

## Short communication

Preparation and characterization of LA/PCL composite fibers containing beta tricalcium phosphate ( $\beta$ -TCP) particlesChan-Hee Park<sup>a,\*,1</sup>, Eun Kyo Kim<sup>a,1</sup>, Leonard D. Tijning<sup>b,\*</sup>, Altangerel Amarjargal<sup>a</sup>,  
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## Abstract

Beta-TCP ( $\beta$ -TCP) particle-containing LA/PCL micro/nanofibers were fabricated via a one-step electrospinning process. The morphology and chemical structure of the composite nanofibers were characterized by FESEM, XRD, and FTIR. Rougher surfaces were observed for the LA/PCL micro/nanofibers containing  $\beta$ -TCP compared to the neat LA/PCL fibers, which could possibly provide extra sites for cell binding. XRD and FTIR confirmed the presence of  $\beta$ -TCP as being firmly deposited on the fibers. After an immersion in distilled water, we observed that the  $\beta$ -TCP-containing composite fibers were more degradable with many damaged and broken fibers compared to the LA/PCL fibers. MTT assay and immersion test showed better cell viability and proliferation, and improved mineralization ability compared to the LA/PCL only mat. Thus, the incorporation of  $\beta$ -TCP and the presence of LA in PCL micro/nanofibers could result to an improved biocompatibility and faster degradation of the composite fibers, which would possibly be useful for tissue scaffold application.

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**Keywords:** Beta tricalcium phosphate; Electrospinning; Lactic acid; Polycaprolactone; Degradation

## 1. Introduction

Beta-tricalcium phosphate ( $\beta$ -TCP) ceramics have been used for organic components and are reported to be equivalent to human bone [1,2]. Some studies reported the positive effect of  $\beta$ -TCP, i.e.  $\text{Ca}_3(\text{PO}_4)_2$ , in promoting bone ingrowth and regeneration [3]. However, calcium phosphate is quite brittle material because of its porous surface. Thus, many recent studies have been conducted to improve the ductility of calcium phosphate using polymers, especially biopolymers. Bang et al. [4] coated

alpha-TCP with PCL and investigated its effect on the mechanical properties and cell response. They concluded that coating alpha-TCP with bioresorbable PCL presents good potential candidate as replacement for cancellous-type artificial bone. Another way of utilizing the biocompatible properties of  $\beta$ -TCP is through decoration or incorporation in a polymeric substrate. Paik et al. [5] fabricated a biomimetic polycaprolactone (PCL) fibrous matrix with gradation in TCP content and found that osteoblast proliferation and osteogenic differentiation favored the fibrous matrix with high TCP content. Eriskien et al. [6] reported the potential of functionally-graded nanocomposite structure of PCL- $\beta$ -TCP fabricated by twin-screw extrusion electrospinning for tissue engineering and bone-cartilage interface formation. Others have fabricated polymeric nanofibrous matrices containing proteins and DNA, wherein the protein and DNA were released upon the degradation of the polymeric matrix. Zhang et al. [7] incorporated  $\beta$ -TCP in a silicone rubber by simple blending and

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concluded that the addition of  $\beta$ -TCP can change the surface microstructure and improve cytocompatibility. Many polymer and ceramic composites have been used for improvement of bone formation [8–10]; however, despite the stimulatory effects of ceramic/polymer composite matrix for osteoblasts growth, the electrospun ceramic/polymer composites are not extensively researched yet for biomaterials application.

Electrospinning process is simple yet effective technique in producing micro to nanofibers [11]. The fabricated nanofibrous matrices have gained tremendous interest, mainly due to the structural similarity to the tissue extracellular matrix (ECM), thus it has wide potential for tissue engineering applications [12]. The ECM should be in a three-dimensional matrix with high porosity and surface area to provide adequate space for cell migration and attachment. Studies have shown positive results of using nanofibrous structures for cell adhesion and growth, and tissue development [13,14]. Moreover, the incorporation of inorganic components in polymeric matrices showed better responses *in vitro* and *in vivo* compared when the components were used individually [15,16]. In this study, we fabricated a spongy, 3D cancellous-bone type structure by electrospinning polycaprolactone (PCL) micro/nanofibers containing lactic acid (LA), and decorated with  $\beta$ -TCP. PCL is a synthetic polymer for medical use, which is biocompatible and biodegradable. It has been widely used in tissue engineering scaffolding because of its properties like flexibility, low density and easy processability. Furthermore, it possesses good mechanical strength, rigidity and heat resistance. However, PCL also suffers from low wettability, and low degradation rate, thus limiting its use in the biomedical field [17]. As a way to improve the properties of PCL, incorporating bioactive component such as LA, which is an important constituent of living organism and is largely used in biological systems, could possibly improve the biocompatibility of PCL and improve its degradation rate. Furthermore, the presence of firmly-deposited  $\beta$ -TCP on LA/PCL fibers would improve their biocompatibility, resulting to potentially better results in tissue engineering application.

## 2. Materials and method

### 2.1. Materials

Polycaprolactone (PCL, Mw = 70,000–90,000, Sigma-Aldrich, USA) and lactic acid, (LA, Showa, Japan) were used to prepare the composite nanofiber mats. A mixture of dichloromethane (DMC, Junsei) and N,N-dimethylformamide (DMF, Samchun) in 4:1 ratio by weight was used as solvent.  $\beta$ -TCP particles (chronOS) were purchased from DePuySynthes (USA) and were used as received.

### 2.2. Electrospinning

LA/PCL solutions were prepared by dissolving 10 wt% PCL containing 20 wt% LA in the DCM/DMF (4:1) solvent system. Varying amounts of  $\beta$ -TCP nanoparticles (1 wt%, 2 wt%) were then incorporated in the PCL/LA solution. The solution was

electrospun using the setup in our previous study [18]. Electrospinning was carried out at 22 kV voltage, a feed rate of 0.3–0.5 ml/h, and a tip-to-collector distance of 10 cm. A 21 G nozzle (inner diameter = 0.51 mm) was used. During electrospinning, the nozzle kept on moving sideways (i.e., horizontally, back and forth) on its axis for a distance of 150 mm and a linear speed of 100 mm/min. All experiments were performed at room temperature. The collected electrospun fiber mats were kept in an oven at room temperature under vacuum for 24 h to remove any residual solvent.

### 2.3. Characterization

The surface morphological structure of the samples was characterized by field emission scanning electron microscopy (FESEM, Hitachi S-4800) and the elemental composition of the surface was checked using an energy dispersive spectrometer (EDS) connected to FESEM. Fourier transform infrared (FTIR) spectra of the samples were obtained using a Paragon 1000 Spectrometer (Perkin Elmer) with a signal resolution of  $1\text{ cm}^{-1}$ . A minimum of 16 scans was obtained and averaged within the range of  $400\text{--}4000\text{ cm}^{-1}$ . X-ray powder diffraction (XRD) analysis was carried out by a Rigaku X-ray diffractometer (Cu  $K\alpha$ ,  $\lambda = 1.54059\text{ \AA}$ ) over Bragg angles ranging from  $10^\circ$  to  $60^\circ$ . pH measurements were carried out using a portable pH meter (Hannah Instruments).

### 2.4. MTT assay

Samples of electrospun composite mats were cut in similar sizes and were sterilized for 1 h by UV light. All samples were washed three times with sterile PBS prior to transfer to individual 96-well tissue culture plates. MC3T3-E1 cells were seeded to the electrospun scaffold surface at a density of  $1 \times 10^4$  cells/well, and were grown in the  $\alpha$ -MEM medium. The culture medium was incubated at  $37^\circ\text{C}$  in a humidified 5%  $\text{CO}_2$  incubator for different days (1, 3, and 7 days), and then subsequently removed and washed twice with sterile PBS. A 100  $\mu\text{L}$  of MTT solution (0.5 mg/ml) was then added to each well. After 3 h incubation at  $37^\circ\text{C}$ , 100  $\mu\text{L}$  of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. The dissolved solution was swirled homogeneously for about 10 min by a shaker. The optical density of the formazan solution was detected by a 96-well plate reader at 575 nm.

### 2.5. *In vitro* calcium compound deposition on nanofibers

The fabricated samples of 20 wt% LA/PCL and the sample containing 1 wt%  $\beta$ -TCP particles were kept in a 24-well plate. An SBF solution (Hank's balanced salt solution containing 0.097 g  $\text{MgSO}_4$ , 0.350 g  $\text{NaHCO}_3$  and 0.185 g  $\text{CaCl}_2$ , Aldrich, H2387-1L) was then added over the mats and incubation was carried out for five days at  $37^\circ\text{C}$  in 5%  $\text{CO}_2$  atmosphere. The temperature was maintained constant throughout the test. The surface appearance and structure of the mats were observed after the immersion test.

### 3. Results and discussion

Here, LA/PCL and  $\beta$ -TCP-LA/PCL nanofibers were fabricated by electrospinning. The loadings of  $\beta$ -TCP in the micro/nanofibers were set at 1 wt% and 2 wt%. The as-spun PCL micro/nanofibers showed solid fibers with interconnected pores, while some were in braided form. Upon the incorporation of LA, the micro/nanofiber diameter showed increasing trend with the increase in LA content from 4 to 20 wt% (data not shown), showing the largest average diameter at 20 wt% LA content and the most fluffy (sponge-type) 3D structure among the samples, thus we decided to use this LA concentration for all tests. As shown in Fig. 1a–c, all nanofibers obtained were very fluffy in structure, and very porous. This kind of structure is similar even at higher contents of  $\beta$ -TCP. One can see that  $\beta$ -TCP particles were uniformly decorated on the micro/nanofibers with rice-like morphology at 1 wt% (Fig. 1b3), and round-like, agglomerated structure at 2 wt% (Fig. 1c3). EDS spectra (inset of Fig. 1b3) on the composite mat confirmed the presence of  $\beta$ -TCP on the micro/nanofibers.

In bone regeneration, scaffolds should have an interconnected pore structure for new bone tissue ingrowth, wherein, the present fluffy, and fibrous structure is suitable. Additionally, scaffolds should have good mechanical properties to withstand loading during bone formation [19]. Here, LA/PCL fibers serve as substrate to provide load transfer for  $\beta$ -TCP particles. The presence of nanoparticles in/on nanofibers is reported to improve the mechanical and thermal properties of the composite material [20].

To further check and investigate the presence of different components in the nanofiber, XRD and FTIR analyses were carried out (see Fig. 2). The XRD spectra (Fig. 2a) showed several new peaks when compared to those of LA/PCL nanofiber, which correspond to the peaks of  $\beta$ -TCP. Peaks at around  $25.7^\circ$  and  $27.7^\circ$  correspond to the  $\beta$ -TCP crystal planes of (1010) and (214), respectively [21]. These new peaks confirm the presence of  $\beta$ -TCP in the composite matrix. The FTIR results showed similar spectra for all samples. However, it could be observed that at  $3000\text{--}3600\text{ cm}^{-1}$ , which is assigned to the stretching vibration of hydroxyl groups ( $\text{OH}^-$ ), there was a broadening of the spectrum

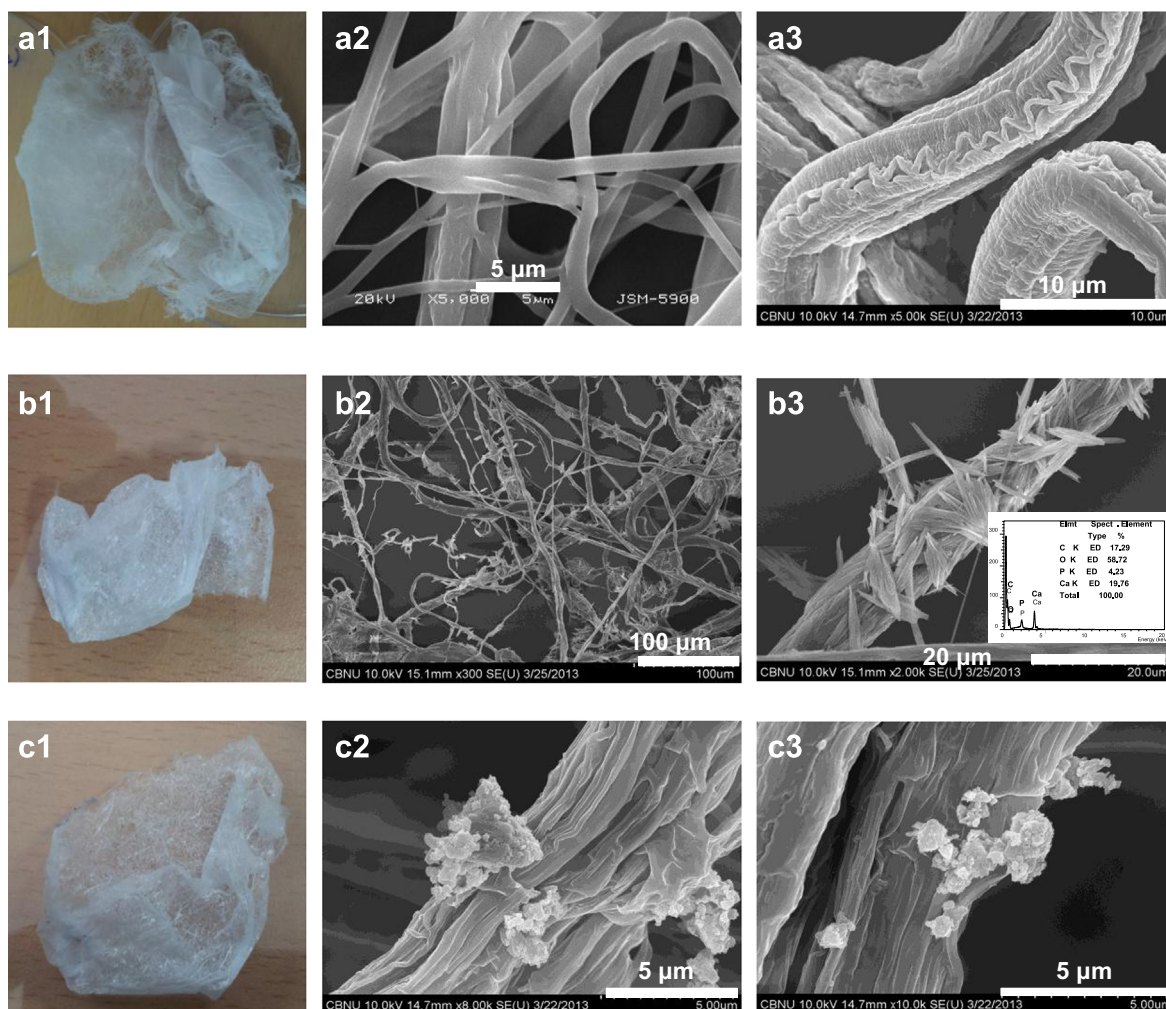


Fig. 1. Photographic (a1–c1) and SEM images of the fabricated electrospun nanofibers made of (a1–a3) LA/PCL, (b1–b3) 1 wt%  $\beta$ -TCP-LA/PCL and (c1–c3) 2 wt%  $\beta$ -TCP-LA/PCL. Inset of b3 shows the EDS spectra.



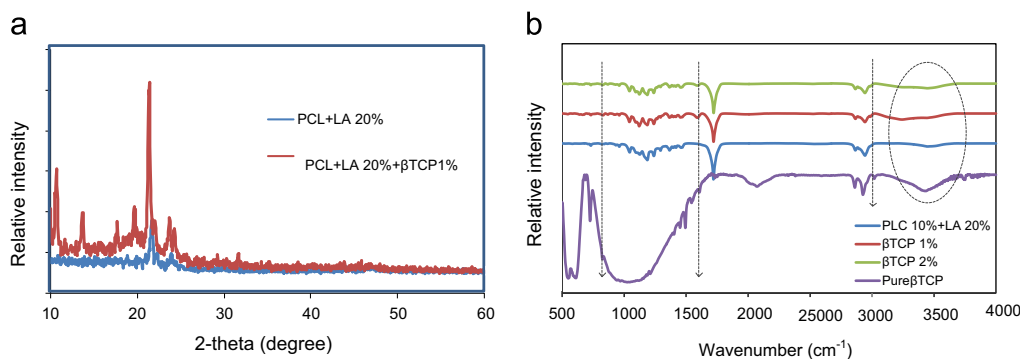


Fig. 2. FTIR and XRD spectra of the prepared samples.

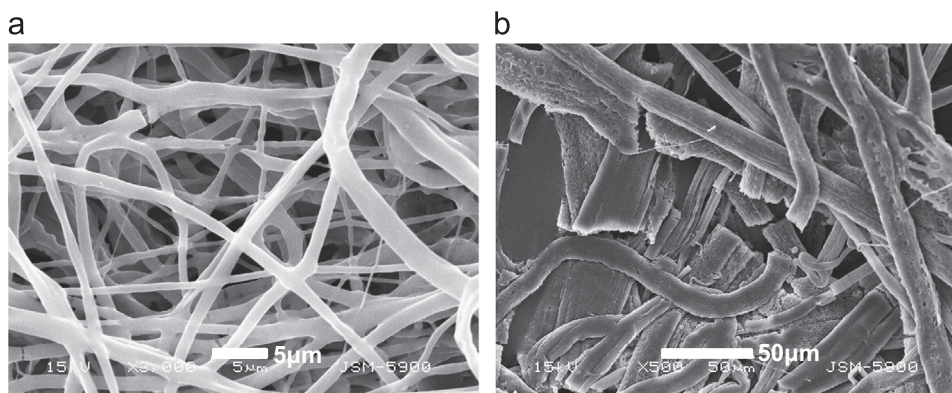


Fig. 3. SEM images of fibers after immersion in distilled water: (a) LA/PCL, and (b) 1 wt%  $\beta$ -TCP-LA/PCL.

for the nanofibers containing  $\beta$ -TCP [22], which could be due to interaction of particles and polymer. New peaks at  $500\text{--}605\text{ cm}^{-1}$ , and  $940\text{--}1120\text{ cm}^{-1}$  are assigned to the orthophosphate ( $\text{PO}_4^{3-}$ ) group.

The incorporation of LA in PCL has improved the degradation property of the LA/PCL nanofibers, wherein degradation depends on the content of LA. In our previous study [23], the LA/PCL fibers started to be broken within 1–3 weeks of immersion in phosphate buffer solution, while neat PCL fibers still did not show any broken fiber even up to nine weeks (results not shown). It revealed that LA can easily accelerate the PCL degradation rate probably by catalyzing of hydrolysis of ester bonds, resulting to the formation of carboxylic acid, thus decreasing the pH of the surroundings. The presence of LA was also found to increase the hydrophilicity of the membrane, which leads to more water absorption promoting degradation of PCL [23]. Our previous study [24] showed that the presence of LA in nylon 6 nanofibers has decreased the pH of water (from 6.1 to 3.2), which makes the surroundings less compatible, thus less cell proliferation was observed. But after treating the LA/nylon-6 nanofibers with  $\text{Ca}(\text{OH})_2$ , the pH remained constant (no decrease) after immersion in distilled water, due to the neutralization effect and the release of calcium ions on the surface. In our present study, to check whether the pH of surrounding fluid is decreased or not, we immersed the 1 wt%  $\beta$ -TCP sample in distilled water for one week, and found that the pH of water did not decrease, but was maintained or slightly increase. This is mainly due to the

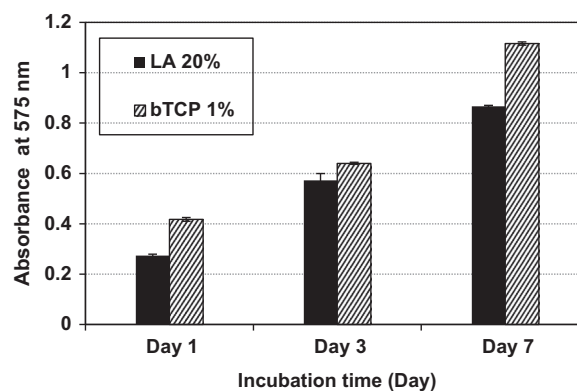


Fig. 4. Cell proliferation and viability through MTT assay for different days of (a) 20 wt% LA/PCL and (b) 1 wt%  $\beta$ -TCP-containing LA/PCL nanofibers.

presence  $\beta$ -TCP on/in the LA/PCL nanofibers, which could have a neutralization effect on LA, thus the pH of the surrounding fluid did not decrease. Checking the SEM images (Fig. 3) of the immersed samples, we observed that  $\beta$ -TCP composite fibers (Fig. 3b) were more degradable with many damaged and broken fibers compared to LA/PCL fibers (Fig. 3a). The incorporation of  $\beta$ -TCP particles has increased the hydrophilicity of the composite fibers, thus there was more water uptake through the pores and the decorated fibers, leading to faster degradation. Thus, the incorporation of  $\beta$ -TCP and the presence of LA in PCL micro/nanofibers could result to improved biocompatibility and faster degradation of

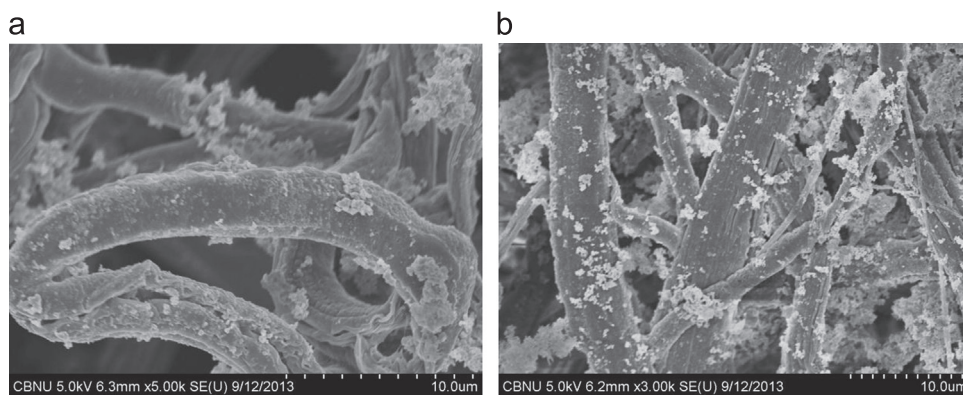


Fig. 5. SEM images of (a) 20 wt% LA/PCL and (b) 1 wt%  $\beta$ -TCP-containing LA/PCL nanofibers after immersion in SBF solution for 3 days.

the composite nanofibers, which would be very useful for tissue scaffold application. Zhang et al. [25] concluded their study that the addition of  $\beta$ -TCP in silicone rubber can change the surface microstructure, thereby improving cytocompatibility.

The composition and surface morphology of fibrous matrix play an important role in cell behavior. The biological role of  $\beta$ -TCP particles on/in LA/PCL nanofibers was assessed in terms of cell adhesion and proliferation. MC3T3-E1 osteoblast cells were cultured for different days (1, 3, and 7 days) on the nanofibrous mats and the cell proliferation was investigated via an MTT assay. (Fig. 4) shows the MTT assay results of the 20% LA/PCL nanofibers and the sample containing 1 wt%  $\beta$ -TCP (we chose only this sample since it has more uniform distribution of  $\beta$ -TCP particles on the surface compared to higher  $\beta$ -TCP content). The 20 wt% LA/PCL mat showed good cell proliferation but has consistently lesser cell viability compared to the sample with 1 wt%  $\beta$ -TCP particles. Cell proliferation constantly got higher at longer culture days suggesting a time-dependent behavior. The presence of  $\beta$ -TCP on the LA/PCL nanofiber has significantly increased the cell proliferation especially after seven days. Fig. 5 shows the SEM images of 20 wt% LA/PCL and LA/PCL containing 1 wt%  $\beta$ -TCP after immersion in SBF solution for three days. This immersion test was used to assess the mineralization ability of the samples. It can be seen that although LA/PCL nanofiber showed good amount of deposited calcium compound; however more calcium compound deposition were still observed on the composite containing 1 wt%  $\beta$ -TCP. The incorporation of LA in PCL has increased its mineralization ability due to the good hydrophilicity and more carboxylic group induced by LA. However, in the case of 1 wt%  $\beta$ -TCP mat, aside from the effect of LA, the loading of  $\beta$ -TCP has improved its mineralization ability. The increase in both cell proliferation and calcium compound deposition on the LA/PCL nanofiber containing 1 wt%  $\beta$ -TCP is attributed to (a) increased protein absorption ability of the composite mat due to the presence of  $\beta$ -TCP, (b) partial dissolution of  $\beta$ -TCP, which releases  $\text{Ca}^{2+}$  and is implied to have significant role in the extracellular signaling by membrane mediated ionic transfer [26], and (c) rougher surface due to protrusions resulting from  $\beta$ -TCP aggregations on fiber surface which might provide

extra sites for cell binding [27]. Erisken et al. [6] reported that the incorporation of  $\beta$ -TCP in PCL nanofibers in a controlled manner resulted to the better mimicking of the compositional and structural characteristics of the bone tissue. The results in the present study suggest the good potential of one-step fabrication of  $\beta$ -TCP-incorporated LA/PCL nanofibers for tissue engineering application with improved bone formation ability and cell viability.

#### 4. Conclusions

In this study, different contents of  $\beta$ -TCP particles were successfully incorporated on/in electrospun LA/PCL micro/nanofibers by facile one-step electrospinning. XRD and FTIR confirmed the decoration of  $\beta$ -TCP on the nanofibers. Slight shifting of peaks was observed in the spectroscopic analysis, which suggest possible interaction between  $\beta$ -TCP and LA/PCL nanofibers. The incorporation of  $\beta$ -TCP has resulted to rougher fiber surfaces, which could add more reactive sites and better biocompatibility. The loading of  $\beta$ -TCP in LA/PCL nanofibers has improved the degradation rate, cell viability and bone formation ability of the composite mat. These improvements are attributed to the increased hydrophilicity, release of calcium ions on the surface, and rougher surface of the  $\beta$ -TCP-containing mat. Here, the incorporation of  $\beta$ -TCP on LA/PCL fibers could change the microstructure and could lead to better degradation and biocompatibility of the composite material.

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