Development and Characterization of a PLA/NHA Composite Scaffold Manufacturated by 3D Printing

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The use of 3D bioprinting techniques for scaffold production represents an innovative approach, enabling the development of biomimetic structures that serve as matrices for new tissue formation. This study investigated the influence of varying nanohydroxyapatite (nHA) particle sizes on the properties of PLA/nHA scaffolds. The results obtained from nanocomposites containing 3% wt. nHA indicated that particle size does not significantly affect surface morphology, mobility, thermal behavior, handling, or cytotoxicity in the analyzed scaffolds.
Keywords: Biocomposite. Scaffold. PLA/nHA. 3D Printing.

The regeneration and repair of bone tissue lesions remain significant challenges in tissue engineering. Consequently, 3D printing has emerged as a promising production technique. By leveraging additive manufacturing, it is possible to achieve high precision and greater freedom in customizing the geometries of extracellular matrices and scaffolds [1].

Studies on materials for bone implants, including metals, ceramics, and polymers, have identified biopolymers as the most widely used material in biomedicine, offering good mechanical support. However, biopolymers exhibit limitations such as low osteogenesis activity, histocompatibility, and an inadequate degradation rate, often leading to unsatisfactory results in bone regeneration [2].

Combining biocompatible materials to mimic bone tissue is essential to address these shortcomings. Hydroxyapatite (hydrated calcium phosphate, Ca/P ratio = 1.67) is a suitable candidate due to its similarity to the mineral phase of bone tissue. In this study, poly(lactic acid) (PLA), a biopolymer, and hydroxyapatite nanoparticles (nHA), a bioceramic, were combined to fabricate PLA/nHA biocomposite scaffolds.

nHA possesses osteoconductivity, osteoinductivity, and bioactivity [3], making it a valuable component in bone tissue engineering.Therefore, this study aimed to develop and characterize PLA/nHA biocomposite scaffolds with varying nHA particle sizes and crystallinity using 3D bioprinting. The resulting scaffolds were analyzed through scanning electron microscopy (SEM), thermogravimetric analysis (TGA), wettability tests, degradation tests, and cytotoxicity assays.

Materials and Methods

Materials

The polymer matrix used was PLA Luminy® from Corbion (Amsterdam, The Netherlands), with the following properties: density of 1.24 g/cm³, melt flow index of 3–8 g/10 min, stereochemical purity of 96% L-isomer, crystalline white pellet appearance, melting temperature (Tm) of 155 °C, and glass transition temperature (Tg) of 55–60 °C. Chloroform (99.8%, Êxodo Científica, Brazil) was used as a solvent for fabricating the PLA/nHA composite.

The hydroxyapatite used was synthesized from the spines of tilapia fish. The synthesized hydroxyapatite underwent heat treatment, and milling time was varied to modify surface area, crystallite size, and crystallinity [4].

Received on 17 September 2024; revised 12 November 2024.
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J Bioeng. Tech. Health 2024;7(4):327-332

Preparation of PLA/nHA Nanocomposite

The nanocomposite was prepared by incorporating 3% wt of nHA relative to the PLA weight. nHA was dissolved in 100 mL of chloroform under magnetic stirring for 30 minutes, followed by dispersion in an ultrasonic bath for 1 hour. The solution was then stirred again while 10 g of PLA was gradually added until completely dissolved (Figure 1).

The resulting solution was poured into a Petri dish and dried at room temperature in an exhaustion hood for 24 hours. The dried composite films were then cut into smaller pieces for 3D printing. Two types of composites, PLA/nHA-1 and PLA/nHA-2 were produced, differing in nHA particle sizes of 6.06 nm and 9.42 nm, respectively [5].

Method

3D Printing of Composite Scaffolds Scaffolds were printed using an Octopus™ 3D bioprinter (3D Biotechnology Solutions, 3DBS). Designs were created using SolidWorks CAD software, and G-code for printing was generated with Simplify3D. Tabe 1 provides the printing parameters.

Thermogravimetric Analysis (TGA)

TGA was performed using a TA Instruments Q50 analyzer (TA Instruments, New Castle, USA) in a temperature range of 25–600 °C, with a heating rate of 10 °C/min under a nitrogen atmosphere. This analysis evaluated the degradation temperature and residue percentage of the samples.

Table 1. 3D printing parameters.

Parameters of print	
Scaffold size	12x12x5 mm
Printing temperature	195 °C
Nozzle diameter	1 mm
Build platform temperature	60 °C
Scaffold fill	90% (±45°)
Layer Size	0.8 mm
Print speed	45 mm/min

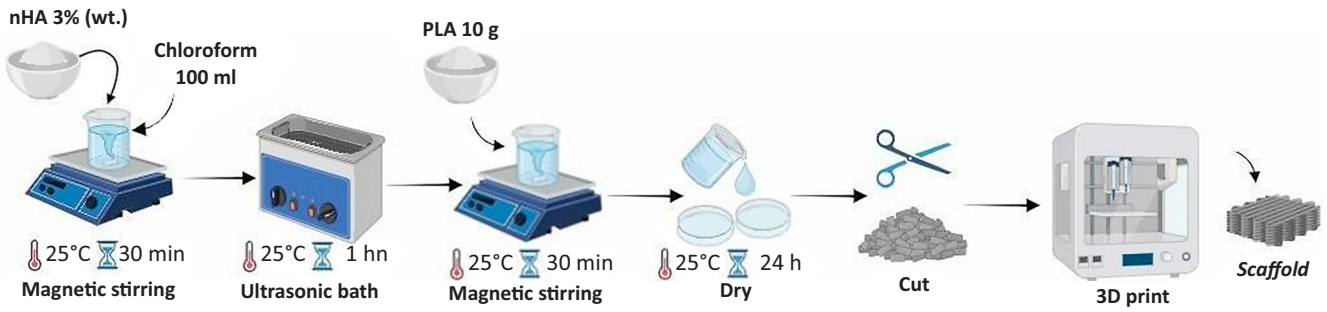
Scanning Electron Microscopy (SEM)

Surface morphology was analyzed using a JSM-6510LV SEM (JEOL, Tokyo, Japan) at an acceleration voltage of 20 kV. Samples were metalized with gold to enhance electron beam dispersion across the scaffold surface.

Wettability Test

Wettability was assessed by measuring the contact angle of water droplets (5 µL) on the sample surfaces using a DSA-25 goniometer (Krüss, Hamburg, Germany). Photos were taken to measure the angles, with tests performed five times at different locations on each sample.

Figure 1. Composite scaffold manufacturing.



In vitro Scaffold Degradation Test

Scaffolds were submerged in a 0.1 M phosphate-buffered solution (PBS) at pH 7.4 (Sigma-Aldrich, Missouri, USA). The PBS volume was calculated using Equation 1, where V is the volume and Sa is the surface area.

$$V = \frac{S_a}{10} \quad (1)$$

Scaffolds were submerged for three months, with weekly weight measurements. Samples were dried at 90 °C before weighing. Weight loss was calculated using Equation 2, where W_0 is the initial mass and W_F is the final mass.

$$WL\ (\%) = \frac{W_0 - W_F}{W_0}\ X\ 100 \quad (2)$$

Cytotoxicity

Cytotoxicity was evaluated using the AlamarBlue® assay (Invitrogen, Carlsbad, CA, USA). Mouse fibroblast L929 cells were cultured in DMEM (Life Technologies) supplemented with 10% FBS and 1% penicillin/streptomycin in a 5% CO_2 incubator at 37 °C.

Sterilized scaffolds were incubated in DMEM for 24 hours before cell seeding at 5×10^5 cells/well. After 72 hours of incubation, resazurin sodium salt was added at 10% v/v, followed by a 4-hour incubation. The supernatant was transferred to a 96-well plate, and metabolic activity was measured at 570 and 600 nm using a multi-plate reader [6]. Tests were conducted in triplicate.

Results and Discussion

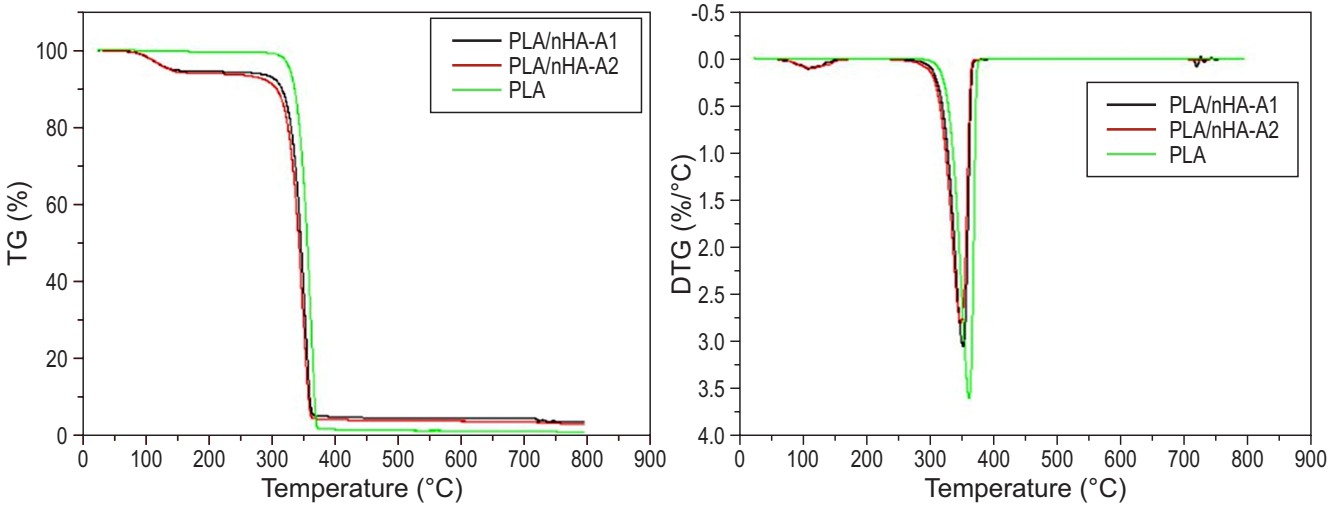
Thermal Analysis

The analysis of TGA was done to assess and validate the method of fabrication of the composite. Figure 2 shows, respectively, the TG (a) and DTG (b), which represent the curves from the samples of PLA, PLA/nHA-A1, and PLA/nHA-A2. In Figure 2 (a), the PLA curve shows only one thermic event, which is the loss of mass of the polymer, and Figure 2 (b) shows that this occurs at approximately 360 ºC.

The composites have the same thermic behavior between each other and can be observed in two thermal events. The first one is approximately 140 ºC, and this loss of mass is because of the evaporation of water from the material, justified because of the hydrophilic properties of the material determined in the wettability test. The second thermal event is approximately 350 ºC, representing the loss of mass in the material.

Also, it is possible to observe that the composites have a residue of approximately 3 % wt. Which is equivalent to a concentration of nHA from the

Figure 2. Thermal analysis of the sample, TGA Curves (a), and DTG curves (b).



fabrication of the composite. The TGA analysis proved that there was an efficient fabrication of the composite without considerable waste of nHA. Furthermore, in comparison with the analysis from Rodovalho and colleagues [5], who have made a study with 10 % wt. nHA, the fabrication of composites is effective independent of the concentration because there isn't considerable loss of bioceramics.

Scanning Electronic Microscopy

The morphological analysis of the filament and the surface of the scaffold was done by an SEM after the 3D printing. SEM of the PLA (a-b), PLA/nHA-A1 (c-d), and PLA/ nHA-A2 (e-f) samples are shown in Figure 3. The filament of the PLA, PLA/nHA- A1 and PLA/ nHA-A2 scaffolds presented in Figure 3 (a,c,e) show the uniformity of the filaments and the porosity. In Figure 3 (d,f), the presence of nHA dispersed evenly along the scaffold surface can be seen by the white points on the surface. With that, the efficiency of the casting method in the dispersion of the nanoparticles in the polymeric matrix grants homogeneity of nHA along the composite scaffold.

Wettability Test

Figure 4 presents the evaluation of the contact angle of the PLA, PLA/nHA-A1 e PLA/nHA-A2, showing that the PLA had a contact angle of 95.5º ± 1.12°, which means that the material has a hydrophobic behavior. The nanocomposites PLA/nHA-A1 and PLA/ nHA-A2 had respectively a contact angle of 70.4° ± 1.92° e 72.7° ± 6.02°, characterizing hydrophilic

Figure 3. SEM of scaffolds (a-b) PLA, (c-d) PLA/nHA-A1, and (e-f) PLA/nHA-A2.

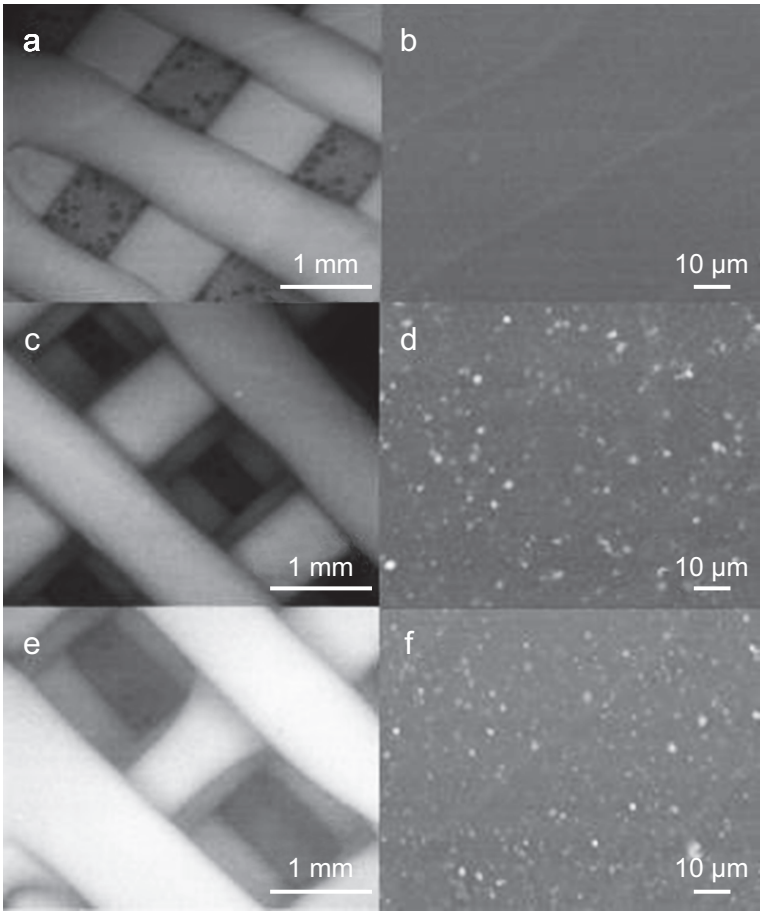
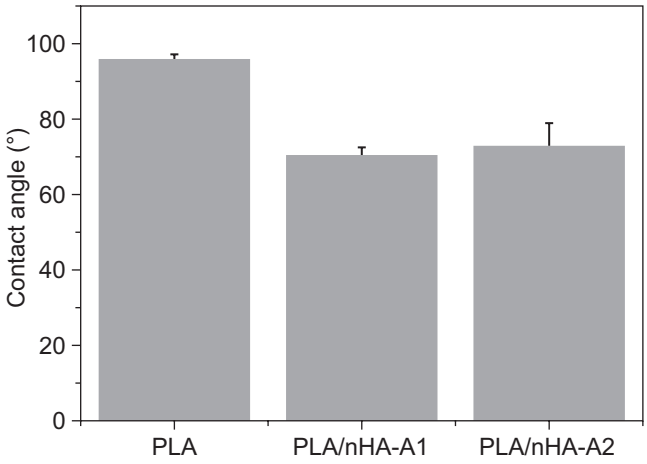


Figure 4. The average contact angle of PLA, PLA/nHA.1, and PLA/nHA.2 scaffolds.



behavior [7,8]. The composites had hydrophilic behavior is caused by the presence of nHA.

In vitro Scaffold Degradation Test

The scaffolds were submerged in PBS for 91 days to evaluate the degradation rate (Figure 5). All of the samples had mass loss, but PLA obtained the lowest mass loss percentage in relation to the composite scaffolds, justified by their hydrophobic behavior. The composite obtained the biggest degradation rate because of its hydrophilic behavior, which is provided by the insertion of nHA, corroborating with the studies of Rodovalho and colleagues [5]. In this study, PLA/nHA-A1 obtained a degradation rate similar to PLA/nHA-A2, showing that the different nHA particle sizes did not impact the degradation rate of the composites.

Cytotoxicity

The results of cellular viability test of the PLA, PLA/nHA-A1 e PLA/nHA-A2 are showed in Figure 6. The viability of scaffold was superior than 90 %, which states that the material didn't have significant toxicity to the cells. The values of cellular viability aren't 100 % viables because the scaffold occupied a space in the surface of the plate and interferes with the cell carpet.

Figure 5. Percentage of mass loss for the PLA, PLA/nHA-1, and PLA/nHA-2 scaffolds during the 91-day degradation period.

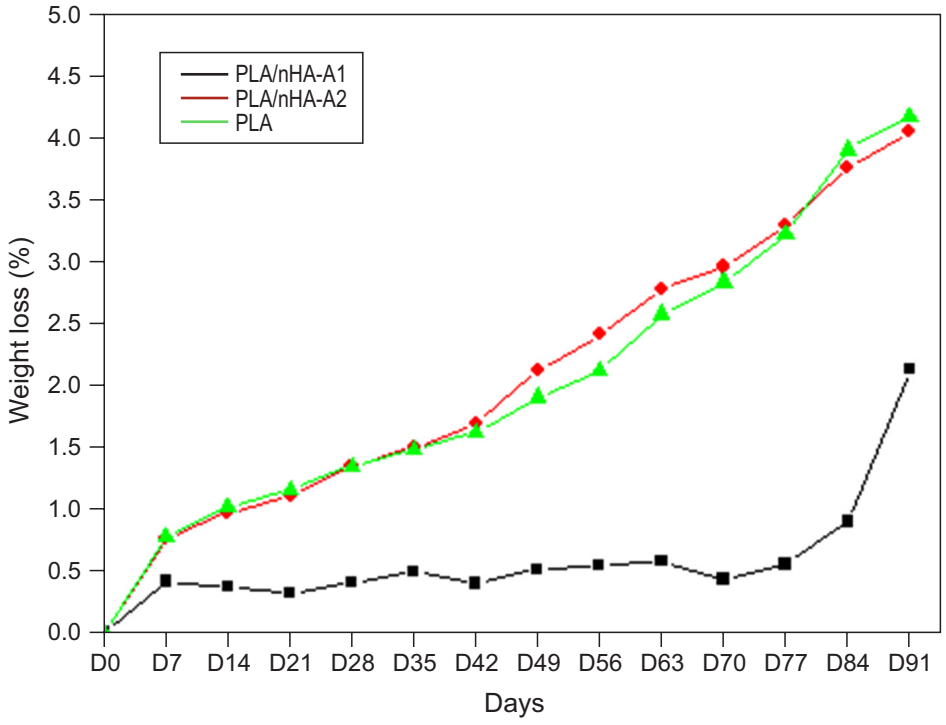
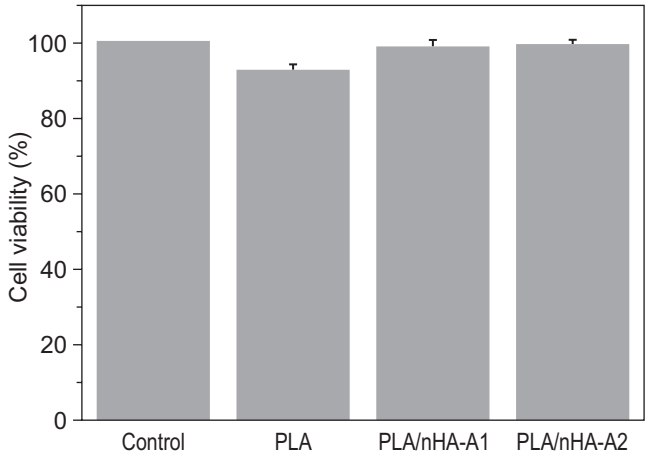


Figure 6. Percentage of cell viability of PLA, PLA/nHA-1, and PLA/nHA-2 scaffolds.



Conclusion

This study had the objective of evaluating the influence of nanoparticles of hydroxyapatite with a concentration of 3 % wt. in the PLA/nHA composite. The wettability test showed that the insertion of nHA in the polymeric matrix with a concentration of 3 % wt. or above provided a hydrophilic behavior to the composite, seeing that the PLA has a hydrophobic behavior. The degradation of the composite scaffolds obtained a bigger loss of mass in relation to the PLA, and this is because of the hydrophilic behavior that the nHA provided to the composite, but the difference of nHA nanoparticles, in this concentration, didn't impact the results. However, the compounds developed showed satisfactory results for future applications in tissue engineering.

Acknowledgments

The authors are grateful to SENAI CIMATEC University Center, Coordination for the Improvement of Higher Education Personnel (CAPES), Research Support Foundation of Bahia State (FAPESB), Research and Innovation Foundation of Santa Catarina State (FAPESC) for their support in the development of this study.

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