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Siqueira, Lilian de; Passador, Fábio Roberto; Lobo,
Anderson Oliveira; Trichês, Eliandra de Sousa

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ORIGINAL ARTICLE

Morphological, thermal and bioactivity evaluation of electrospun PCL/β-TCP fibers for tissue regeneration

Lilian de Siqueira[1]*, Fábio Roberto Passador[2], Anderson Oliveira Lobo[3] and Eliandra de Sousa Trichês[1]

[1]Laboratório de Biocerâmica – BIOCERAM, Instituto de Ciência e Tecnologia – ICT, Universidade Federal de São Paulo – UNIFESP, São José dos Campos, SP, Brasil

[2]Laboratório de Tecnologia de Polímeros e Biopolímeros – TecPBio, Instituto de Ciência e Tecnologia – ICT, Universidade Federal de São Paulo – UNIFESP, São José dos Campos, SP, Brasil

[3]Laboratório Interdisciplinar de Materiais Avançados – LIMAV, Departamento de Engenharia de Materiais, Universidade Federal do Piauí – UFPI, Teresina, PI, Brasil

**lilian.siqueira@unifesp.br*

Abstract

Electrospinning is a simple and low-cost way to fabricate fibers. Among the various polymers used in electrospinning, polycaprolactone (PCL) stands out due to its excellent biodegradability and biocompatibility. However, PCL has some limitations such as low bioactivity, hydrophobic surface, and long *in vivo* degradation. Calcium phosphate ceramics have been recognized as an attractive biomaterial. They are bioactive and osteoinductive, and some are even quite biodegradable. Different contents of particles of beta-tricalcium phosphate (β-TCP) were incorporated in polymer matrix to form fibers of PCL/β-TCP composites by electrospinning for possible application in tissue regeneration. The presence of β-TCP particles promoted some changes in the thermal properties of the fibers. The immersion of PCL/β-TCP 8 wt-% fibers in simulated body fluid (SBF) caused the formation of a denser and homogeneous apatite layer on its surface.

Keywords: *electrospinning, fibers, polycaprolactone, scaffolds, tricalcium phosphate.*

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1. Introduction

The use of electrospun fibers in biomedical applications as scaffolds has increased in the last years because these fibers offer a range of attractive features such as high surface area, high porosity, and ease of incorporation of functional components (bioactive nanoparticles, drug, gene, enzyme, etc.)[1]. Electrospinning is regarded as a simple and versatile top-down approach for fabricating uniform ultra-fine fibers in a continuous process and at long length scales[2] by applying an electrostatic field to a polymer solution driven by high voltage supply between a needle tip and the collector[3].

Among the biodegradable polymers used in the electrospinning, the synthetic aliphatic polyesters, such as poly (lactic acid) (PLA), poly (glycolic acid) (PGA), and polycaprolactone (PCL), stand out[4,5]. However, these polymers have some limitations such as low bioactivity, hydrophobic surface, and long *in vivo* degradation[6]. The incorporation of inorganic particles into the polymer matrix or coatings of polymer matrix are alternatives to improve these limitations[7].

Calcium phosphate bioceramics are recognized as an attractive biomaterial because their chemical composition is similar to the mineral component of bone. Moreover, they are bioactive and biodegradable, with show osteoinductive properties. Among the ceramics of calcium phosphate, beta-tricalcium phosphate (β-TCP) $[Ca_3(PO_4)]_2$ stands out for its osteoconductive activity[8]. Fibers of PCL and its composites have been obtained successfully as shown in the literature by the works of Lu et al.[9], Ribeiro et al.[10], and Hassan et al.[3,11]. Recently, Park et al.[12] prepared and characterized composite fibers of lactic acid and PCL (LA/PCL) containing β-TCP nanoparticles (1-2 wt-%) by electrospinning. The incorporation of β-TCP in LA/PCL fibers could change the microstructure and could lead to a better degradation and biocompatibility of the composite mat. In another study, Kim and Kim[13] generated highly porous electrospun 3D polycaprolactone/β-TCP biocomposites (with 5, 10 and 15 wt-% of β-TCP particles) for tissue regeneration using modified wet-electrospinning supplemented with a femtosecond laser. The fabricated scaffolds demonstrated improved mechanical properties and relatively high cellular activities compared to other methods like rapid-prototyped.

In the literature there are reports of tissue engineering applications of electrospun fibers of PCL and PCL/β-TCP. However, most of these works have focused on the biological tests. Therefore, we fabricated and characterized electrospun fibers of PCL and PCL/β-TCP composites with different

contents of β-TCP that exhibited interesting structural properties. In this work, we investigated how the addition of β-TCP particles influenced the thermal behavior of the polymer matrix; for example, the crystallinity change as related to the degradation time. The relationships between structure (e.g., morphology and fiber diameter) and property (e.g., thermal and bioactive properties) were established and elucidated in detail.

2. Materials and Methods

2.1 Materials

The polymer used in this work was PCL supplied by Sigma-Aldrich Company Ltd. (USA) with 80 kDa of molecular mass. The solvents used were chloroform [$CHCl_3$, 99%] and methanol [CH_3OH, 99%], both supplied by Synth Acessórios e Equipamentos para Laboratório (Brazil). The β-TCP was produced in the Laboratory of Bioceramics (BIOCERAM) of the Federal University of Sao Paulo using the following reagents: calcium carbonate [$CaCO_3$, 99%] and bibasic anhydrous phosphate [$CaHPO_4$, 99%], both supplied by Synth Acessórios e Equipamentos para Laboratório (Brazil).

2.2 Synthesis and characterization of β-TCP particles

The β-TCP powder was synthesized by a solid-state reaction, as described elsewhere[14]. Briefly, a 2:1 molar ratio mixture of $CaHPO_4$ and $CaCO_3$ was calcined at 1050 ºC (Oven, Inox Line/3000 3P, EDG Equipamentos e Controles Ltda., Brazil) for 6 h followed by milling in a horizontal ball mill (Ball mill, MA500, Marconi Equipamentos para Laboratório Ltda., Brazil) during 48 h (alumina milling media of 6 mm of diameter and ball/powder weight ratio of 10:1) and then ground in a high energy ball mill (Planetary mill, Pulverisette 5, Fritsch Company Ltd., Germany) for 4 h (rotation 250 rpm, alumina balls with 2 mm of diameter). The resulting powder was analyzed by laser light diffraction (Laser Particle Size Analyzer, CILAS 1190L, Cilas Company Ltd., France) and presented a mean particle size of 1.13μm (D_{50} value) and a particle size distribution between 0.04μm (D_{10} value) and 4.0μm (D_{90} value), where D = diameter.

2.3 Preparation of PCL/β-TCP composites by electrospinning

The PCL was first dissolved in chloroform under ultrasound dispersion for 30 min; after its complete dissolution, methanol was added, forming a 75/25 v/v the solvent solution[10]. The PCL final concentration in this solution was 1.2 g/ml. To produce the fibers of PCL/β-TCP composites, the particles of β-TCP (D_{50} = 1.13 μm) were first mixed with chloroform under ultrasound dispersion for 30 min. The PCL solution was then added to the β-TCP suspension to form a 75/25 ratio solvent and after complete dissolution, methanol solvent was added. The same ratio of solvents was also used to prepare solutions containing just the β-TCP particles (75/25 v/v). The final suspensions were maintained under agitation for at least 12 h until complete homogenization. The final concentrations of β-TCP in the suspensions were 0.01, 0.05, and 0.08 g/ml. The samples were named PCL/β-TCP 1 wt-%, PCL/β-TCP 5 wt-% and PCL/β-TCP 8 wt-%, according to the β-TCP content. To assemble the electrospinning apparatus we used: a high voltage source (High Voltage Power Supply, Series 230R, Bertan Ind. e Com. de Máquinas, Brazil), a static collector, attached to an insulating rod claw holding a syringe containing the polymer solution, the needle (Inbras® 0.8 mm diameter and 25 mm length), and a glass syringe with a volume of 20 ml with the plunger. The working distance was maintained at 10 cm from the collector, and the applied voltage was 12 kV, with the fibers collected as randomly oriented.

2.4 Fibers characterization

The morphology of the fibers of neat PCL and the PCL/β-TCP composites with different contents of β-TCP was evaluated by scanning electron microscopy (SEM, EVO MA10, ZEISS Company Ltd., Germany). The mean diameter of the fibers was measured using the ImageJ 6.0 software. The data were obtained from 30 fibers and expressed as mean ± standard deviation.

The chemical composition of the samples was investigated using attenuated total reflection (ATR) with an FT-IR Spectrometer (FTIR, Nicolet iS5, Thermo Fisher Scientific Inc., USA) with a scanning range of 400 to 4000 cm^{-1} and resolution of 4 cm^{-1}.

The thermal behavior of the samples was analyzed using differential scanning calorimetry (DSC, 204 F1 – Phoenix, NETZSCH Group, Germany). A first heating scan was done from 25 to 120 °C, at 10 °C/min to obtain the glass transition and crystalline melting temperatures of the samples.

Degree of crystallinity (Xc) for the first heating was calculated according to Equation 1.

$$Xc(\%) = \frac{\Delta H_m}{\Delta H_{m\infty} \cdot \phi_{PCL}} x100 \quad (1)$$

Where: ΔH_M is the melting enthalpy of the sample, $\Delta H_{M\infty}$ is the melting enthalpy of a 100% crystalline sample and ϕ_{PCL} is the mass fraction of PCL in the fiber. For the PCL samples, $\Delta H_{M\infty}$ is 136 J/g[15].

In vitro bioactivity of the samples was evaluated by immersing them in 15 ml of the Simulated Body Fluid solution[16] (1.5 SBF, pH 7.4) at 37 ºC. After given times of 7, 14 and 21 days of immersion, the samples were extracted from the SBF and analyzed by SEM (EVO MA10, ZEISS Company Ltd., Germany) and X-Ray diffraction (XRD, X'pert Powder, PANalytical Co., Ltd., Netherlands) operating at 45 kV/40 mA (CuK_α, λ = 0.154 nm); the samples were scanned with steps of 0.02º between 2θ = 20 and 50º.

3. Results and Discussion

Figure 1 shows SEM micrographs of the fibers. The fibers of PCL were smooth, homogeneous, and without defects (Figure 1a and b). When 1 wt-% of β-TCP particles was added, some clusters appeared within the polymeric fibers indicating that these particles were incorporated into the polymer matrix (Figures 1c and d). The fiber diameters of

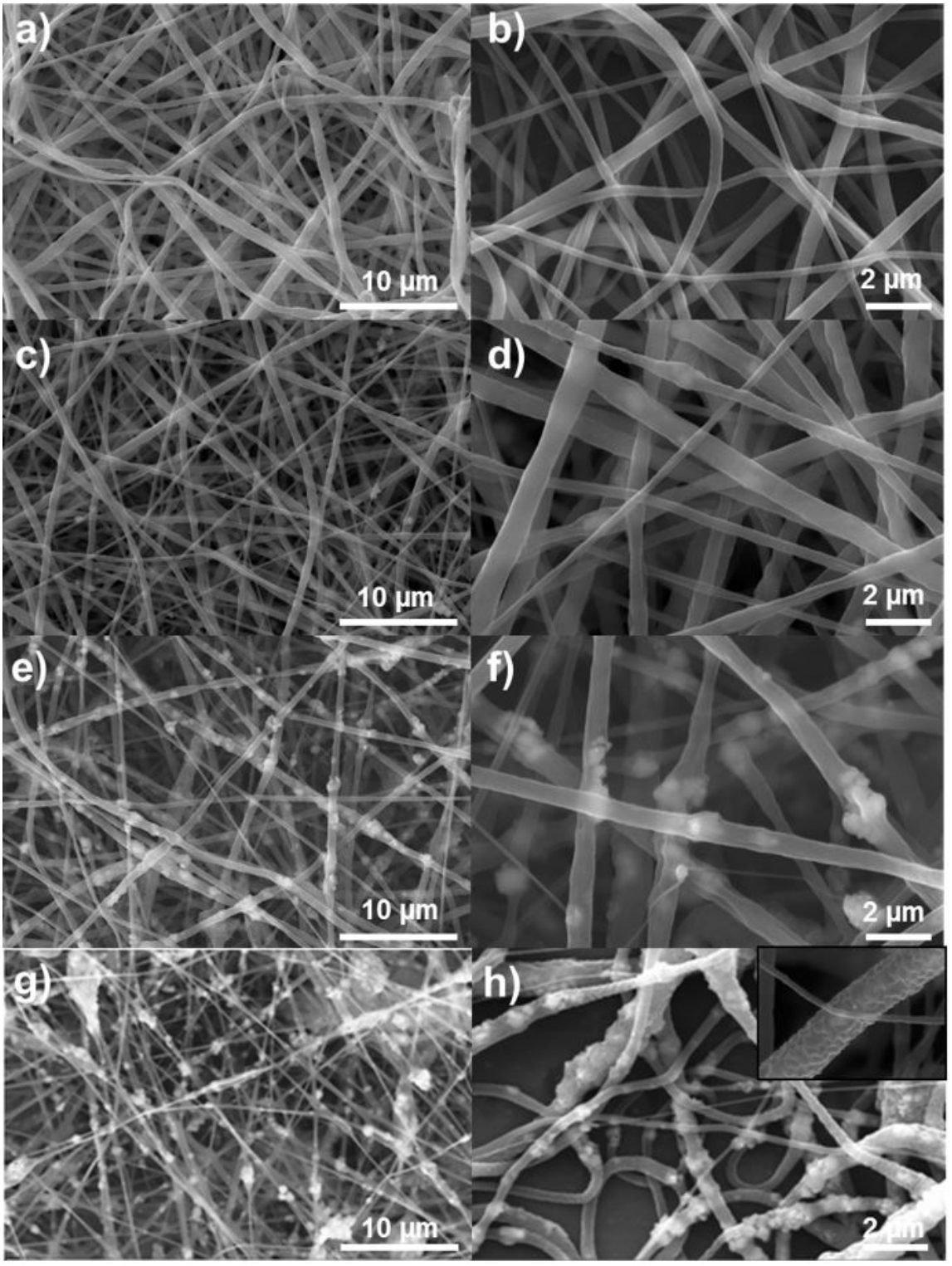

Figure 1. SEM micrographs of the fibers: (a, b) PCL; (c, d) PCL/β-TCP 1 wt-%; (e, f) PCL/β-TCP 5 wt-%; and (g, h) PCL/β-TCP 8 wt-%.

PCL/β-TCP decreased with addition of 1 wt-% of β-TCP (640 ± 20 nm) when compared to PCL fibers (774 ± 49 nm). This physical phenomenon may be associated with the difference in the density between the filler and the polymer matrix. In addition, with increasing content of β-TCP, the presence of a larger number of agglomerates within the fibers (Figures 1e and f) was observed. However, with the addition of 8 wt-% of β-TCP, the presence of agglomerates

of larger size within the fibers (Figure 1g) and changes in the roughness of the fibers (Figure 1h) can be seen. PCL fibers with the addition of 5 and 8 wt-% of β-TCP showed an increase in the average diameter with values of 867 ± 40 nm and 726 ± 110 nm, respectively.

FTIR analyses of the functional organic groups in the polymers and in the β-TCP are shown in Figure 2. In the case of the PCL, the main absorbance band at 1723 cm^{-1} corresponds to carbonyl stretching[17] while the bands at 1294, 1240.2, and 1162.2 cm^{-1} correspond to the stretching vibrations of the C-O-C groups. In all spectra, except for the PCL and PCL/β-TCP 1 wt-% fibers, the bands at 557 and 615 cm^{-1} correspond to the absorption bands related to PO_4^{3-}, which is an indication of incorporation of the β-TCP in the polymeric fibers, showing that a physical interaction occurred between them. When β-TCP particles were present at 8 wt-%, an increase in the intensity of the β-TCP absorption bands was observed.

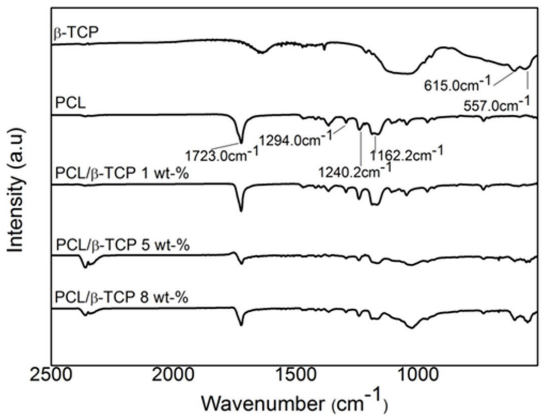

Figure 2. FTIR spectra of fibers of PCL and PCL/β-TCP composites with different contents of β-TCP.

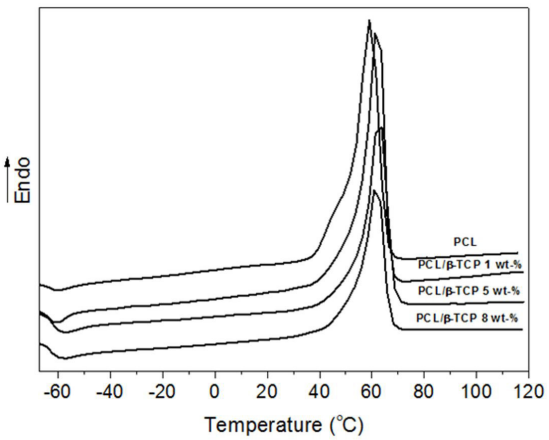

Figure 3. DSC thermograms of the fibers.

Figure 3 shows the DSC thermograms of the fibers and Table 1 shows the thermal parameters calculated from these thermograms. The crystalline melting peak occurred in the first heating at 61.2 °C, 62.2 °C, 62.7 °C, and 61.8 °C for the samples of PCL, PCL/β-TCP 1 wt-%, PCL/β-TCP 5 wt-%, and PCL/β-TCP 8 wt-%, respectively The glass transition temperature values of the different samples remained virtually unchanged.

The degree of crystallinity was modified by the addition of β-TCP particles. Increasing the content of β-TCP decreased the degree of crystallinity. This result can be considered representative and can be associated with particle behavior, which acted as obstacles for the diffusion of the macromolecules toward the growing crystal surface (Table 1). It is a positive result because it favors the degradation process, remembering that the PCL scaffold or implant takes many months or even years to degrade *in vitro* or *in vivo*[6,18,19].

Bioactivity, one of the most desirable properties of the materials for bone tissue regeneration, can be inferred by the formation of bone-like apatite on the surface of the materials in contact with the SBF solution *in vitro*. Figure 4a,b,c,d shows micrographs of the fibers after the bioactivity assay. The presence of the typical HA globular morphology on the surface of all of the electrospun fibers can clearly be seen. However, when 8 wt-% of β-TCP was incorpored to the polymeric matrix, a more homogeneous layer of HA can be observed on the surface of the fibers.

The XRD pattern of the fibers before and after the biomineralization process can be observed at right in Figure 4. For all fibers, two distinct diffraction peaks were observable, at $2\theta = 21.5°$ and $2\theta = 23.9°$, indexed to the (110) and (200) planes respectively of the orthorhombic crystal structure of PCL[20]. The peaks at 31.2° and 34.5° correspond to the diffraction peaks of the β-TCP (JCPDS 009-0169) while the peaks at 26, 32, and 45.6° were related to HA (JCPDS 74-0565).

The obtained results demonstrated the fibers were very promising for a possible application in tissue engineering. Among all the compositions studied it is suggest that the fibers of PCL/β-TCP with 8 wt-% of β-TCP are the most suitable due to improvement in its morphological and bioactive properties.

Table 1. Thermal parameters of the fibers of PCL and PCL/β-TCP composites with different contents of β-TCP.

Material	*Tg* (°C)	*Tm* (°C)	*ΔHm* (J/g)	*Xc* (%)
PCL	-63.2	61.2	71.3	52.4
PCL/β-TCP 1 wt-%	-63.4	62.2	67.7	50.3
PCL/β-TCP 5 wt-%	-61.4	62.7	51.0	39.5
PCL/β-TCP 8 wt-%	-61.4	61.8	40.1	32.0

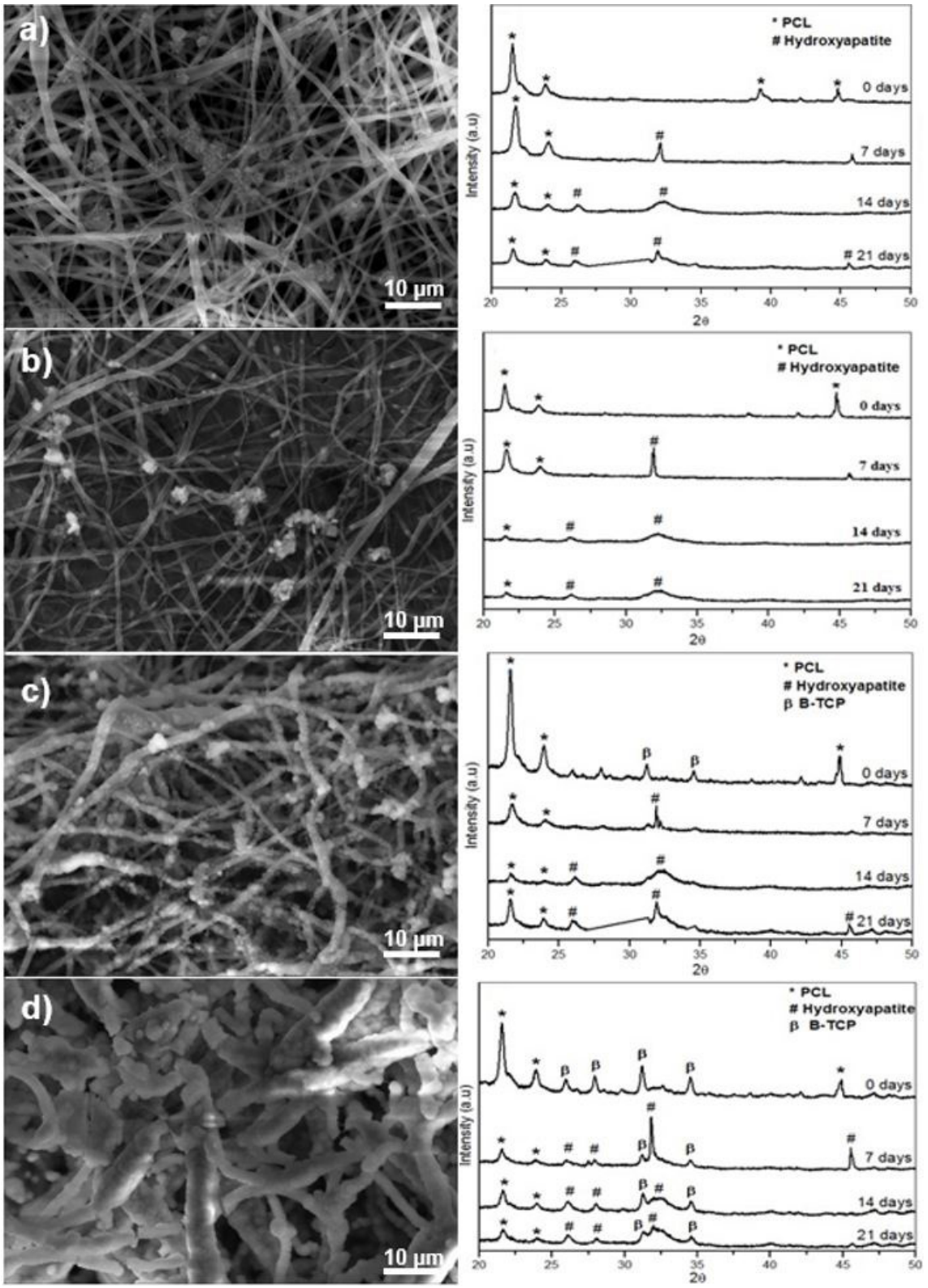

Figure 4. Micrographs of the fibers: (a) PCL; (b) PCL/β-TCP 1 wt-%; (c) PCL/β-TCP 5 wt-%; and (d) PCL/β-TCP 8 wt-% after biomineralization for 21 days. Right: XRD patterns.

4. Conclusions

Fibers of PCL and PCL/β-TCP with 1, 5 and 8 wt-% of β-TCP were successfully produced by electrospinning. The best fibers obtained for future applications in tissue engineering were the PCL/β-TCP 8 wt-%, due to its lower degree of crystallinity and better bioactive properties.

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