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Electrospun-modified nanofibrous scaffolds for the mineralization of osteoblast cells

J. Venugopal,[1] Sharon Low,[2] Aw Tar Choon,[2,3,4] A. Bharath Kumar,[1] S. Ramakrishna[1]
[1]*Nanoscience and Nanotechnology Initiative, Division of Bioengineering, National University of Singapore, Singapore*
[2]*StemLife Sdn BhD, 50450 Kuala Lumpur, Malaysia*
[3]*Department of Laboratory Medicine, Changi General Hospital, Singapore 529889*
[4]*School of Medicine and Health Sciences, Monash University Malaysia, Johor Bahru 80100, Malaysia*

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Abstract: Biocompatible polycaprolactone (PCL) and hydroxyapatite (HA) were fabricated into nanofibrous scaffolds for the mineralization of osteoblasts in bone tissue engineering. PCL and PCL/HA nanofibrous surface were modified using oxygen plasma treatment and showing 0° contact angle for the adhesion and mineralization of osteoblast cells. The fiber diameter, pore size and porosity of nanofibrous scaffolds were estimated to be 220–625 nm, 3–20 μm, and 87–92% respectively. The ultimate tensile strength of PCL was about 3.37 MPa and PCL/HA was 1.07 MPa to withstand the long term culture of osteoblasts on nanofibrous scaffolds. Human fetal osteoblast cells (hFOB) were cultured on PCL and PCL/HA surface modified and unmodified nanofibrous scaffolds. The osteoblast proliferation rate was significantly ($p < 0.001$) increased in surface-modified nanofibrous scaffolds. FESEM showed normal phenotypic cell morphology and mineralization occurred in PCL/HA nanofibrous scaffolds, HA acting as a chelating agent for the mineralization of osteoblast to form bone like apatite for bone tissue engineering. EDX and Alizarin Red-S staining indicated mineral Ca^{2+} and phosphorous deposited on the surface of osteoblast cells. The mineralization was significantly increased in PCL/HA-modified nanofibrous scaffolds and appeared as a mineral nodule synthesized by osteoblasts similar to apatite of the natural bone. The present study indicated that the PCL/HA surfacemodified nanofibrous scaffolds are potential for the mineralization of osteoblast for bone tissue engineering. © 2007 Wiley Periodicals, Inc. J Biomed Mater Res 00A: 000–000, 2007

Key words: electrospun nanofibers; mineralization; hydroxyapatite; polycaprolactone; bone regeneration

INTRODUCTION

Bone tissue engineering is a rapidly expanding research area providing new and promising approach for bone repair and regeneration. Several requirements have been considered for engineering bone by choosing a cell type that matures/differentiates into bone cells with proper form and phenotype, regulating the growth factors and designing an ideal scaffold.[1] An ideal scaffold must be biocompatible both in bulk and degraded form, exhibit a porous, be interconnected and of permeable structure to permit ingress of cells and nutrients should exhibit appropriate surface structure and chemistry for cell adhesion and proliferation.[2] Several techniques aim to produce a scaffold that can mimic the architecture of natural extracellular matrix (ECM). Natural ECM is composed of various protein fibrils and fibers interwoven with a hydrated network of collagen and glycosaminoglycan chains.[3] This network structure serves as a scaffold which can support tensile and compressive stresses by the fibrils and hydrated networks. Besides providing an appropriate microenvironment of cells, ECM is responsible for transmitting signals to cell membrane receptors that reach nucleus via intracellular signaling cascades. Therefore, the fibrillar and porous structure of ECM have a great influence on cell functionality, mainly on cell adhesion, proliferation and migration.

Recently, electrospinning processes have attracted a great deal of attention as a way to mimic the structure of natural ECM by means of producing fibers down to 5 nm.[4,5] This technique is used to fabricate

Correspondence to: Dr. J. Venugopal; e-mail: engjrv@nus.edu.sg
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nanofibrous structures from natural and synthetic polymers, such as collagen,[6] chitosan,[7] silk fibroin,[8] and poly(DL-lactide-*co*-glycolide),[9,10] poly(lactide),[11] polyurethane[12] and polycaprolactone.[13] Moreover, blending of synthetic and natural polymers allows a control degradation rate of the system: as the degradation kinetics of a bioartificial blend increase with increasing the natural polymer amount, the blend composition can be adjusted to make a scaffold degradation rate match with the growth rate of regenerating tissue.[14] The produced polymeric nanofibrous networks have been proposed for engineering many different tissues. For instance, Li et al. reported that electrospun PCL membranes could promote chondrocyte proliferation and provide maintenance of chondrogenic phenotype.[15]

Several scaffold materials have been investigated for tissue engineering bone and cartilage, including hydroxyapatite (HA), poly(α-hydroxyesters) and natural polymers such as collagen and chitin. HA implants or coatings provide good adhesion of cells, due to their surface chemistry and have been shown to enhance osteoblast proliferation and differentiation.[16] Osteoblasts initiate biomineralization in a rapid and reproducible manner which would greatly facilitate exploring the variables that regulate the initiation of mineralization. PCL mats could be useful as a scaffold to support differentiation of mesenchymal stem cells cultured in specific differentiation media.[17] Marra et al. reported that PCL is a comparable substrate for supporting cell growth resulting from two-dimensional bone-marrow stromal cell culture.[18] In this research, we studied PCL-based bioartificial blends, which are biodegradable and biocompatible synthetic polymer with good mechanical properties,[19] which may undergo enzymatic degradation through hydrolysis of its ester bonds by lipases.[20] The main applications of PCL in biomedical field include tissue engineered skin,[13] drug delivery systems,[21] axonal regeneration,[22] and scaffolds supporting fibroblasts[23] and osteoblasts growth.[24] This work is aimed to study electrospun-modified PCL nanofibrous scaffold, considered as a substitute for bone formation, following addition of HA nanoparticles for the mineralization of osteoblasts for bone regeneration.

MATERIALS AND METHODS

Materials

Human fetal osteoblast cells (hFOB) were obtained from the American Type Culture Collection (ATCC, Arlington, VA). Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 (HAM), fetal bovine serum (FBS), antibiotics and trypsin-EDTA were purchased from GIBCO Invitrogen, USA. Polycaprolactone (MW 80,000) was from Aldrich Chemical Company, USA. CellTiter 96® AQ_{ueous} one solution was purchased from Promega, Madison, WI, USA. Crystalline hydroxyapatite (30.25 ± 4.43 nm) was generously provided by the Department of Metallurgical and Materials Engineering, Indian Institute of Technology, Chennai, India.

Fabrication of nanofibrous scaffolds and characterization

Polycaprolactone (9% w/w) and PCL/HA (1:1) were dissolved in methanol and chloroform (1:3) under stirring conditions for 2 days. PCL/HA nanofibers were fabricated by an electrospinning process, 2 mL/hr employing an applied voltage of 12 kV using a high voltage power supply (Gamma High Voltage Research, USA). The ground collection plate of aluminum foil was located around 12 cm from the needle tip. A positively charged jet was formed from the Taylor cone and nanofibers were sprayed onto the grounded aluminum foil target.[25] Cover slips of different sizes were spread on the aluminum foil target to collect nanofibers to investigate biocompatibility by culturing cells as well as to observe the structure and properties of nanofibers. Nanofibers collected on aluminum foil were used for characterization and assessment of tensile strength. These nanofibers were sputter coated with gold (JEOL JFC-1200 Fine Coater, Japan) and visualized by field emission scanning electron microscopy (FESEM, FEI-OUANTA 200F, Netherland) at an accelerating voltage of 10 kV for characterization. The diameter of the fibers was measured with FESEM images using Image J software (National Institutes of Health, USA). The pore size was measured by bubble point pressure and the porosity was estimated by weight and volume of the sample.[26]

Mechanical characterization of nanofibrous scaffolds

Tensile properties of electrospun nanofibrous scaffolds were determined with a tabletop MicroTester (Instron 5845, USA) using low force load cell of 10 N capacity. Strip shaped specimens (30 × 6 mm^2) were tested at a crosshead speed of 10 mm/min. The ambient condition was controlled to be 25°C and 74% humidity. At least six samples were tested for each type of electrospun nanofibrous scaffolds. Ultimate strength, Young's modulus as well as tensile elongation were calculated based on the generated tensile stress-strain curves.

Air plasma treatment and wettability of nanofibrous scaffolds

Air plasma treatment was conducted to plain PCL and PCL/HA nanofibrous scaffolds by electrode less radio frequency glow discharge plasma cleaner (Model: PDC-001, Harrick Scientific Corporation, USA). The samples placed on a glass slide were stably put in the chamber of plasma

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cleaner. Plasma discharge was applied to samples for 1 min with radio frequency power set as 30 W under vacuuming condition. Water contact angle of PCL and PCL/HA nanofibrous scaffolds before and after plasma treatment were measured using a contact angle measurement system (VCA Optima XE Video Contact Angle System, Crest Technology, Singapore) mounted with a CCD camera at an ambient temperature. The nanofibrous scaffolds were carefully placed on a sample stage and a single drop of distilled water was applied to the mesh surface to measure the angle and averaged.

hFOB cell culture

Human fetal osteoblast cells were cultured in DMEM/F12 medium (1:1) containing 10% FBS in 75 cm^2 flasks. The osteoblast culture was maintained at 37°C in a humidified CO_2 incubator for 6 days and fed every 3 days and hFOB were harvested from 3rd passage cultures by trypsin-EDTA treatment and replated. Populations of cell lines used in this experiment were between passages 4 and 5.

Processing of hFOB cells for FESEM

Human fetal osteoblast cells were seeded (2×10^4 cells/cm^2) on PCL, plasma-treated PCL (PCL-P), PCL/HA, and plasma-treated PCL/HA (PCL/HA-P) nanofibrous scaffolds on 24-well plates. After 6 days of the experiment hFOB grown on scaffolds were washed with PBS to remove non-adherent cells and then fixed in 3% glutaraldehyde for 3 hr at room temperature, dehydrated through a series of graded alcohol solutions and finally dried into hexamethyldisilazan overnight. Dried cellular constructs were sputter coated with gold and observed under FESEM at an accelerating voltage of 10 kV.

MTS assay for hFOB cells proliferation

Human fetal osteoblast cells were seeded (2×10^4 cells/cm^2) on PCL, PCL-P, PCL/HA, and PCL/HA-P nanofibrous scaffolds on 24 well plates. Cell proliferation was monitored after 2, 4, and 6 days by MTS assay (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt). In order to monitor cell adhesion and proliferation on different substrates, the number of cells was determined by using the colorimetric MTS assay (CellTiter 96® AQ_{ueous} Assay). The mechanism behind this assay is that metabolically active cells react with a tetrazolium salt in MTS reagent to produce a soluble formazan dye that can be observed at 490 nm. The cellular constructs were rinsed with PBS followed by incubation with 20% MTS reagent in serum free medium for 3 hr. Thereafter, aliquots were pipetted into 96-well plates and the samples read in a spectrophotometric plate reader at 490 nm (FLUOstar OPTIMA, BMG Lab Technologies, Germany).

Mineralization of hFOB

Alizarin Red-S (ARS) is a dye which binds selectively calcium salts and widely used for calcium mineral histochemistry. ARS staining was used to detect and quantify mineralization.[27] Nanofibrous scaffolds with hFOB cells (construct) were washed five times in PBS and fixed in ice cold 70% ethanol for 1 hr. These constructs were washed three times with dH_2O and stained with ARS (40 m*M*) for 20 min at room temperature. After several washes with dH_2O, the constructs were observed under optical microscope and the stain was desorbed with the use of 10% cetylpyridinium chloride (sigma) for 1 hr. The dye was collected and absorbance read at 540 nm in spectrophotometer (Thermo Spectronic).

Energy dispersive X-ray analysis

Surface morphology of the materials was studied by using scanning electron microscopy (JEOL–JSM–5500, Germany) at an accelerating voltage of 20 kV and the specimens were coated with gold in an automatic sputter coater. Representative areas of the nanofibrous scaffold sample surface were observed at the magnification of ×200 and occasionally higher magnification. Energy dispersive X-ray analysis (EDX) was carried out by using JEOL-EX-23000 BU (Germany) analyzer. The electron beam was used to scan small areas to obtain compositional information from well defined regions of the nanofibrous scaffolds.

Statistical analysis

All quantitative results were obtained from triplicate samples. Data was expressed as the mean ± SD. Statistical analysis was carried out using the unpaired Student's *t*-test. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Characterization of nanofibrous scaffolds

Electrospun PCL and PCL/HA nanofibrous scaffolds showed nonwoven, bead free, porous and interconnected fibrous structure (Fig. 1). HA (50%) was loaded to PCL (50%) nanofibers and these HA embedded within the nanofibers formed nanofibrous matrix for bone tissue engineering [Fig. 1(b)]. The fiber diameter of PCL and PCL/HA nanofibers was around 226–358 nm and 352–625 nm respectively. Fiber diameter increased with the concentration of polymer solution and diameter of the syringe needle. The pore size of PCL and PCL/HA nanofibrous scaffolds was around 3–18 μm, 4–20 μm and porosity was estimated as 87% and 92% respectively. F1

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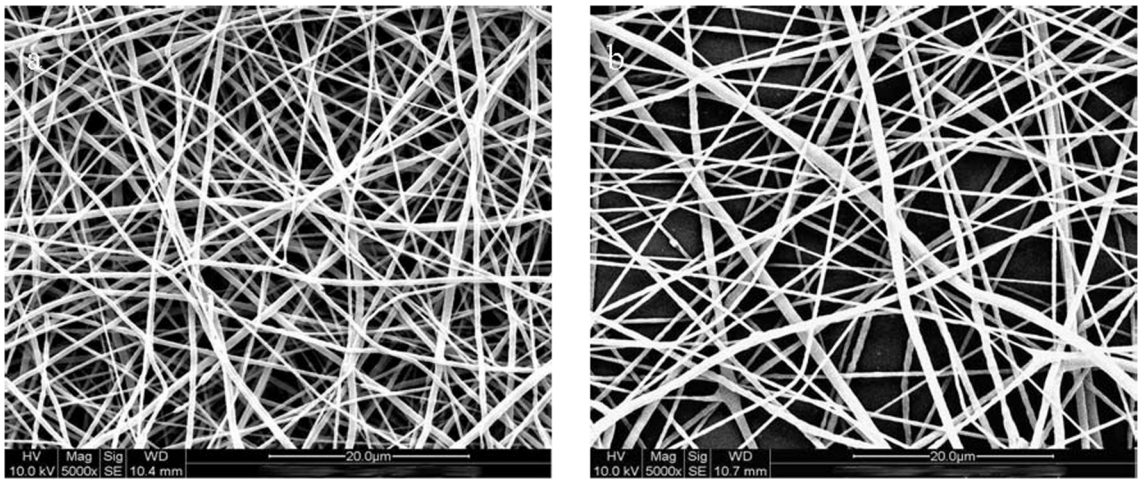

Figure 1. FESEM micrographs of (a) PCL, (b) PCL-HA nanofibers.

Mechanical properties such as tensile modulus, ultimate tensile stress and ultimate strain were evaluated for PCL and PCL/HA nanofibrous scaffolds (Fig. 2). Tensile properties, Young's modulus, and strain at break are summarized in Table I. The tensile strength of PCL and PCL/HA nanofibrous scaffolds was around 3.37 and 1.07 MPa respectively. HA blended with PCL showed no improvement of tensile property of scaffold compared to PCL nanofibrous scaffold. With regard to elongation, the blended HA did not reduce the extension ability but maintained the stability of PCL nanofibrous scaffolds.

Air plasma treatment is a surface selective process and has a significant effect on the surface energy as reflected by contact angle measurements. It modified the polymeric nanofibrous surfaces for adhesion promotion, enhanced wettability, and reduced surface friction and tackiness. The purpose of contact angle instrument is to analyze the surface roughness or heterogeneity of solid substrate and/or deposition of solutes (surfactant, polymers) from the liquid onto the solid surface. Water contact angle of PCL, PCL/HA before and after plasma treatment was measured using a contact angle measurement system mounted with a CCD camera at ambient condition. These nanofibrous scaffolds contact angle was found to be of more or less same wettability: 130.98° and 124.73° respectively (Table II). After plasma treatment, nanofibrous scaffolds absorb water immediately to show the wettability (0°) and suitable for the adhesion of cells.[28]

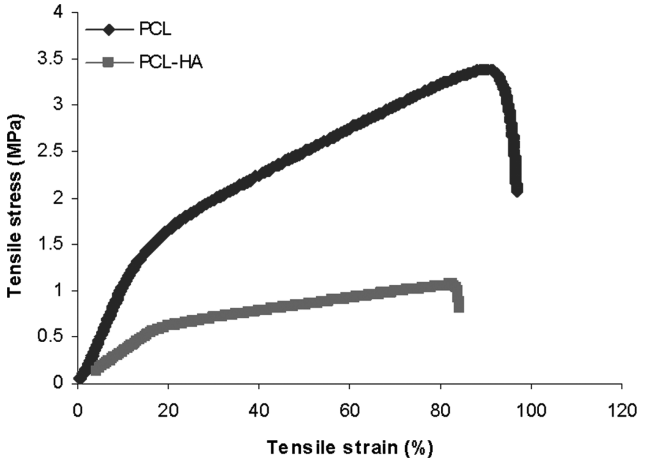

Figure 2. Stress-strain curve for the electrospun nanofibrous scaffolds of PCL, PCL-HA under tensile loading. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Interaction of osteoblasts and nanofibrous scaffolds for mineralization

Human fetal osteoblast cell proliferation on scaffolds (PCL, PCL-P, PCL/HA, PCL/HA-P) were evaluated at 2, 4, and 6 days of *in vitro* culture (Fig. 3). The cell proliferation was significantly ($p < 0.001$) increased on day 4 and 6 on PCL, PCL/HA-modified nanofibrous scaffolds compared to unmodified samples. Percent level of cell growth increased up to 35%, 31% on days 4 and 6 in PCL/HA-modified nanofibrous scaffolds. Cells are observed spanning the gaps of PCL/HA nanofibrous scaffolds and

TABLE I
Tensile Properties of Electrospun Nanofibrous Scaffolds

Sample	Tensile Strength (MPa)	Young's Modulus (MPa)	Strain at Break (%)
PCL	3.37	10.82	102
PCL-HA	1.07	3.52	82

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TABLE II
Contact Angles of Different Electrospun Nanofibrous Scaffolds (Contact Angle in Degrees)

Plasma	PCL	PCL-HA
Before	130.98	124.73
After	0	0

growing across the entire scaffolds after 6 days of cell culture. Osteoblasts grown on plasma treated and untreated PCL nanofibrous scaffold showed limited mineralization after 6 days of the experiment [Fig. 4(a,b)]. After plasma treatment, PCL/HA nanofibrous scaffold surface morphology was dramatically changed to hydrophilic condition for the adhesion of cells to synthesize and secrete ECM to form nodules for bone formation [Fig. 4(d)]. HA possesses the effective affinity for regulating cell function and promoting osteogenesis and mineralization of bone. Cells formed layers on the surface of plasma treated samples and synthesized to secrete their own ECM of collagen for mineralization. Figure 5 shows at different magnifications cell secreting proteins as minerals in the form of apatite-like morphology within 6 days and proved this nodule through ARS staining.

Calcium mineralization was quantitatively measured after elution of ARS staining and showing higher staining intensity on PCL/HA-modified nanofibrous scaffolds compared to PCL nanofibrous scaffold (Fig. 6). The mineralization was increased significantly ($p < 0.001$) after 10 days of osteoblast culture on PCL/HA-modified nanofibrous scaffolds (Fig. 7). EDX spectroscopy was used to determine the presence of mineral calcium and phosphorous in PCL/HA nanofibers, PCL-Osteoblast and PCL/HA-Osteoblast scaffolds (Fig. 8). As shown in Figure 8(c) peaks of Ca and P were observed as nodules in specific area of osteoblast cell layers on nanofibrous scaffolds (Table III); similar nodules were not observed in PCL nanofibrous scaffolds [Fig. 8(b)].

DISCUSSION

Tissue engineering is highly depended on the properties of scaffold and used as a cell carrier and exchange of nutrients. Fabrication of scaffolds in bone tissue engineering generally required scaffold degradability, biocompatibility, high surface area/volume ratio, osteoconductivity and suitable mechanical properties. PCL is a nontoxic, synthetic aliphatic polyester and soft tissue biocompatible material widely used as a drug delivery devices and recently developed as a bone graft substitute in bone tissue engineering because it undergoes slow hydrolytic degradation into natural metabolites.[24,29] A crucial point for a scaffold to be successful in bone tissue engineering, is the combination of structural/mechanical properties of polymer structure and biological activities, all of them playing a critical role in cell seeding, proliferation and new tissue formation. Calcium phosphate materials have been widely used as bone substitutes in dentistry as well as orthopedic and reconstructive surgery. This group of materials exhibits high level of biocompatibility and osteoconductivity and binds directly to bone tissues. Of the various calcium phosphates, HA has received considerable attention because its mineral composition is close to natural bone. However, HA is less soluble than other calcium phosphates such as tricalcium phosphate and consequently remains in the body longer and impedes new bone replacement.[30,31]

The present study is to examine the efficacy of PCL with HA electrospun nanofibrous scaffolds inducing the expression of osteoblast phenotype and subsequent development of mineralized tissue formation for bone tissue engineering. A mineralized matrix was synthesized only by cells grown on PCL/HA combined nanofibrous scaffolds, while mineral deposits were not detected in cultures grown on PCL nanofibrous scaffolds alone (Fig. 4). Electrospinning of PCL/HA mixture produced highly porous nanofibrous structure and also has a high surface area, flexibility for surface modification and sufficient mechanical strength for handling. This fibrous architecture mimicked the natural ECM and assisted in maintaining a normal phenotype of the cells. Surface topography of nanostructured substrate plays a critical role in regulating initial cell behaviors, such as cell adhesion, which can also influence cellular viability and proliferation.[32] In plasma treatment, hydroxyl groups produced on the surface of nanofibrous structure to modify the surface resulted in decreased surface contact angle leading to the

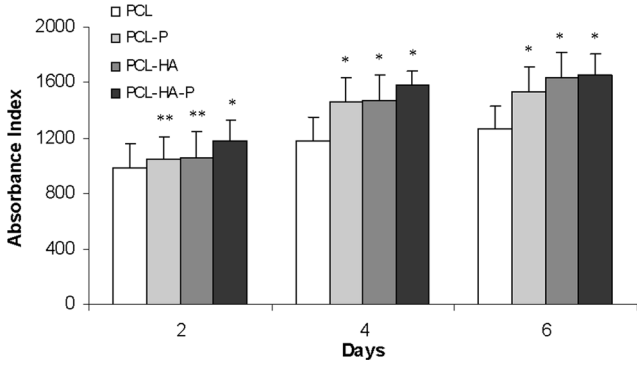

Figure 3. The hFOB proliferation on PCL, PCL-P, PCL-HA, and PCL-HA-P nanofibrous scaffolds ($n = 6$). Bar represent means ± SD. Asterisks indicate significant difference in measurement when compared to PCL nanofibrous scaffold (*$p < 0.001$).

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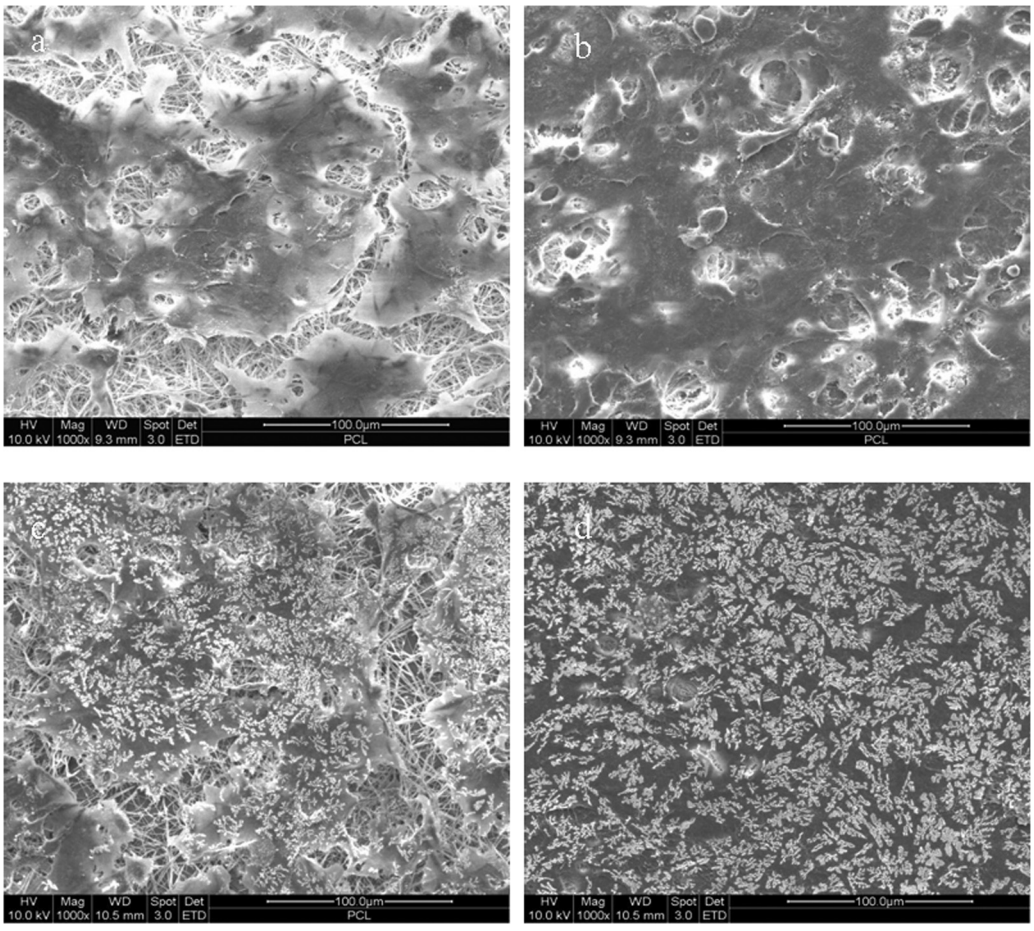

Figure 4. FESEM micrographs of the interaction between hFOB and nanofibrous scaffold after 6 days of culture. (a) PCL-Osteoblasts, (b) PCL-P-Osteoblasts, (c) PCL-HA-Osteoblasts, (d) PCL-HA-P-Osteoblasts.

adhesion and proliferation of osteoblast cells. In water contact angle measurement, plasma-treated nanofibrous scaffolds showed rapid penetration of a water drop into the scaffolds and increased the wettability (Table II). The surface charge, wettabilty, and topography influence the cell adhesion process to vastly different extents.[28]

The pore size and porosity of nanofibrous scaffolds are suitable for spreading and holding the cells by filapodia like structure for the proliferation and secretion of ECM. Porous nanofibrous scaffolds for tissue engineering should have high porosity to allow accommodation of large number of cells, as well as large interconnected pores to facilitate uniform distribution of cells and diffusion of oxygen and nutrients.[33] The present results reveal that the proliferation of hFOB significantly ($p < 0.001$) increased in modified nanofibrous scaffolds compared to unmodified nanofibrous scaffolds. PCL nanofibrous scaffold shown to be significantly less number of cell adhesion and proliferation because of less hydrophilic properties of the scaffold. The increased cell proliferation on plasma treated PCL/HA nanofibrous scaffolds for modifying the surface to hydrophilic nature and HA provides support for the mineralization of ECM proteins (Figures 3 and 6).

The ability to form an ECM that can undergo regulated mineralization is the ultimate phenotypic expression of an osteogenic tissue. Two patterns of mineral deposition on an ECM have been described: (i) matrix vesicle mediated mineral initiation and (ii) heterogeneous nucleation of mineral crystals on collagen, perhaps with the involvement of some noncollagenous glycoproteins that bind Ca^{2+} and collagen.[34,35] An osteogenic cell culture model that mineralizes in a rapid and reproducible manner would greatly facilitate exploring the variables that regulate the initiation of mineralization. The current study demonstrates that the cultured osteoblasts rapidly form an apatite-like, calcium phosphate mineral associated with cells and their surrounding ECM. The osteoblasts support collagen matrix synthesis and bone nodule formation on the surface of cells with the activation of HA (Fig. 5). A subsequent period of matrix maturation, collagen synthesis

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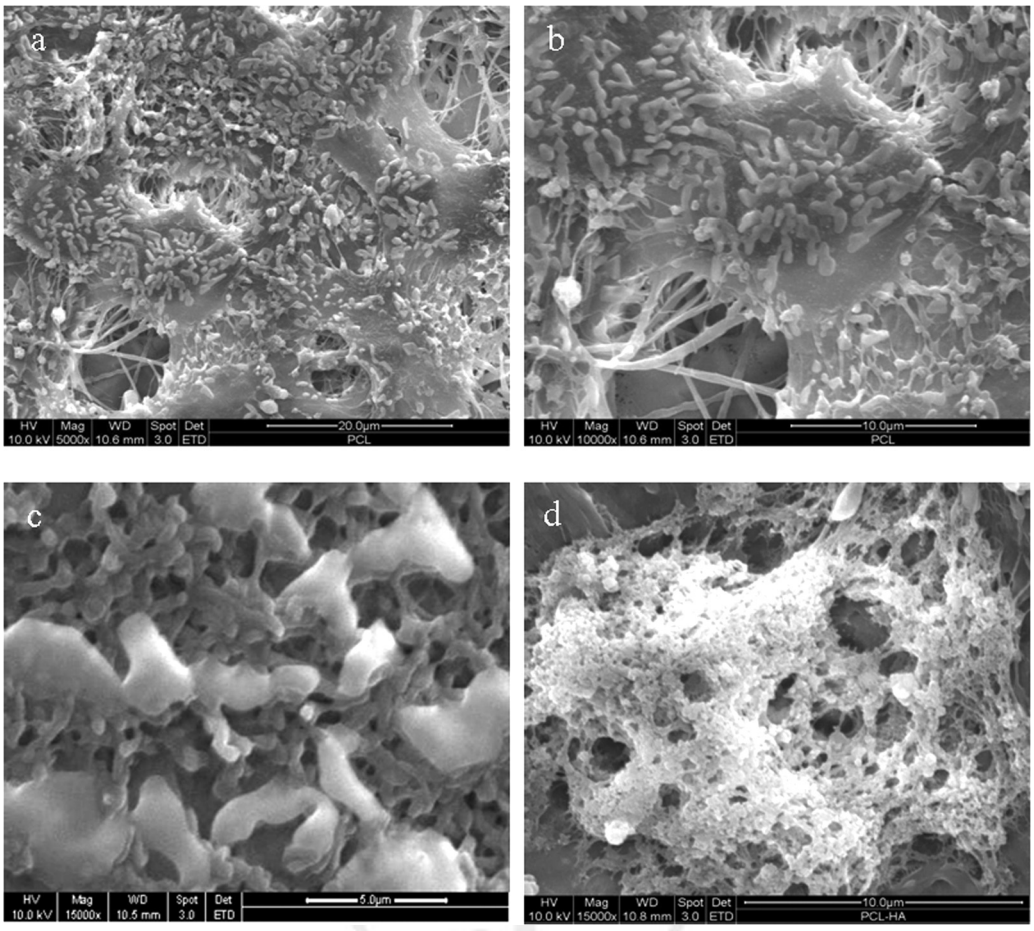

Figure 5. Mineralization of hFOB on PCL-HA-P nanofibrous scaffolds at different magnifications: (a) mineral deposition 5 k (6 days), (b) mineral deposition 10 k (6 days), (c) mineral deposition 15 k (6 days), (d) apatite-like morphology of natural bone 15 k (10 days).

and alkaline phosphatase expressions are elevated and ECM organized in the form of minerals for bone regeneration. Mineralization occurs through the deposition of HA in association with peak expression of bone matrix proteins osteocalcin, osteopontin and bone sialoprotein.[36,37]

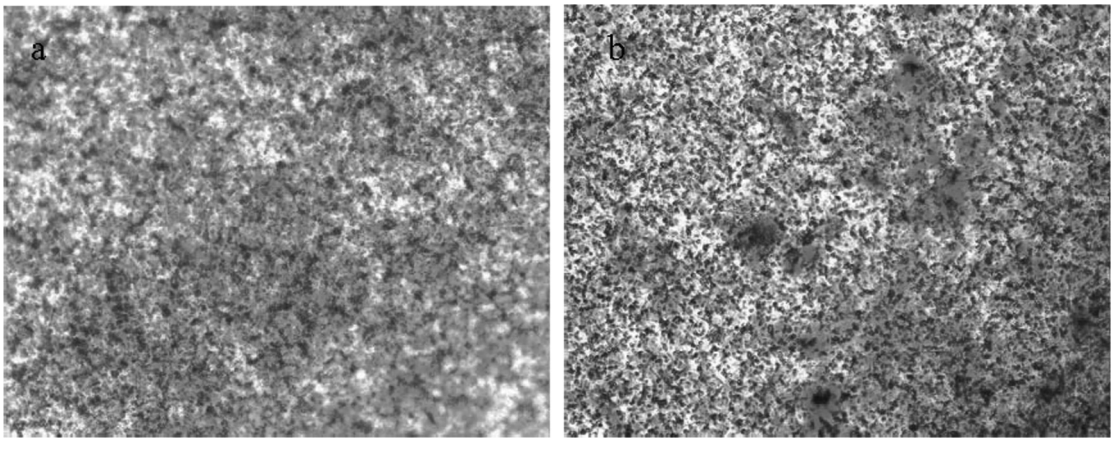

Figure 6. Alizarin Red-S staining for calcium mineralization in hFOB on (a) PCL-P, (b) PCL-HA-P nanofibrous scaffolds after 6 days of culture (×200). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

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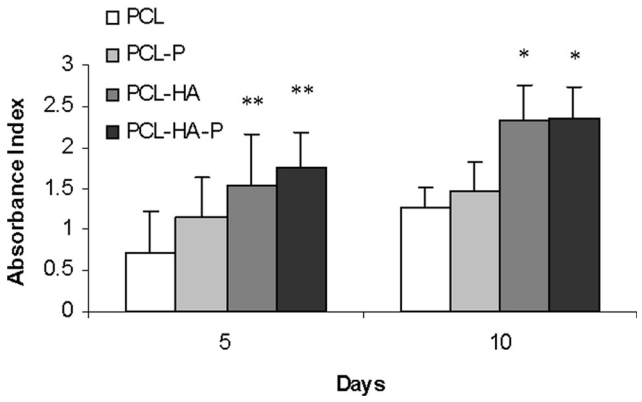

Figure 7. Alizarin Red-S staining of mineral deposition in hFOB on PCL, PCL-P, PCL+HA, and PCL+HA-P nanofibrous scaffolds (*$p < 0.001$, **$p < 0.05$).

FESEM images showed that the osteoblasts covered entire surface area of modified nanofibrous scaffolds and started to fill the spaces after 4–6 days of culture. Figure 4(b,d) shows confluent cell growth on plasma-treated PCL and PCL/HA nanofibrous scaffolds compared to unmodified nanofibrous scaffolds and showing normal cell morphology. Calcification occurred at the nucleation site of matrix vesicles present in the lacunae of mineralizing bone. They are believed to accumulate Ca^{2+} and inorganic phosphate, which served as a nucleating agent for the formation of hydroaxyapatite, the main organic component of bone. Hydroxyapatite acts as a chelating agent for the mineralization of bone in modified nanofibrous scaffold. Figure 5(a–c) shows at different magnifications mineral formation on the surface of osteoblast cell layers within 6 days and the morphology is similar to HA of the natural bone after 10

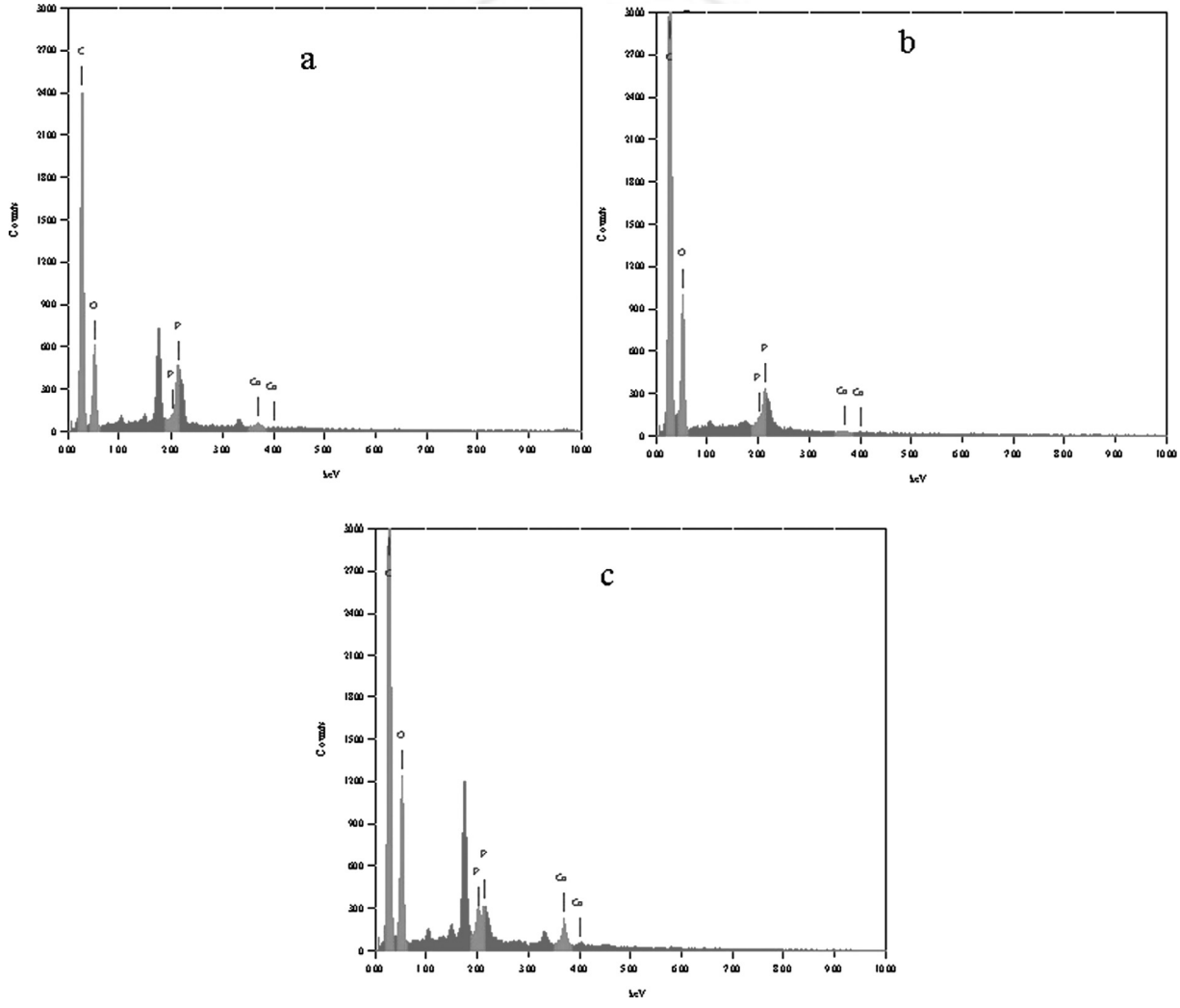

Figure 8. EDX measurement for the detection of mineralization; (a) PCL+HA nanofibrous scaffold, After 6 days of hFOB culture, (b) In control nanofibrous scaffolds (PCL-P) without HA showing low level of minerals compared to HA blended nanofibrous scaffolds, (c) EDX detected significantly higher levels of phosphorus and calcium on PCL-HA-P nanofibrous scaffolds. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

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TABLE III
Weight and Atomic Percentage of O, P, Ca Elements in PCL-HA nanofibers, PCL-Osteoblast, PCL-HA-Osteoblasts for the Mineralization of Bone

	PCL-HA Nanofibers		PCL-Osteoblasts		PCL-HA-Osteoblasts	
Element	Atomic %	Weight %	Atomic %	Weight %	Atomic %	Weight %
O	36.74	30.55	23.72	29.26	26.50	31.96
P	0.31	0.13	0.25	0.33	0.29	0.67
Ca	0.54	0.18	0.05	0.14	0.50	1.51

days [Fig. 5(d)]. In the case of metabolic (cell-mediated) calcification, deposition of salts has to be preceded by the formation of an inorganic extracellular compartment.[38] To be representative for bone formation, mineralization must be osteoblast mediated: a collagen type I containing extracellular compartment has to be formed, on which, via extrusion of membrane-bound vesicles loaded with crystals, mineralization was formed on the surface of the cells.

FESEM and EDX results indicated that the cells are able to attach and grow on PCL nanofibrous scaffolds in the presence of HA to form the mineralized tissue, which primarily consists of calcium and phosphorous deposits (Fig. 8). EDX results were further supported by qualitative and quantitative analysis of ARS-calcium expression, with the highest calcium concentration measured on PCL/HA-modified nanofibrous scaffolds (Figs. 6–8). Morphological evidence demonstrated large number of extracellular mineral deposits on the basal surface of cells that contained crystalline structures associated with a fibrous organic matrix; relatively lot of mineral deposits were detected on the surface of osteoblast cells.

CONCLUSION

We fabricated electrospun biocompatible nanofibrous scaffolds with PCL and HA for the mineralization of osteoblasts in bone tissue engineering. These nanofibrous scaffolds are highly porous and modified the surface area to hydrophilic, to offer biomimicking structure for cell growth and synthesis of biomineral in the form of mineralized bone nodule within 10 days of culture for bone tissue regeneration. Nanofibrous scaffolds also provide more structural space for the adhesion, accommodation, proliferation and mineralization of osteoblast cells and enable the efficient exchange of nutrients and metabolic waste. The PCL/HA nanofibrous scaffolds treated with plasma is to enhance the wettability and thus accelerated the biodegradation rate of nanofibrous scaffolds. We believe that the electrospun PCL-modified nanofibrous scaffolds with HA hold great potential for the mineralization of osteoblasts in bone tissue engineering applications.

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