Production of Polycaprolactone/Hidroxyapatite Scaffold for Application in Tissue Engineering

Joanne G. A. Mendes[1*], João Pedro de A. Caribé[1], Adillys M. da C. Santos[1], Willams T. Barbosa[2], Josiane D. V. Barbosa[2], Luis Alberto Loureiro dos Santos[3], Liciane Sabadin Bertol[3], Imarally V. de S.R. Nascimento[1]

[1]Federal University of Recôncavo da Bahia (UFRB), Center for Science and Technology in Energy and Sustainability (CETENS)-(UFRB), Feira de Santana, Bahia; [2]SENAI CIMATEC University Center; Salvador, Bahia; 3Federal University of Rio Grande do Sul (UFRGS); Porto Alegre, Rio Grande do Sul, Brazil

The study aims to produce fibrous polycaprolactone (PCL) scaffolds with hydroxyapatite (HA) incorporation using the Solution Blown Spinning (SBS) technique for application in tissue engineering. The scaffolds were characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), contact angle, and thermogravimetric analysis (TG). The images obtained by SEM showed that the fibers have a diameter on a micrometer scale. FTIR spectra indicated physical and chemical interactions between PCL and HA. TG analysis showed the presence of HA, which provided an anticipation of the PCL degradation temperature. We obtained micrometric fibers of PCL and PCL/HA with different orientations. The technique used is promising for making scaffolds.
Keywords: Tissue Engineering. Composites. Scaffolds.

Introduction

Tissue engineering seeks to create functional tissues from biocompatible materials and living cells to improve the quality of life of people who suffer bone fractures or trauma. The development of three-dimensional porous scaffolds has gained attention since they can promote support for cells to adhere, grow, and proliferate to form the neo-tissue [1].

The development of hybrid systems of biodegradable polymers, such as polycaprolactone (PCL), and ceramics, such as hydroxyapatite (HA), is an approach commonly used in bone tissue engineering to assist bone recovery [2]. PCL has advantageous mechanical properties, while HA is biocompatible and promotes osseointegration. Combining PCL and HA makes it possible to obtain a material with mechanical resistance and bioactive properties, favoring the formation of bone tissue [3,4].

Despite the number of techniques available to manufacture 3D scaffolds, solution-blown spinning (SBS) has attracted the attention of researchers because of its simplicity and advantages, as high production rate and low cost. The SBS uses a high-speed air stream to promote fiber production from a precursor solution. Therefore, the study's objective was to develop and optimize the preparation of PCL and PCL/HA scaffolds by SBS, seeking to obtain homogeneous fibers efficiently and economically.

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Address for correspondence: Joanne G. A. Mendes. Av. Centenário, 697 - Sim, Feira de Santana - BA. Zipcode: 44042-280 E-mailmendesanne736@gmail.com.

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Materials and Methods

Perstorpo supplied PCL (Mw = 80,000 g/mol) as pellets. Nanosized HA was synthesized and kindly donated by the biomaterials laboratory (LABIOMAT) of the Brazilian Center for Research in Physics (CBPF) - UFRGS. The solvent used was glacial acetic acid (GAA), with a molar mass of 60.052 g/mol supplied by Exodus Scientific.

Preparation of Scaffolds

PCL and PCL/HA scaffolds were produced by preparing PCL solutions in 6 mL GAA at different polymer/solvent ratios (see Table 01). The solutions were vigorously stirred at 40 °C for 1 h. Further, the solutions were stirred for 2 h at room temperature. For the PCL/HA scaffold, the nanosized HA powder was added into the PCL acid solution (after the first hour) and then sonicated for 10 min. The solution was further mixed for 2 h. Finally, the precursor solutions were spun into fibers by SBS to produce

the hybrid scaffolds, employing the following conditions: 6 mL/h feed rate, 20 - 30 Psi spinning pressure (Table 1), and a working distance of 25 cm. It was used a static collector.

Characterization

X-ray diffraction analysis was carried out using the Empyrean PANalytical instrument. Angular range from 2-75 (2θ) with 0.01 step/time. 40 kV and 40 mA. The diffraction intensities of maxima of hydroxyapatite were used to measure the crystallinity ratio of the samples and were defined according to Annex D of ABNT NBR ISO 13779-3:2020. FTIR analysis was carried out in a Perkin Elmer Spectrum device, version 10.4.2, in the 400 to 4000 cm^{-1} range, using KBr disks. A HITACHI Scanning Electron Microscope, model TM3000, assessed the nanostructured hydroxyapatite's morphology and the scaffolds produced. Measurements of the diameter of the fibers were performed with the help of the ImageJ software, taking 25 measurements per sample. Contact angle measurement was performed by the sessile drop procedure using 5 x 5 cm samples for 3 minutes. A 3 µL sessile drop of water was deposited on each specimen's surface with a micropipette. The drop was captured with the Kruss Goniometer equipment, model DSA-25. Five measurements were taken and averaged to obtain the final contact angle value. The thermal stability of the scaffolds was evaluated by thermogravimetric analyses (TA Instruments DSC model Q3 was used instruments, New Castle, USA) using a thermal protocol in the range 25 - 800 °C, a heating rate of 10 °C /min, and in a nitrogen atmosphere.

Results and Discussion

Characterization of Sample Hydroxyapatite

Figure 1 shows the XRD of the nanosized HA calcinated at 1000° for 15 h. The diffraction pattern corresponds to hydroxyapatite of chemical formula $Ca_5OH(PO_4)^3$, as the ICDD 01-084-1998 card corroborates. Also, the nanosized HA is of high purity and with a degree of crystallinity of 82.5%. No peaks related to calcium oxide or α- or β-tricalcium phosphate were detected. Therefore, the hydroxyapatite can be considered stoichiometric, with the Ca:P ratio at 1.667.

Figure 1. X-ray diffraction analysis of HA.

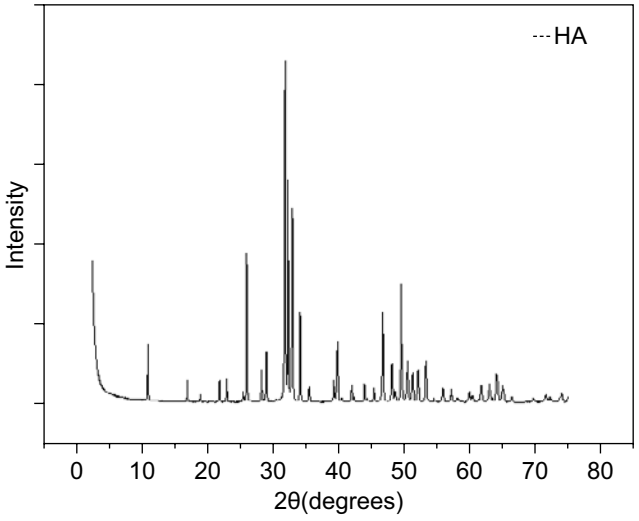


Table 1. Studied variations used to produce the scaffolds.

Sample	Concentration (%)	Pressure (PSI)	Polymer mass (g)	HA (g)
PCLA	18	20	1.0865	-
PCLB	22	20	1.327	-
PCLC	18	30	1.0891	-
PCLD	22	30	1.3244	-
PCLHA	22	20	1.3134	0.0395

Figure 2 shows the FT-IR analysis for the nanosized HA. The bands at 3571 cm^{-1} and 632 cm^{-1} were attributed to the stretching and bending of the hydroxyl group (OH^-) constituting the crystalline structure. The vibrations related to the phosphate group (PO_4^{3-}) were assigned to the bands at 1089 cm^{-1} and 1046 cm^{-1} (asymmetric stretching), 962 cm^{-1} (symmetric stretching), 602 cm^{-1} and 572 cm^{-1} (asymmetric stretching P-O) and 474 cm^{-1} (O-P-O flexion). The bands mentioned above are characteristic and confirm the chemical composition of HA. The absorption bands at 3454 cm^{-1} and 1636 cm^{-1} are characteristic of hydroxyl vibrations relative to adsorbed water [5-7].

In Figure 3, we observe the HA morphology evaluated by SEM. The image shows that the HA

Figure 2. Fourier transform infrared spectrum of the nanosized HA.

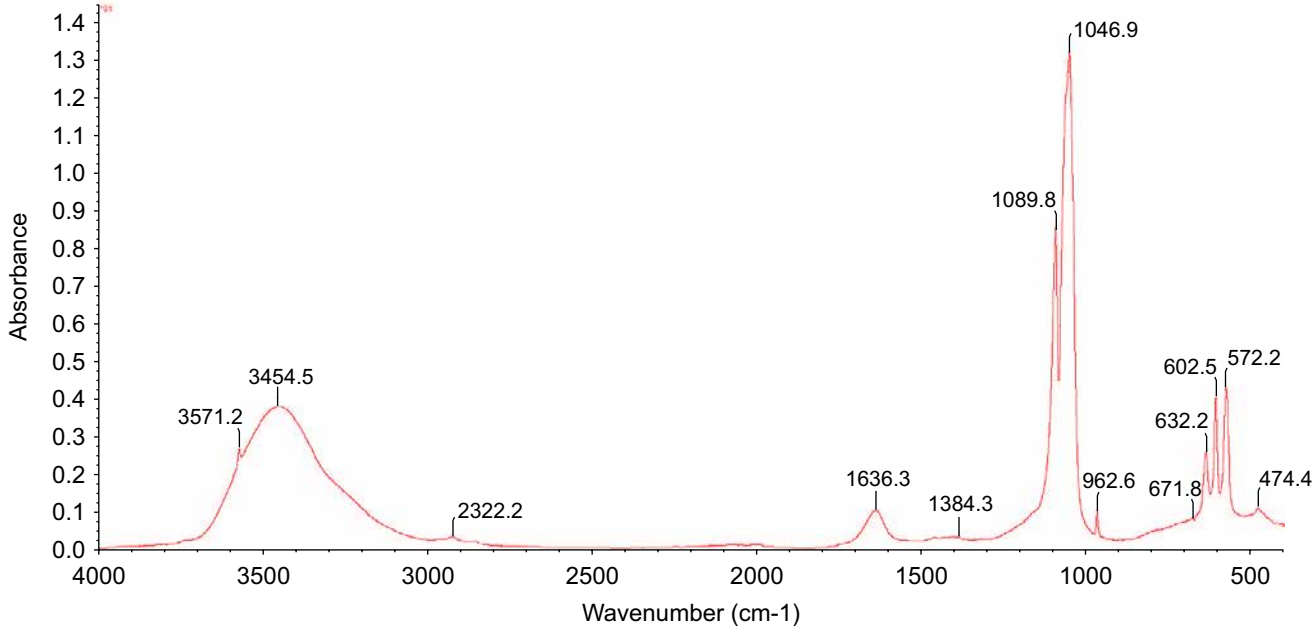
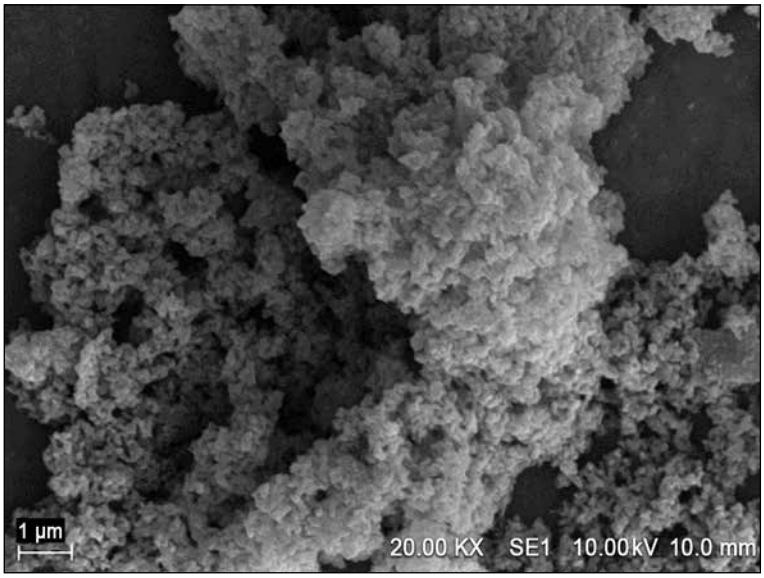


Figure 3. SEM from hydroxyapatite.



is formed by agglomerates of smaller particles, on the order of nanometers, with an elongated shape.

Figure 4 shows the infrared spectra of the obtained PCL and PCL/HA samples and their characteristic bands. The samples show a similar profile with slight differences, suggesting the combination of pure polymer and PCL/HA, causing some overlapping bands. These bands show slight variations in intensity and small shifts. The characteristic bands of PCL are observed at 2943 cm^{-1} (asymmetrical axial strain of the CH_2 group) and 2864 cm^{-1} (symmetrical axial strain of the CH_2 group). Additionally, the bands at 1720 cm^{-1} and 1161 cm^{-1} correspond to the elongation of the absorbance group, representing the symmetric vibration of O-C-O [8]. The band located at 601 cm^{-1}, along with the bands at 631 cm^{-1} and 574 cm^{-1}, correspond to the vibrational modes of the PO_4^{3-} group attributed to HA, indicating physical and chemical interactions between the PCL carboxyl groups and the HA [8,9].

Figure 5 presents micrographs of the scaffolds obtained. The macro appearance after the spinning process is similar to a cotton-wool-like structure. However, micrographs with 2000x magnification reveal the presence of many "beads" (beads) in all samples, which may be attributed to the incomplete solvent evaporation. According to SEM histograms, fibers (a) and (c) have mean diameters of approximately 0.6 µm and 0.3 µm, respectively, due to the application of different pressures. The same pattern was observed in samples (b) and (d), with mean diameters of 0.78 µm and 0.45 µm, respectively. The diameter variation can also be observed when comparing samples (a), (b), (c), and (d) due to the change in the solution concentration. The incorporation of bioceramics results in an increase in the average diameter of the fibers due to the increase in the viscosity of the solution.

Figure 6 shows that most of the samples have contact angles greater than 90°, indicating a hydrophobic character of the scaffolds thanks to the PCL matrix. HA in the fibers increases the surface roughness, resulting in contact angles of around 128°, compared to 119° for the sample without HA [10].

Figure 7 demonstrates the thermal stability of samples. For the PCL, the mass loss was around 90.31%, with the maximum degradation rate at a temperature of 396°C. Char is formed as a residue. In the case of the PCL/HA hybrid, the mass loss

Figure 4. FTIR of PCL and PCL/HA fibers.

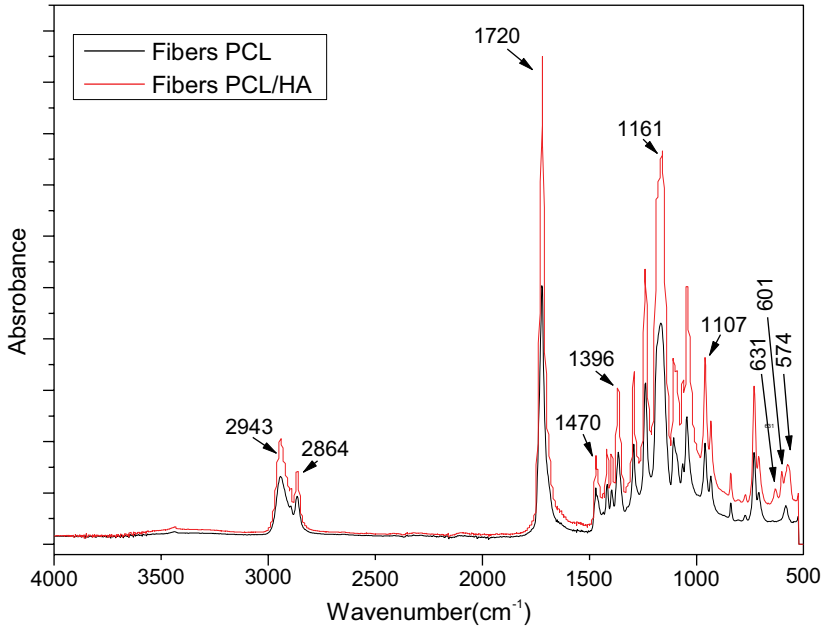


Figure 5. SEM of PCL and PCL/HA fibers with different concentrations.

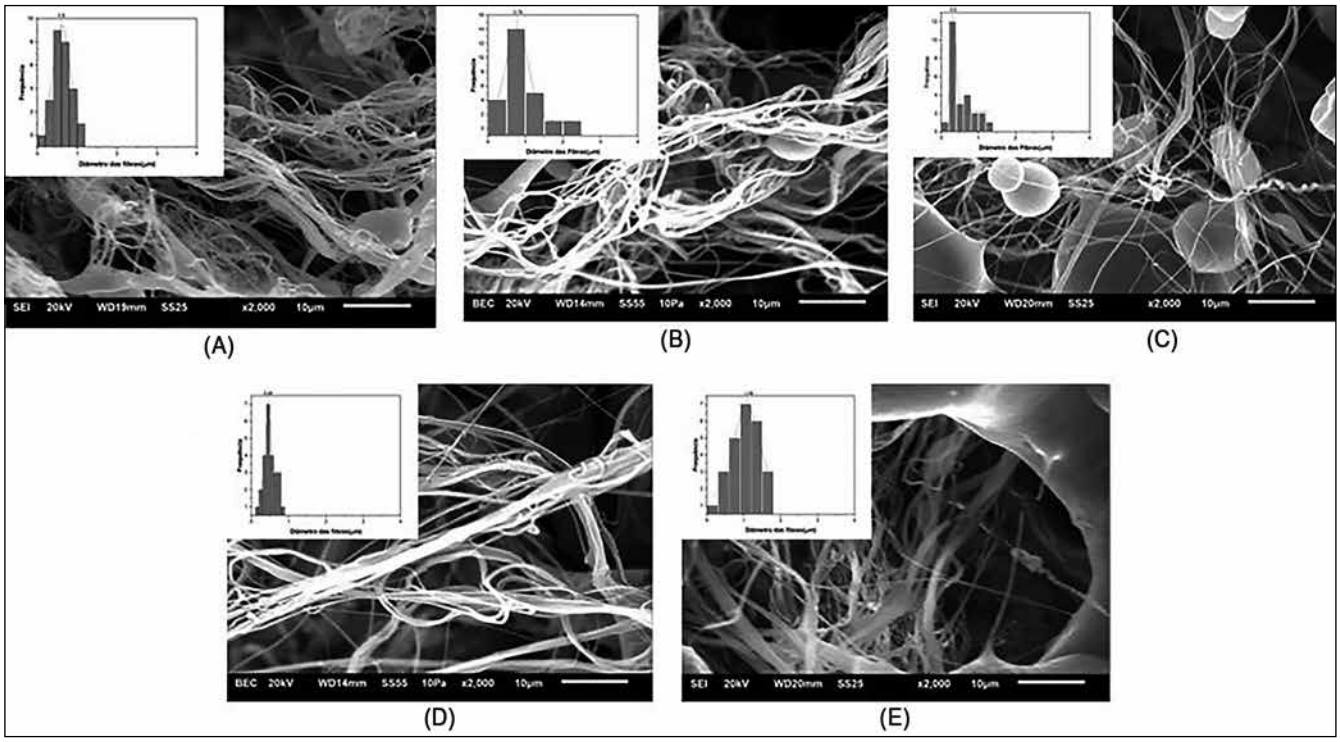


Figure 6. The contact angle of PCL and PCL/HA fibers.

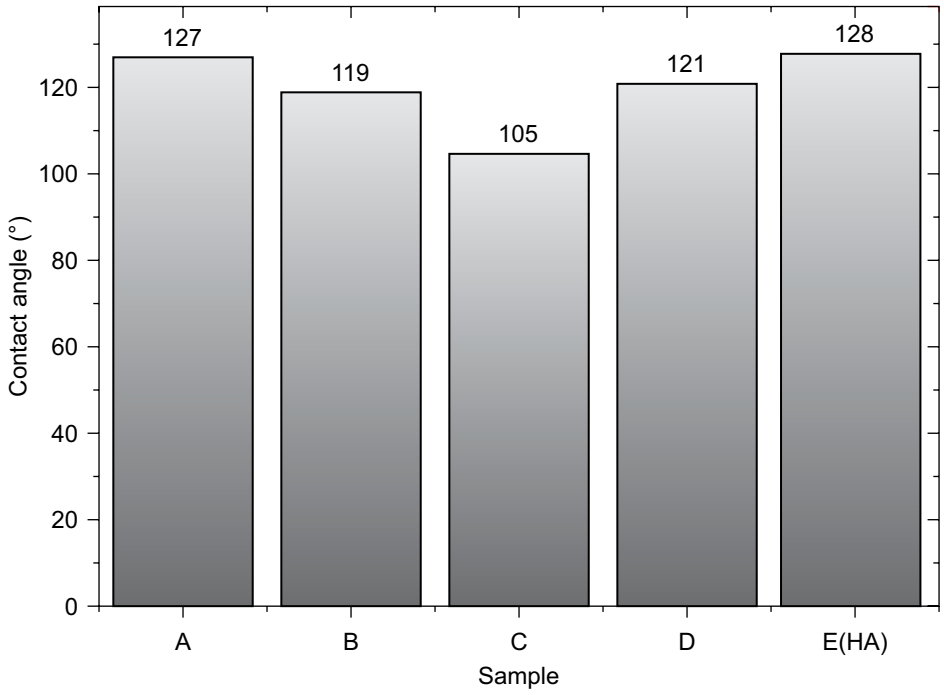
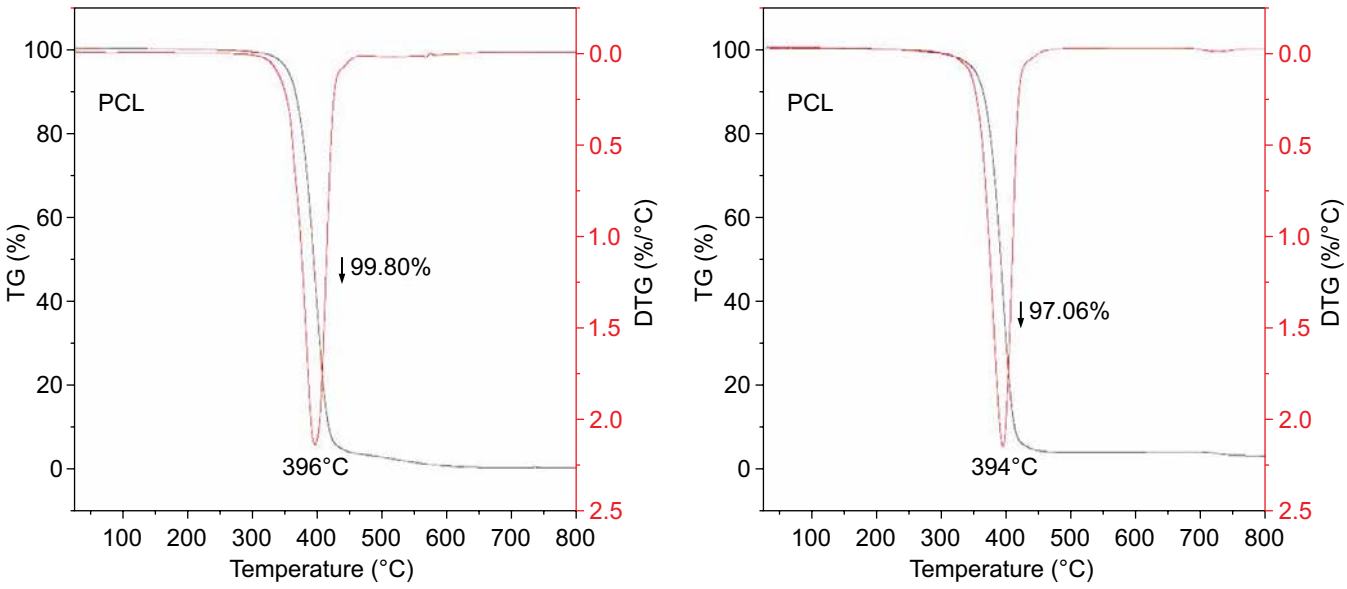


Figure 7. Thermographic analysis of PCL and PCL/HA fibers.



was approximately 75.81%, and the temperature for maximum degradation rate was shifted to 385°C. The addition of HA resulted in anticipation of the polymer thermal degradation. Also, the residue formed can be attributed to inorganic HA and char formed.

Conclusion

In this work, fibers of different polycaprolactone (PCL) orientations with hydroxyapatite (HA) incorporation were produced using the blow-spinning technique, a promising approach for scaffold fabrication. HA, as an inorganic component, is advantageous due to its chemical properties. SEM analyzes observed the presence of "beads" due to incomplete evaporation of the solvent. FTIR spectra indicated possible physical and chemical experiments accommodated between PCL and HA. X-ray diffraction analysis confirmed the crystalline structure of HA. These results suggest that the SBS technique combined with HA can contribute to developing high-quality and high-performance biomedical implants. However, further studies are needed to improve processing conditions and evaluate the tissue response to the scaffolds produced.

Acknowledgments

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