Line Instruments, Inc.). Cells were then suspended and seeded on every matrix. The cell-seeded matrices were cultured in the humidified incubator at 37°C with 5% CO₂.

2.7. Cell morphology

After 3 days of cell culture, the cell-seeded matrices were removed from the culture plates and washed with PBS for three times. The samples were fixed with 3% glutaraldehyde in PBS at 4°C for 24 h. After being thoroughly washed with PBS, the samples were treated with 1% osmium tetroxide in 0.1 mol/l cacodylate buffer for 1 h, and then washed with PBS. The samples were dehydrated sequentially in 50%, 60%, 70%, 80%, 90%, and 100% ethanol for 30 min each and then dried in 100% hexamethyldisilazane (HMDS). The dried samples were cross-sectioned, sputter-coated with gold, and observed under an SEM (Philips XL30 FEG) at 10 kV.

2.8. Proliferation assay

For cell proliferation assay, 5×10^3 cells were seeded on each matrix in 48-well tissue culture plates. MTS assay was carried out at days 1, 4, and 10 after cell seeding. Cell proliferation was examined using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay kit (Promega, Madison, WI, USA). Briefly, 200 μ l fresh medium and 40 μ l CellTiter 96 Aqueous One Solution Reagent were added to each well, after being incubated at 37°C for 1.5 h, the solutions were transferred into 96-well cell culture plates. The absorbance was then read at 490 nm with a microplate spectrophotometer.

2.9. Alkaline phosphatase (ALP) assay

For osteogenic differentiation assay, 2×10^4 cells were seeded on each matrix in 24-well tissue culture plates. 24 hours after cell seeding, complete medium supplemented with 50 mg/ml ascorbic acid and 10 mM β -glycerol phosphate was added. The medium was changed every other day. ALP activity was measured at 7 and 14 days. ALP was extracted and detected using the EnzoLyte pNPP Alkaline Phosphatase Assay Kit (AnaSpec, San Jose, CA, USA). The cell-seeded matrices were homogenized in 400 μ l lysis buffer provided in the kit. The cell suspension was centrifuged at $10,000 \times g$ at 4°C for 15 min. Supernatant was collected for ALP assay using p-nitrophenyl phosphate (p-NPP) as a phosphatase substrate and alkaline phosphatase provided in the kit as the standard. The amounts of ALP in the cells were measured at 405 nm and normalized against total protein content.

2.10. Statistical analysis

All experiments were conducted at least three times and all values are reported as the mean $\pm$ standard deviation. Statistical analysis was carried out using Student's t-Test (assuming unequal variance). The difference between two sets of data was considered statistically significant when $p < 0.05$.

3. Results

3.1. The diameter of nanofibers

The diameters of PLLA nanofibers fabricated using electrospinning of different polymer concentrations are shown in Figure 2. The average fiber diameter significantly increases with increasing polymer concentration.

3.2. The effect of fiber diameter on the rate of mineralization

In both mineralization processes, the amounts of calcium phosphate on the PLLA matrices increase with increasing mineralization time (Figure 3). However, the fiber diameter has distinct effects on mass increase of the PLLA matrices for the two different mineralization processes. Figure 3a shows the mass increase of matrices produced from varying PLLA concentrations versus electrodeposition time at 3V and 60 °C. For a fixed deposition time, the increase in fiber diameter results in an increase in deposition rate. For example, the mass increase of PLLA matrices with an average fiber diameter of 1363 nm (prepared from a 12 wt% solution) was about 116% after 60 min, whereas the mass increase of PLLA matrices with an average fiber diameter of 211 nm (prepared from a 6 wt% solution) was about 43% after 60 min. Figure 3b shows the mass increase of matrices with varying fiber diameters after incubation in $1.5 \times$ SBF at 37 °C for various time periods. In general, increasing fiber diameter reduces mass increase rate of the matrices. A 418% mass increase was obtained for 6 wt% PLLA matrices after incubation for 30 days, whereas only 145% mass increase was obtained for 10 wt% PLLA matrices within the same incubation time. As for 10 wt% PLLA matrices, 99% mass increase was obtained after electrodeposition for 60 min, which was roughly equivalent to that of the similar matrices after incubation in $1.5 \times$ SBF for 12 days.

Therefore, these two mineralized matrices were selected for subsequent cell culture experiments because the two types of matrices had similar calcium phosphate contents.

3.3. Mineralized fiber formation

In a previous study [45], we reported that the morphology of calcium phosphate coating could be tuned by altering the processing parameters (including electrolyte temperature, electric potential, and deposition time) in the electrodeposition process. Figure 4 shows the morphological difference of calcium phosphate deposited on the surface of 10 wt% PLLA nanofibrous matrices between the two different mineralization processes. A flake-like calcium phosphate coating was formed on top of the nanofibrous mesh when electrodeposition was conducted at 3V and 60°C for 60 min, as shown in Figure 4a and 4b. When SBF process was used, the calcium phosphate crystals were deposited around the individual nanofibers, resulting in core-shell mineralized fibers. Figure 4c-f shows representative SEM images of the same matrices mineralized in 1.5× SBF for 12 and 30 days, respectively. After 12 days of incubation in SBF, the formed calcium phosphate was equivalent in mass to that by electrodeposition for 60 min. The SEM images (Figures 4c versus 4e; Figures 4d versus 4f) indicate that the fiber diameter increases significantly with increasing incubation time, and the matrix's overall porosity decreases with the incubation time. After 30 days of incubation, the nanofibers were fully covered by a thicker mineral layer, but the matrix's fibrous structure was maintained (Figure 4e). The magnified SEM image (Figure 4f) revealed that the mineral layer was mainly composed of amorphous apatite and a small quantity of mineral granules, and the thickness of mineral layer was around 1~2 μm .

3.4. Calcium Phosphate Characterization

As mentioned previously, all matrices for cellular evaluation were electrospun from 10 wt% PLLA solution; both types of mineralized matrices had similar degrees of mineralization. Figure 5 shows the XRD spectra of the two kinds of mineralized PLLA matrices with a similar mineral content (about 50%). The XRD data suggests that samples prepared by electrodeposition contain a mixture of dicalcium phosphate dihydrate (DCPD) and HAp. The peak (020) at $2\theta = 11.7^\circ$ is from DCPD, while diffraction peaks at $2\theta = 26^\circ$, 31.9° , and 33° correspond to (002), (211), and (300) planes of HAp. In addition, the XRD spectra of mineralized matrices also exhibited peaks at $2\theta = 16.1^\circ$ and 18.4° , corresponding to the characteristic peaks of PLLA. The XRD spectra of the SBF modified matrices contain peaks centered at $2\theta = 26^\circ$, 31.9° and 45.4° , which are characteristic of carbonated HAp [46].

The typical XPS spectra of the neat PLLA matrix (prepared from a 10 wt% PLLA solution), electrodeposition-modified PLLA matrix (ED-PLLA matrix), and the SBF-modified PLLA matrix (SBF-PLLA matrix) are shown in Figure 6. The Ca2p/P2p ratios for the ED-PLLA matrix and SBF-PLLA matrix were 1.21 and 1.26, respectively, which are significantly lower than that of the stoichiometric HAp (Ca/P=1.67). The mineral in the SBF-PLLA matrix contained a low-crystallinity carbonated HAp, whereas the mineral in the ED-PLLA matrices contained a mixture of HAp and calcium-deficient calcium phosphate DCPD (Ca/P=1.0). For the mineralized matrices, besides the expected Ca, P, and O peaks, a C1s peak was observed, which is likely from PLLA.

3.5. Cellular behavior

Figure 7 shows the cellular morphology of MC3T3-E1 cells grown on the mineralized and un-mineralized matrices after 3 days of culture. It should be noted that both the neat-PLLA matrix (Figure 7b) and the SBF-PLLA matrix (Figure 7f) showed a visible fibrous morphology. In contrast, the PLLA fibers in the ED-PLLA matrix were covered by flake-like calcium phosphate (Figure 7d). Cells adhered well on all the matrices, and no distinct

morphological difference of the cells was observed on these matrices. However, the proliferation rates of MC3T3-E1 cells on the neat and mineralized nanofibrous PLLA matrices were different (Figure 8). After 10 days of culture, cell numbers on the SBF-PLLA matrices and the ED-PLLA matrices were significantly higher than those on the neat PLLA matrices, whereas there was not a significant difference between the SBF and ED modified PLLA matrices.

Figure 9 shows the ALP activity of MC3T3-E1 cells cultured on different nanofibrous matrices after 7 and 14 days in culture. ALP activities of the cells on both ED and SBF modified matrices were higher than those on unmodified PLLA matrices at both 7 and 14 days. However, differences between the ALP activities of the ED and SBF mineralized PLLA matrices were not statistically significant.

4. Discussion

The development of calcium phosphate-containing nanofibrous matrices has broadened the scope of bone tissue engineering matrices, mimicking both nanoscale architecture and chemical composition of native bone ECM [30, 31, 47]. SBF incubation represents a widely used and an effective way to introduce bone-like apatites onto nanofibrous matrices. However, the process is time-consuming and usually requires a few weeks to complete. Hence, the electrodeposition technique for rapid mineralization of nanofibrous matrices was recently developed in our lab [45] and is compared with the SBF incubation technique in terms of mineralization rate, mineral structure, formation mechanisms, and biological effects on osteogenic cells in this work.

The significant mass increase of mineral deposition on nanofibrous matrices can be achieved using either method (Figure 3), but there is a dramatic difference in the required time between the SBF and the electrodeposition methods. Formation of a calcium phosphate layer on the surface of a nanofibrous matrix takes only one hour using the electrodeposition method, whereas it takes many days using the SBF method. Interestingly, the diameter of nanofibers has distinctly different effects on the deposition rate of the two different mineralization methods. For electrodeposition, the increase in fiber diameter results in a faster deposition rate. On the contrary, the increase in fiber diameter results in a slower deposition rate for the SBF method. This phenomenon may be attributed to the different deposition mechanisms involved in the two methods. In the case of electrodeposition, the calcium phosphate deposition is aided by electrochemical reactions on the cathode surface that increase the local pH value and consequently result in the super-saturation of calcium phosphate at the vicinity of the cathode. The PLLA nanofibers overlaid on the cathode serves as an effective substrate for calcium phosphate deposition, which allows positively-charged ions migrate towards the cathode due to the high porosity between the nanofibers. The electrical current densities are not equal on the outer surface and inner surface of the electrospun fibers. The electrical current density on the surface that face the ion movement (outer surface) is higher than that on the other surface (inner surface), which was corroborated by the data of a porous electrode [41]. Thus, calcium phosphate is easier to deposit on the outer surface due to a higher electrochemical reaction rate. Additionally, a locally concentrated alkaline environment at the vicinity of PLLA nanofibers may activate carboxyl groups by partially hydrolyzing the PLLA in the initial stage of electrodeposition [45]. The activated anionic groups on the fiber surface are favorable for enrichment of calcium ions and calcium phosphate nucleation [30, 33]. Since a supersaturation state is maintained by the applied electric field during electrodeposition process, rapid crystal growth can be achieved, resulting in the formation of larger crystals on the fiber surface. The fibers of larger diameters provide larger surface areas on individual fibers, which, we hypothesize, allow for the development of more stable mineral nuclei and growth of larger

mineral particles, leading to an increased overall deposition rate during electrodeposition. However, unlike the electrodeposition process, all the nanofibers are exposed to essentially the same un-accelerated deposition conditions (ionic strength, pH value, etc.) during the SBF incubation, leading to a slower overall deposition rate. The nucleation sites compete equally for calcium and phosphate ions. Thereby a non-directional uniform coating with a smaller crystal size is formed on the surface of each fiber during incubation in SBF. Smaller diameter fibers provide a larger total surface area than large diameter fibers, leading to the faster mineral deposition rate in the SBF incubation process.

Also, the two mineralized matrices exhibit clear differences in their mineral morphology. The surface morphology of ED-PLLA matrices could be controlled by adjusting the processing conditions such as the deposition potential and the electrolyte temperature. In our previous study [45], a low deposition potential of 2V created a lower super-saturation condition in the vicinity of the nanofibers, leading to the deposition of sparse and large size apatite particles. A moderate deposition potential of 3V increased the degree of super-saturation, which not only allowed for the formation of more mineral nuclei on the surface of nanofibers (heterogeneous nucleation), but also competitively reduced the mineral nucleation in the electrolyte (homogeneous nucleation), providing a favorable environment for a thicker compact apatite layer formation. A further higher electrical deposition potential triggered hydrogen bubble formation, resulting in porous apatite formation. The electrolyte temperature also had significant effect on the thermodynamic stability and solubility of calcium phosphate [41]. The mineral layer formed at a lower temperature of 25°C had an amorphous nest-like structure, whereas the mineral layer was composed of flake-like and needle-like crystals when the electrolyte temperature was increased to 60°C and 80°C, respectively [45]. Therefore, the morphology of the deposited calcium phosphate can be regulated by the processing conditions using the electrodeposition technique.

In contrast, the mineral layer produced by SBF technique was more homogeneous and was mainly composed of a lower crystallinity apatite. The mineralized matrices still kept the visible fibrous structure, where a mineralized fiber had a core-shell structure with polymer fiber as the core and calcium phosphate as the shell. Figure 10 schematically illustrates the formation of different calcium phosphate layer structures on nanofibrous matrices by using the two different mineralization techniques (electrodeposition and SBF incubation).

The XRD and XPS results confirmed that the electrodeposited mineral (3V and 60°C) contained a mixture of DCPD and HAp, while the mineral formed during SBF incubation was mainly composed of a lower crystallinity carbonated HAp. However, there was no significant difference in the overall Ca/P ratio between the two kinds of mineral layers formed. In this study, we selected conditions to coat the electrospun matrices with similarly large amounts of CaP for the convenience of characterizing the deposited mineral structure more easily and of identifying the symmetrical “core-shell” deposition (SBF method) or unsymmetrical “carpeting-like” deposition (electrodeposition method) at later stages. When needed, the conditions can be altered to achieve desired degrees of mineralization to maintain both the nanofibrous structure and partially mineralized composition, which is part of our ongoing studies that aim at generating advanced 3D pore network structure, maintaining an optimal fiber size, and achieving desired mineral composition and morphology.

MC3T3-E1 cells were cultured on these matrices. The cell attachment, proliferation, and osteogenic differentiation were examined. No substantial difference in cell morphology was identified among the three types of matrices after 3 days in culture. Significant increases in cell proliferation rates were observed on both types of mineralized matrices compared to neat PLLA matrix after 10 days in culture. ALP activity is an early marker of osteoblast

differentiation [48]. The MC3T3-E1 cells grown on both types of mineralized matrices exhibited significantly higher ALP activity than those on the unmineralized matrix after 7 and 14 days in culture, indicating that both types of calcium phosphate coating promoted the osteogenic differentiation of MC3T3-E1 cells. However, more detailed studies, especially on scaffolds with designed 3D pore network, are needed to further evaluate the effect of deposited calcium phosphate coatings by the two different methods on the proliferation and differentiation of stem and osteogenic cells.

5. Conclusions

The electrospun PLLA fibrous thin matrices (prepared with 6, 8, 10 and 12 wt% PLLA solutions) were mineralized using either electrodeposition or simulated body fluid incubation. While larger diameter fibers (such as 1363 nm) accelerate calcium phosphate deposition rate compared to smaller diameter fibers (such as 211 nm) during electrodeposition, the larger diameter fibers reduce the calcium phosphate deposition rate compared to the smaller diameter fibers during SBF incubation. Compared to simulated body fluid incubation, electrodeposition is substantially more rapid in forming a mineral layer on the surface of electrospun fibrous matrices. Furthermore, the morphology and chemical composition of the formed mineral layer can be controlled by applying different processing conditions such as electrical deposition potential and electrolyte temperature during the electrodeposition. Both types of mineralized PLLA fibrous matrices enhanced cell proliferation and osteoblastic differentiation of MC3T3-E1 cells as compared to the control, the neat PLLA fibrous matrices. Therefore, electrodeposition is a fast and versatile technique to fabricate mineralized fibrous polymer matrices. Since eletrospinning method is limited in designing pore shape or controlling pore size independently from fiber size, systematic studies of 3D pore design and structural optimization of calcium phosphate coating on nanofibrous scaffolds fabricated from a phase-separation method combined with a templating method are ongoing in our lab. These scaffolds and their effect in supporting stem cells for bone regeneration will be reported in the future.

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Figure 1. A schematic diagram of experimental setup for fabricating mineralized nanofibers by combining electrospinning and electrodeposition methods

Figure 2.
The diameters of electrospun fibers from various PLLA solution concentrations.

Figure 3. Mass increases of matrices electrospun from different PLLA concentrations versus time: (a) Using the electrodeposition method at 3V and 60°C; (b) Using the SBF incubation method in $1.5 \times$ SBF at 37°C.

Figure 4. SEM micrographs of mineralized PLLA (10 wt%) matrices. (a) Electrodeposition at 3V, 60°C for 60 min, (b) the magnified image of (a), (c) mineralized in 1.5× SBF for 12 days, (d) The magnified image of (c), (e) Mineralized in 1.5× SBF for 30 days, (f) The magnified image of (e).

Figure 5.
XRD patterns of mineralized PLLA matrices (--DCPD, -- HAp).

Figure 6.
XPS survey spectra of un-mineralized and mineralized PLLA matrices.

Figure 7.

SEM micrographs of MC3T3-E1 cells grown on the mineralized and un-mineralized nanofibrous PLLA matrices after 3 days in culture: (a) Neat PLLA matrices, (b) The magnified image of (a), (c) ED PLLA matrices (3V, 60°C for 60 min), (d) The magnified image of (c), (e) SBF PLLA matrices (SBF incubation for 12 days), (f) The magnified image of (e).

Figure 8. Proliferation of MC3T3-E1 cells on the surface of the mineralized and un-mineralized nanofibrous PLLA matrices after 1, 4 and 10 days in culture. Data were expressed as mean $\pm$ SD (n = 3). Significant difference between groups is indicated by * ($p < 0.05$ compared with the neat PLLA matrix group).

Figure 9.

ALP activity of MC3T3-E1 cells cultured on different nanofibrous matrices after 7 and 14 days in culture. Data were expressed as mean $\pm$ SD ($n = 3$). Significant difference between groups is indicated by * ($p < 0.05$ compared with the neat PLLA matrix).

Figure 10.

Schematic drawing of calcium phosphate coating formation on nanofibrous PLLA matrices using different mineralization methods: (a) Electrodeposition, in which relatively large crystals such as flower-like and flake-like deposits can be formed; (b) SBF incubation, where the prepared mineral layer is relatively uniform and mainly composed of lower-crystallinity apatite, each mineralized fiber exhibited a core-shell structure with polymer fiber as the core and apatite as the shell.