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Influence of Hydroxyapatite on Extruded 3D Scaffolds

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Abstract

Ideal scaffolds for tissue engineering must mimic the complex characteristics of natural tissues and their mechanical performance. In this work, Polycaprolactone (PCL) and composite Polycaprolactone/Hydroxyapatite (PCL/HA) (75/25) scaffolds were produced by using an extrusion-based process called BioExtruder. The structures were characterized regarding the chemical, thermal, morphological and mechanical properties, in order to investigate the effect of HA addition in the polymeric scaffolds. Results show that the incorporation of HA in the 3D structures improve both the thermal and mechanical properties.

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Keywords: Tissue Engineering; Scaffold; Polycaprolactone; Hydroxyapatite; Extrusion.

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1. Introduction

Nowadays, there is a great interest in the development of new materials and processes to obtain optimized scaffolds for tissue engineering applications. An ideal scaffold should be characterized by biocompatibility, biodegradability, appropriate mechanical properties, interconnectivity pores with appropriate size to retain cells, and allow exchanges of nutrients and waste products.

Scaffolds can be produced using non-additive or conventional processes and additive biomanufacturing techniques. Non-additive techniques include solvent casting, freeze-drying, phase separation, gas foaming, melt moulding and particle-leaching [1]. The main limitations of these techniques concern the poor control over the porosity, pore size, spatial distribution and interconnectivity, which leads to a non-homogenous distribution of cells throughout the construct, insufficient vascularization, and non-uniform tissue growth. Moreover, some of these techniques also require the use of toxic solvents, preventing the fabrication of constructs containing living cells and biological molecules [1,2] Additive biomanufacturing techniques were introduced in the biomedical field to address the limitations of non-additive ones. These techniques provide precise control over the scaffold architecture, shape, porosity, pore size and pore interconnectivity in a wide range of materials [2,3].

In the tissue engineering field, additive technologies were used to produce scaffolds with optimized external shape and predefined internal morphology allowing a good control of pore size and pore distribution favouring mimic the extra cellular matrix (ECM) that facilitate cellular attachment, differentiation and proliferation [1,4].

The bone tissue can be damaged due several factors, such as congenital abnormalities, diseases, injuries, or traumas. To repair this tissue the most common therapeutics used are the autografts or allografts. However, these methods present limited viability, due to the possibility of disease transmission, poor biocompatibility and the risk of implantation failure. Recently, polymer/ceramics scaffolds were used as alternative strategies in tissue engineering, in which bioactive ceramic particles are embedded [5]. Composite systems, based on polymer and ceramic materials, offer the possibility to produce constructs with increased mechanical properties and bioactivity Polycaprolactone (PCL) is the most extensively biodegradable and noncytotoxic polymer used as biomaterial to produce scaffolds for tissue engineering, due to its suitable mechanical properties and appropriated bioresorption rate for bone tissue regeneration [6]. Though, PCL has a hydrophobic nature and its poor surface wetting and interaction with biological fluids result in poor cell adhesion and proliferation. In order to enhance mechanical properties, PCL is often used as polymer matrix in composites including osteogenic and osteoinductive inorganic phases, such as hydroxyapatite (HA), which is the main mineral component of bone tissues and confers its high bioactivity to the polymer-based composite promoting bone regeneration [7].

In this work, was explored the influence of HA incorporation in PCL scaffolds as a preliminary study, nanofibres will be added in the future, produced by electrospinning, in order to obtain hybrid structures with most optimized structure for bone regeneration.

In this research work, 3D scaffolds composed of PCL and HA were produced and the effect of HA particles in the thermal, mechanical, chemical and morphological properties investigated. The main aim of this work is to optimize the properties of PCL/HA scaffolds, for subsequent combination with nanofibres produced by electrospinning in order to obtain hybrid structures with enhance mechanical and biological properties for bone tissue engineering applications.

2. Materials and methods

2.1. Materials

The polymer used in this study was PCL (CAPA 6500) with Mw of 50 000 g/mol from Perstorp Caprolactones Limited (Cheshire, UK). The PCL is a semi-crystalline aliphatic polymer that has a slower degradation rate 12 – 24 month. It has a low glass transition temperature at – 60°C, a melting temperature at about 60°C, and a high thermal stability. Also was used HA with particle size around 5 µm from Altakitin Company (Aveiro, Portugal).

2.2. Methods

2.2.1. Scaffold fabrication

The PCL/HA mixture was prepared through the melt blending methodology, in which PCL was melt at 100°C and added 25 wt% of HA. The scaffolds fabrication was performed through the Dual-Bioextruder equipment developed by the Centre for Rapid and Sustainable Product Development of the Polytechnic Institute of Leiria (Fig. 1).

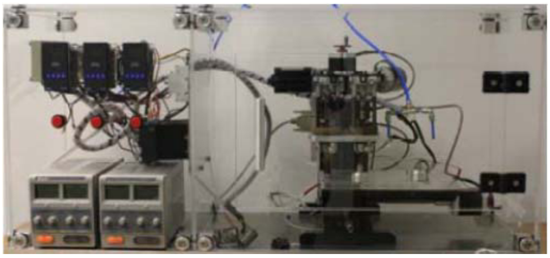

Fig. 1. Dual-Bioextruder System.

The container where loaded with PCL or with PCL/HA and heated to 90°C and 110°C, respectively. The molten polymers were then extruded through a nozzle (23G of diameter) compressed by air pressure, the wire is deposited layer by layer at speed of 130 mm/min (Fig 2) [8].

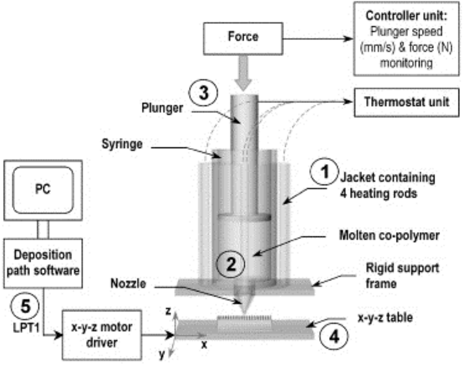

Fig. 2. The 3D deposition device consisted of five main components: (1) a thermostatically controlled heating jacket; (2) a molten copolymer dispensing unit consisting of a syringe and nozzle; (3) a force controlled plunger to regulate flow of molten co-polymer (4); a stepper motor driven x-y-z table; and (5) a positional control unit consisting of stepper-motor drivers linked to a personal computer containing software for generating fiber deposition paths [8].

The cylindrical structures (diameter 3.2mm x height 1.4mm) were prepared with 0°/90° architecture and pore size of approximately 150µm. The processing parameters are indicated in table 1.

Table 1. Processing parameters of the scaffolds.

Parameters	PCL	PCL/HA
Filament Distance (FD, µm)	450	450
ST: Slice Thickness (ST, µm)	280	280
Filament Width (FW, µm)	300	300
Deposition velocity (DV, mm/s)	130	130
Screw rotation velocity (SRV, rpm)		
Heating temperature (HT, ºC)	90	90

2.2.2. Polymer characterisation

Thermal properties were determined using the Simultaneous Thermal Analyzer, STA 6000 system (PerkinElmer, USA). Samples of approximately 10mg were placed into ceramic pans and the tests were performed in a dry nitrogen atmosphere (flow rate of 20mLmin-1).

Fourier Transform Infrared Spectroscopy (FTIR) was used to assess the composition of the samples, in particular to verify if the processing induces chemical changes. The FTIR Alpha-P by Brucker Company was used to perform the tests.

The structures were tested using a Skyscan microtomograph (MicroCT) model 1174 by Brucker Company (Bruxelles, Belgium). The samples analysed were scanned with rotation step of 0.5 degree, exposure time of 1900 ms, source voltage of 50 kV, source current of 800 uA and image pixel size of 6.60 µm.The microCT is a no-destructive analysis that allow the visualization of internal and external morphology of the samples as well as evaluate some parameters as volume of porosity, quantification of particles, 3D measurements, among others. Through this equipment, it is possible to perform a mechanical characterization, compression and tensile tests, the maximum of the load cell is 440N. In this work, morphological and compression tests were performed, where the scaffolds was in dry state, and the tests was performed up to a strain value of 50% of sample height.

3. Results and discussion

3.1. Thermal analysis

According to the thermogram, it is possible observe that PCL scaffold present a melting temperature (Tm) of 68ºC and the degradation temperature of 388ºC, for PCL/HA scaffolds the Tm is 72ºC and degradation temperature is 399ºC, the HA incorporation increase the melting and degradation temperature. Fig. 3 shows the thermal behavior of PCL and PCL/HA scaffolds, the differences in melt temperature could suggest that the aggregation of HA affect the structure cristallinity [9].

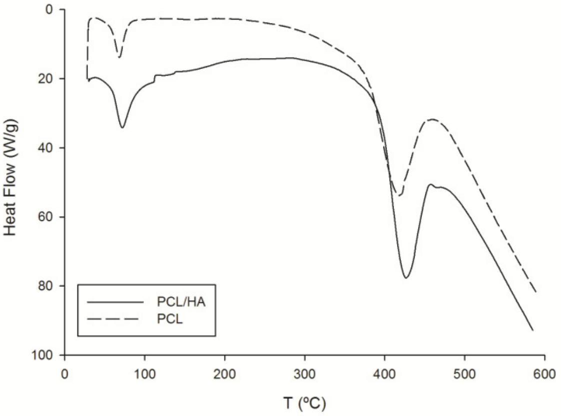

Fig. 3. Thermal curves of the scaffolds.

Through STA analysis, it was possible to evaluate the weight loss for which structures, PCL scaffold present 99.5% of weight loss while the composite scaffold PCL/HA present 96.8%. A lower value of weight loss in the PCL/HA curve may be due to the presence of HA, which have not significant changes at the temperature of 600ºC, the weight loss corresponding to the PCL and the residual mass corresponding to the HA.

3.2. FTIR analyses

FTIR was used to test the chemical composition of PCL grain and the 3D scaffolds. Fig. 4 shows the spectra for the both PCL and PCL/HA structures as well as the PCL grain. In the PCL scaffold and PCL grain curves it is possible observe a peak in 1720.7 cm^{-1}, corresponding to the C=O bond characteristic in Esters, between 750 and 1500 cm-1 we can observe some peaks corresponding to a CH2 groups of PCL chain. Lastly, it can be observed two peaks with 2863.69 cm^{-1} and 2941.57 cm^{-1}, corresponding to the CH bond [10]. The spectrum of PCL/HA present absorption bands at 1041, 600, 568 and 453 cm^{-1}, which are assigned to the PO4-3. The peaks at 840 cm^{-1}, 1417 and1460 cm^{-1} are characteristics of CO3-2, which is an important mark of carbonate group entering into the apatite [9,11]. Chemical characterization indicates that the extrusion processing does not induced chemical changes in the materials structure.

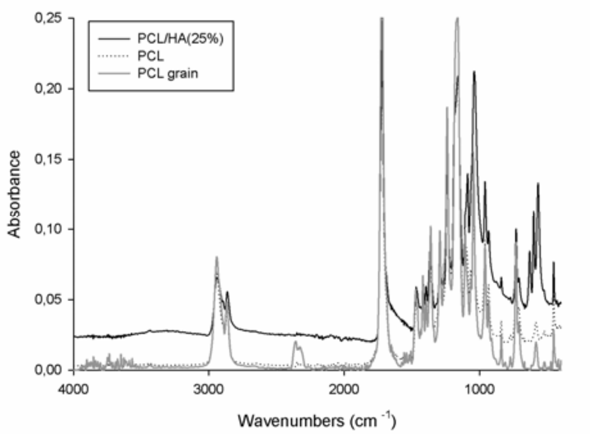

Fig. 4. FTIR spectrums.

3.3. MicroCT

3.3.1. Morphological analysis

The analyses of structures through microCT were performed to provide information about the internal and external morphology of the 3D scaffolds such as the porosity volume and pore size. According to Fig. 5, it is possible observe that the PCL/HA scaffold present higher surface roughness than the PCL scaffold, due to the presence of HA particles at the surface. MicroCT results also show that both PCL and PCL/HA scaffolds present interconnected pores throughout the layers. The PCL scaffolds present 58.1% of porosity and 299,83±25.06 of filament diameter, while the PCL/HA scaffolds present 80.6% of porosity and 325 ±25. of filament diameter.

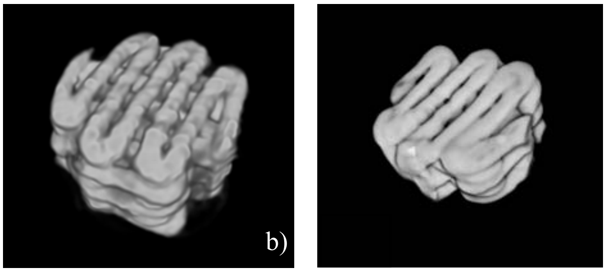

Fig. 5. 3D MicroCT images (a) PCL/HA and (b) PCL.

In Fig 6, the HA particles can be clearly visualized, Fig 6 b) showing 3D hydroxyapatite particles and its dispersion.

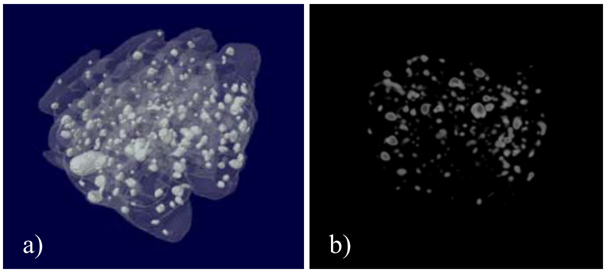

Fig. 6. MicroCT images of HA particle dispersion.

3.3.2. Mechanical analysis

Compression tests were performed in order to evaluate the influence of HA addition in 3D scaffolds. According to Table 2, it is possible to observe that the structures with HA present better mechanical properties than the scaffolds without this ceramic. The mechanical properties of both structures present values appropriate for cortical bone regeneration. This tissue present for maximum stress a range of 60 -160 MPa and for compressive modulus a range of 3-30 GPa, thus the structures produced mimic the cortical bone in terms of mechanical properties [11].

Table 2. Mechanical properties.

Material	Compressive Modulus E (MPa)	Maximum Stress σ max (MPa)
PCL	305,97±30,4	61,585±2,354
PCL/HA	464,94±33,3	64,865±4,43

4. Conclusions

Results show that the scaffolds of PCL/HA are promising for tissue engineering applications. The incorporation of HA particles in the matrix of PCL improves the mechanical and thermal properties of the structure. These structures present interconnectivity pores and a homogeneous dispersion of HA particles across the scaffold.

Acknowledgements

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