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SYNTHESIS AND CHARACTERIZATION OF POLYMERIC SCAFFOLDS LOADED WITH BIOCERAMICS

Atos T. Matos[1], Pâmela B. G. Mamede[1], Marcele F. Passos[2]*

[1] *Graduation in Bioprocess engineering, Institute of Biological Sciences, Federal University of Pará, Belém, Brazil.*
[2] *Postgraduate Program in Materials Science and Engineering, Institute of Biological Sciences, Federal University of Pará, Belém, Brazil.*
** Corresponding author's email address: cellepassos@ufpa.br*

ABSTRACT

Fractures and bone anomalies affect millions of people annually, resulting from accidents, illnesses, or aging, significantly impacting their quality of life. Due to this growing demand, research in bone tissue engineering has grown to develop biomaterials that promote bone tissue regeneration. Polymeric fibers of polycaprolactone/polylactic acid (PCL/PLA) and polycaprolactone/polyhydroxybutyrate-co-valerate (PCL/PHBv) were developed by Rotary Jet Spinning technique, with the addition of bioceramics (HB). The scaffolds obtained showed high porosity (from 82% to 86%) and fiber diameter of 21,40 ± 10,72 µm for PCL/PLA/HB and 12,64 ± 5,59 µm for PCL/PHBv/HB. The incorporation of bioceramics into the fibers was proven by morphological analysis by optical microscopy and chemical groups by Fourier-Transform infrared spectroscopy. The fibers obtained have potential for application in tissue regeneration but require additional evaluations in the future.

Keywords: Bone regeneration. Biomaterials. Scaffolds. Bioceramics.

1 INTRODUCTION

The Brazilian Association for Bone Assessment and Osteometabolism (ABRASSO) has reported that one in four individuals dies within the first few months following surgical procedures related to hip fractures. Researchers are exploring various biomaterials to address the growing demand for quality orthopedic treatments.[1] Biomaterials are synthetic or natural materials used in whole or in part to regenerate or replace tissue, organ, or body function, improving the quality of life of the patient.[2] Several studies are in progress to develop three-dimensional synthetic/natural biomaterials, also known as scaffolds[3,4], to promote the regeneration of both soft and bone tissues by mimicking the natural extracellular matrix.[5] These scaffolds support cellular adhesion, promote proliferation, and fill bone defects with minimal cellular toxicity.[6] Among the different scaffold production techniques, Rotary Jet Spinning (RJS) has gained more attention in recent years.[7] This technique involves propelling a polymer solution through small orifices using centrifugal force as the driving force.[8] A wide variety of synthetic or natural biocompatible polymers, such as polylactic acid (PLA)[9], polycaprolactone (PCL)[10], and polyhydroxybutyrate-co-valerate (PHBv)[3], are used in the formation of these fibers for tissue regeneration. PLA is a biocompatible synthetic polymer widely used to manufacture wound dressings and drug delivery systems.[9] PCL is highly compatible for blending with other polymers, increasing the variety of possible combinations.[11] Conversely, PHBv demonstrates excellent piezoelectric properties, contributing to the modulation of cellular activity during the bone tissue regeneration process.[3]

Recent studies suggest that the use of bioceramics such as hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) in scaffolds, known for being biocompatible and osteoconductive, can amplify their beneficial effects.[12,13] These bioceramics are calcium phosphate materials like those found in dental enamel and bone. Individually or combined, these inorganic additives can significantly enhance the mechanical properties of scaffolds.[14] Once in the human body, these materials dissolve in body fluids, leading to a local increase in Ca^{2+} and PO_4^{3-} ions at the bone/implant interface, causing precipitation and deposition of superficial apatite nanocrystals. This layer can adsorb extracellular environment proteins, which support cellular growth, fixation, and differentiation.[15] Therefore, this study aimed to develop PCL/PLA and PCL/PHBv scaffolds using the RJS technique, loaded with a mixture of hydroxyapatite and βTCP (HA/βTCP 60/40), and to evaluate their morphology using optical microscopy and the functional groups of the materials using Fourier-transform infrared spectroscopy (FTIR).

2 MATERIAL & METHODS

PCL (80k KDa, density 1.145 g/cm³) and PHBv (2% valerate) were commercially acquired from Sigma-Aldrich (Merck), while polylactic acid (PLA) was acquired in filament form from GTMax3D. The bioceramic (Osteosynt®) hydroxyapatite/βTCP 60/40 (HB) was bought from EincoBio. The PCL/PLA (70/30) polymer solution was prepared at room temperature, completely dissolving the polymers in dichloromethane under continuous stirring. The PCL/PHBv polymer solution was prepared by completely dissolving PHBv in chloroform at 60 °C under continuous stirring, then dissolving PCL at room temperature in the same solution, obtaining a solution with a concentration of 20% (m/v) of total PCL/PHBv mixture (polymer ratio of 87.5%/12.5%). PCL/PLA and PCL/PHBv fiber meshes were synthesized in an RJS system, adapted from patent BR101012008404-0 A2 using a microgrinder as the rotor, with the following configurations: 4 mL solution compartment with a 0.5 mm outlet hole, 22 cm distance from the outlet hole to the collector, 11.5 cm outlet hole height, cylindrical collector with a diameter of 5.2 cm and height of 17 cm, solution subjected to 2700 RPM rotation and room temperature of 26 °C. For the bioceramic incorporation, scaffolds were cut into 1x1 cm dimensions and 1.08 ± 0.04 mm average thickness, then added to a 3% w/v HB dispersion in 96% ethanol and stirred for 30 min at 80 RPM in a homogenizer (Benfer BHS-300), followed by drying in a forced-circulation oven at 40 °C for 24 h. Material morphology was analyzed using a biological optical microscope (Quimis, Brasil). Fiber diameter distribution was analyzed using

Statistica 12 software. Porosity analysis was conducted in triplicate using the gravimetric method by cutting scaffolds with bioceramics and without into 1x1 cm dimensions and submerging them in 96% ethanol for 24 h. Porosity (ε) was calculated using Equation (1), where $\rho_{apparent}$ is the apparent density of the scaffold and ρ_{PCL} is the density of PCL.

$$\varepsilon = 1 - \rho_{apparent} / \rho_{PCL} \quad (1)$$

FTIR analysis of materials was carried out using the ATR (Attenuated Total Reflectance) method in the 400-4000 cm-1 range on a Bruker Vertex 70v spectrometer at an ambient temperature of 25 ºC.

3 RESULTS & DISCUSSION

Optical microscopy (Figure 1) revealed that the material exhibited a disordered pattern in fiber deposition on the collector, a characteristic of the RJS technique known for being a rapid and high-yield fiber production approach but challenging in fiber deposition control.[16] Fibers had an average diameter of 17,95 ± 6,04 µm for PCL/PLA, 21,40 ± 10,72 µm for PCL/PLA/HB, 11,54 ± 4,84 µm for PCL/PHBv, and 12,64 ± 5,59 µm for PCL/PHBv/HB, with fibers containing PHBv in the composition showing smaller diameters compared to those with PLA. This is probably due to the low molecular mass of PHBv, which provides low entanglement of molecular chains, leading to smaller fiber diameters.[17] HB particles were also observed on the fiber surface, absent in pure fiber microscopy (Figure 2).

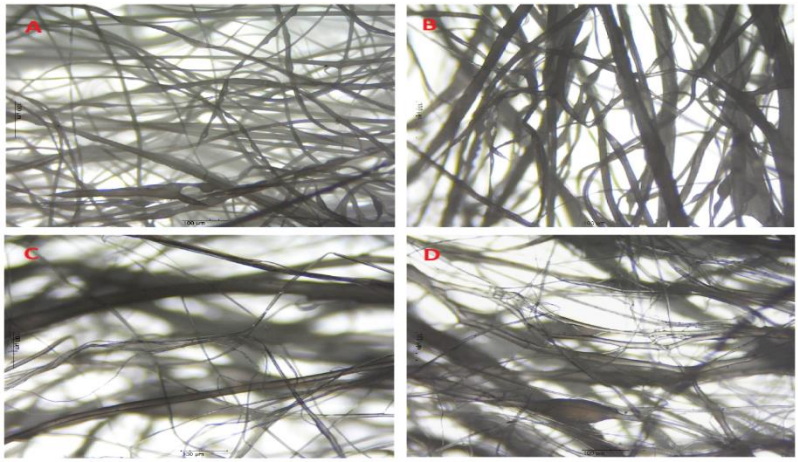

Figure 1. Optical microscopy of PCL/PLA (A), PCL/PLA/HB (B), PCL/PHBv (C), and PCL/PHBv/HB (D) fibers at 40x magnification.

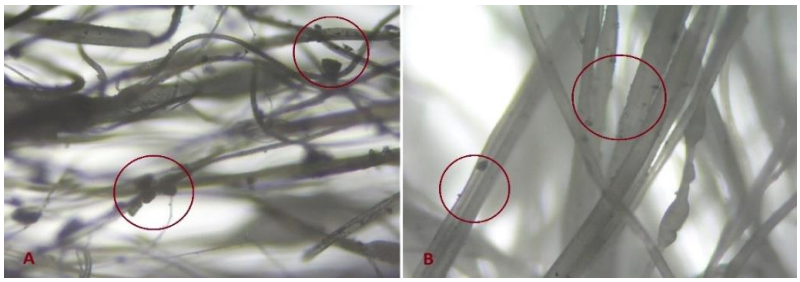

Figure 2. Optical microscopy of PCL/PHBv/HB (A) and PCL/PLA/HB (B) fibers. Highlighted in red are fibers with bioceramics on the surface at 100x magnification.

Table 1 shows scaffold volumes with their incorporated HB mass (difference between final and initial mass), ethanol absorbed volume after 24 h, and porosity of each sample. Average porosity for PCL/PLA fibers was 84.04 ± 4.29, for PCL/PLA/HB was 85.95 ± 2.02, for PCL/PHBv was 86.02 ± 0.08, and for PCL/PHBv/HB was 82.61 ± 2.48. High porosity, as observed in the scaffolds produced, according to some authors, helps promote better cellular infiltration and nutrient flow in the extracellular matrix but also influences the mechanical properties of the material.[18]

Table 1 Characteristics of the scaffolds with porosity (average ± standard deviation).

Pure Samples	Volume (cm^3)	Absorbed ethanol (mL)	Average Porosity (%)	Samples with HB	Volume (cm^3)	Mass of HB (mg)	Absorbed ethanol (mL)	Average Porosity (%)
PCL/PLA	0.108	0.150	84.04 ± 4.29	PCL/PLA	0.106	1.3	0.175	85.95 ± 2.02
PCL/PLA	0.108	0.125		PCL/PLA	0.104	0.8	0.125	
PCL/PLA	0.110	0.125		PCL/PLA	0.114	1.3	0.150	
PCL/PHBv	0.110	0.125	86.02 ± 0.08	PCL/PHBv	0.120	1.0	0.125	82.61 ± 2.48
PCL/PHBv	0.106	0.125		PCL/PHBv	0.106	1.1	0.175	
PCL/PHBv	0.110	0.150		PCL/PHBv	0.104	1.3	0.125	

FTIR-ATR spectra analysis (Figure 3) showed a change in fiber spectra profiles with bioceramic addition compared to those without. Bands at 570 and 600 cm^{-1} for PCL/PLA and PCL/PLA/HB, and 574 and 602 cm^{-1} for PCL/PHBv and PCL/PHBv/HB are characteristic of asymmetric stretching vibration of PO_4^{-3} groups present in the HA-βTCP mixture.[19] An increase in signal intensity in 432 and 1044 cm^{-1} was also observed, caused by asymmetric stretching vibration of PO_4^{-3} groups. A weak signal was also observed at 616 cm^{-1} (Figure 3A), which is characteristic of the terminal OH group of hydroxyapatites with lower steric hindrance.[20] The presence of these functional groups can confirm the presence of bioceramics in the fibers.

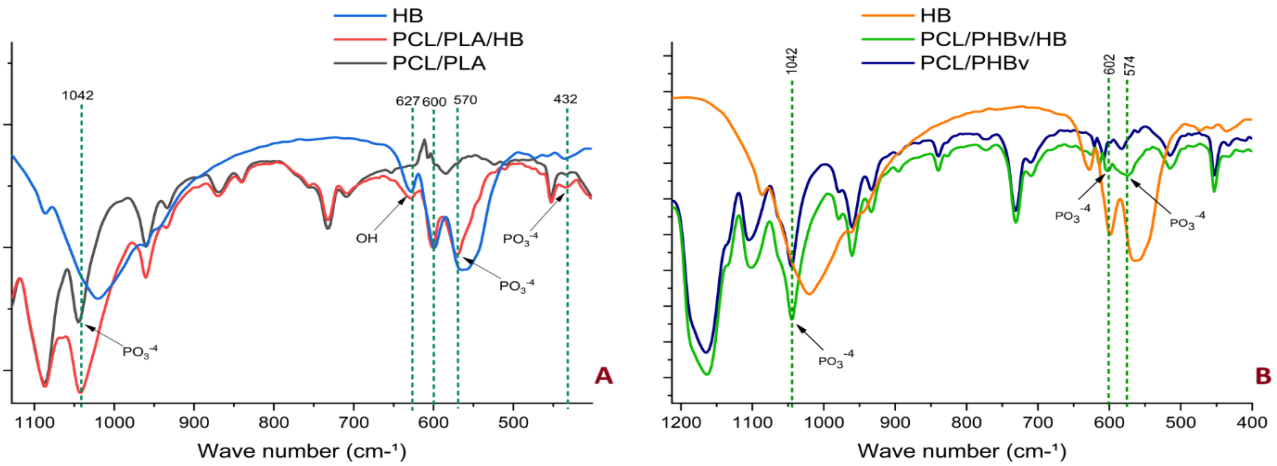

Figure 3. FTIR spectra of PCL/PLA and PCL/PLA/HB (A), PCL/PHBv, and PCL/PHBv/HB (B) scaffolds.

4 CONCLUSION

Based on FTIR-ATR spectra analysis and optical microscopy, the incorporation of HA-βTCP in the scaffolds was successful. The porous scaffolds, composed of biocompatible polymers/bioceramics, demonstrate potential for bone tissue regeneration applications. However, further studies are needed, including bioceramic incorporation yield optimization, mechanical testing of the fibers, and cellular viability analysis.

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