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Characterisation of PCL and PCL/PLA *scaffolds* for tissue engineering

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Abstract

This paper investigates the use of PCL and PCL/PLA *scaffolds* produced using a novel additive biomanufacturing system called *BioCell Printing*. PCL/PLA blends were prepared using melt blend and solvent casting techniques. *Scaffolds* with 0/90° architecture and 350 μm of pore size were morphologically evaluated using scanning electron microscopy and atomic force microscopy. Biological tests, using osteosarcoma cell line G-63, were performed using the Alamar Blue Assay and Alkaline Phosphatase Activity.

Results show that the *BioCell Printing* system produces *scaffolds* with regular and reproducible architecture, presenting no toxicity and enhancing cell attachment and proliferation. It was also possible to observe that the addition of PLA to PCL *scaffolds* strongly improves the biomechanical performance of the constructs.

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1. Introduction

Scaffolds are used in tissue engineering as a physical and biological support for seeding cells, and transplanting them into an organism [1-11]. *Scaffolds* are critical as they serve different purposes: allow cell attachment, proliferation and differentiation, deliver and retain cells and growth factors, enable diffusion of cell nutrients and oxygen, and enable the establishment of an appropriate mechanical and biological environment for tissue regeneration in an organised way [1-11].

Poly (ε-caprolactone) (PCL) is extensively used to produce *scaffolds* for tissue engineering applications due to its biocompatibility, biodegradability, structural stability and mechanical properties. However, PCL presents low bioactivity and surface energy (high hydrophobicity), leading to reduced cell affinity and small tissue regeneration rates. This paper describes a

strategy to reduce these limitations by combining PCL with Poly-lactic acid (PLA) in order to produce less hydrophobic constructs with adjustable degradability and appropriated mechanical properties. PCL/PLA mixtures, prepared by solvent casting and melt blending, were used to produce the *scaffolds* by using an additive biomanufacturing system called *BioCell Printing* [12]. *Scaffolds* were characterised from both morphological and biological views.

2. Materials and Methods

2.1. Materials

PCL is a semi-crystalline linear aliphatic polyester susceptible to undergo autocatalyzed bulk hydrolysis and slow degradation [13]. PCL has a low melting point (≈60°C), which enables easy processing and a low glass

transition temperature ($\approx 60^{\circ}\text{C}$), which allows high elasticity at room and body temperature [7]. PLA is a biocompatible and resorbable aliphatic polyester characterized by its high melting temperature ($\approx 160^{\circ}\text{C}$) and glass transition temperature ($\approx 60^{\circ}\text{C}$) [14].

The PCL used in this research work was Capa 6500 (Perstorp, UK), and the PLA was PLA 2002D (Cargill Down, USA).

2.1.1 Physical blending

The blending process of PCL and PLA (50/50 wt%) was performed using two different methods: melt blending and solvent casting. In both methods, the blends were prepared using 55g of each material. The melt blending method was carried out using a Plastograph[®] EC mixer (Brabender[®] GmbH & Co. KG, Germany) at 160°C and 40 rpm, during 5 min. Once the films produced, they were cut and placed in desiccators to avoid material degradation.

In the second method, PCL and PLA were solubilized in chloroform using two different glass containers kept under stirring (300rpm) during 8 hours. Once completely dissolved, both solutions were mixed, in order to produce the PCL/PLA blends. The mixture was placed into a petri disk to produce thin films. The solvent was slowly evaporated in a laboratory fume hood. The residual solvent was removed by vacuum drying during 24 hours. Finally, similarly to the first method, the films were cut in small pieces, and placed into desiccators to avoid material degradation.

2.2. Methods

2.2.1 Scaffold Fabrication

PCL and PCL/PLA *scaffolds* were produced using the *BioCell Printing* system (Fig. 1), being developed by the Centre for Rapid and Sustainable Product Development of the Polytechnic Institute of Leiria (Portugal). This is a novel additive biomanufacturing system that enables the integration and synchronization of the different stages of production and culture of 3D matrices with reduced manual intervention [12]. Depending on the chosen strategies (acellular or cellular *scaffolds*), a precision robotic arm transfers the 3D *scaffolds* between the construction area (zone 1) to zone 2, where they are sterilized. After sterilization, *scaffolds* are homogeneously seeded with cells using a robotic dispenser (zone 3). Finally, 3D constructs with embedded or seeded cells are cultured *in vitro* under dynamic conditions in the bioreactor (zone 4). The integration of the different stages into a single device significantly reduces the risk of contamination and increases productivity and the possibility of direct clinical application.

All *scaffolds* were produced using a lay down pattern of 0/90, pore size of $350\ \mu\text{m}$ and slice thickness of $280\ \mu\text{m}$

μm , as shown in Fig. 2. The adopted fabrication strategy is illustrated in Fig.3. PCL was processed at 80°C while PCL/PLA was processed at 180°C . In both cases deposition velocity was $17\ \text{mm/s}$.

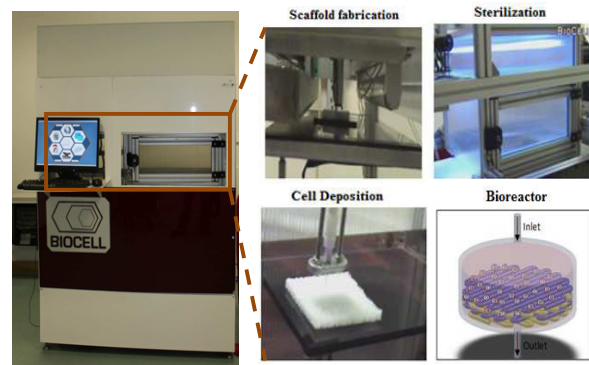


Fig. 1. Biocell Printing system.

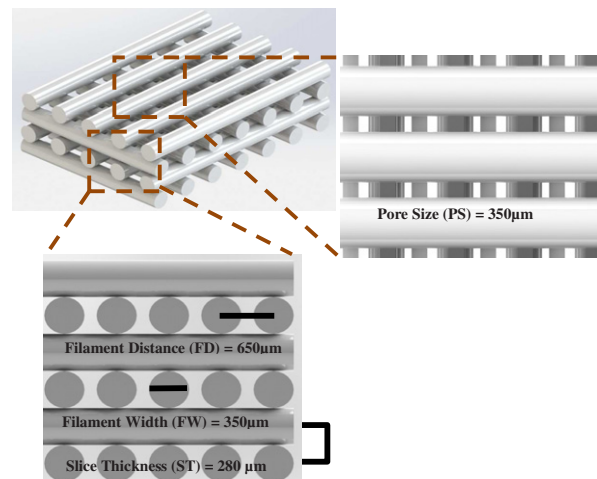


Fig. 2. Design parameters.

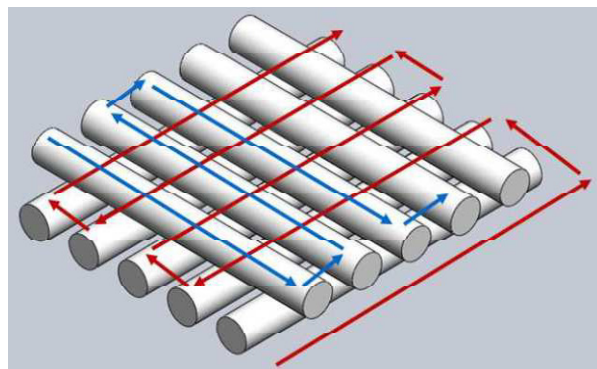


Fig. 3. Scaffold's fabrication strategy.

2.2.2 Morphological characterization

Two techniques were considered for the morphological characterization of the produced *scaffolds*: scanning electron microscopy (SEM) and atomic force microscopy (AFM).

SEM tests were performed using the FEI QUANTA 600F system (FEI Company, USA). All *scaffolds* were cut in blocks of 4.0 mm of length (l), 4.0 mm of width (w) and 8.0 mm of height (h_0), and the images were obtained from frontal face and side cutting. All dimensions were measured using the software Image J. The average and standard deviation from 10 measurements were reported for each sample group.

AFM uses a flexible cantilever as a type of spring to measure the force between the tip and the sample and was used to determine surface roughness. All measurements were obtained using the NanoWizard system (maximum scan range: 100x100 μm ; z-range: 15 μm ; spring constant: 2N/m) with TopViewOptics software (JPK instruments, Germany).

2.2.2 Biological characterisation

Biological studies were performed on PCL and PCL/PLA *scaffolds*. Two methods were considered: the Alamar Blue Assay and Alkaline Phosphatase Activity. Alamar blue was used to measure cell viability and proliferation. This is a sensitive oxidation-reduction indicator that fluoresces and changes colour upon reduction mitochondrial enzymes [15-17].

Samples were sterilized with 70% ethanol/water solution for 24 hours, washed with PBS 0.01M, pH 7.4 and exposed to U.V. light during 40 minutes. 3D *scaffolds* (5 mm \times 5 mm \times 3.36 mm) were seeded with MG-63 cells, using a density of 17×10^3 cells/sample. The number of viable cells is correlated with the magnitude of dye reduction and is expressed as a percentage of Alamar Blue reduction.

Alkaline phosphatase (ALP) is one of the most important osteoblast differentiation marker protein [18]. Alkaline Phosphatase Activity measurements were used to evaluate the osteogenic expression of MG-63 cells, since ALP is an early marker for the osteogenic cell differentiation. A density of 70×10^3 cells/sample was used and a specific biochemical assay was applied to measure ALP activity (SensoLyte® pNPP Alkaline Phosphatase Assay Kit).

3. Results and Discussion

3.1 Morphological characterisation

Fig. 4 shows the SEM micrographs of PCL and PCL/PLA *scaffolds*. From this figure it is possible to observe that all *scaffolds* present a well defined internal geometry and uniform pore distribution. The values

obtained for the pore size (PS), filament width (FW), slice thickness (ST) and filament distance (FD) are summarized in Table 1.

Results show that PCL *scaffolds* present values close to the theoretical ones (input values). In the case of PCL/PLA *scaffolds*, it is possible to observe that the method used to prepare the blends determines the geometrical characteristics of the final *scaffolds*. The differences observed for PCL *scaffolds* and PCL/PLA *scaffolds* are due to viscosity differences, caused by the higher temperature required to extrude PCL/PLA *scaffolds* (180°C).

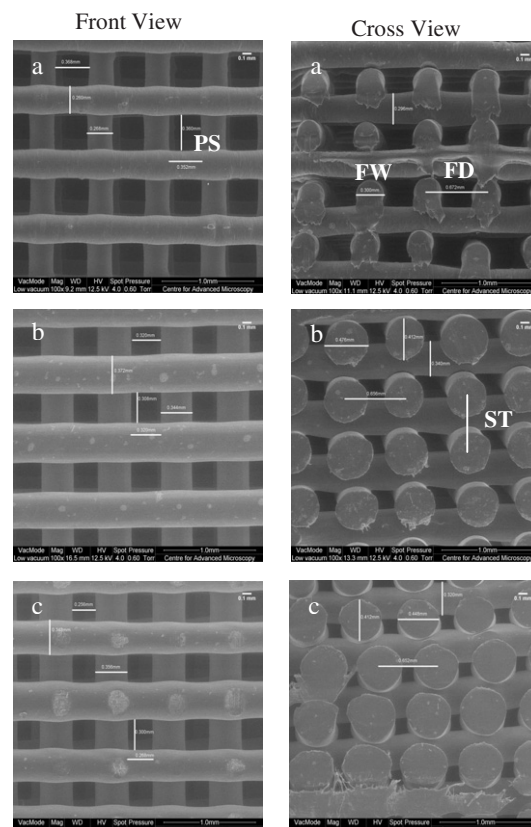


Fig. 4. SEM micrographs of the *scaffolds*. a) PCL *scaffold*; b) PCL/PLA *scaffold* - blends prepared by melt blending; c) PCL/PLA *scaffold* - blends prepared by solvent casting.

Table 1. Design parameters (average of 10 measurements with \pm standard deviation).

	Theoretical values [μm]	PCL <i>scaffold</i> [μm]	PCL/PLA <i>scaffold</i> - blends prepared by melt blending [μm]	PCL/PLA <i>scaffold</i> - blends prepared by solvent casting [μm]
PS	350	$354,4 \pm 3,9$	$311,6 \pm 17$	294 ± 12
FW	300	$310 \pm 9,5$	$414,8 \pm 12$	434 ± 27
ST	280	$263 \pm 13,7$	$288 \pm 14,8$	$367 \pm 27,7$
FD	650	$647,2 \pm 27$	678 ± 37	651 ± 10

AFM measurements (Fig. 5) show that *scaffolds* produced with blends prepared by melt blending present smooth surfaces. In this case it is also possible to observe the presence of nanoscale fibres on the surface of the *scaffold* filaments. The nanoscale topography of the scaffolds produced with blends prepared by solvent casting is favourable for cell attachment, proliferation and differentiation.

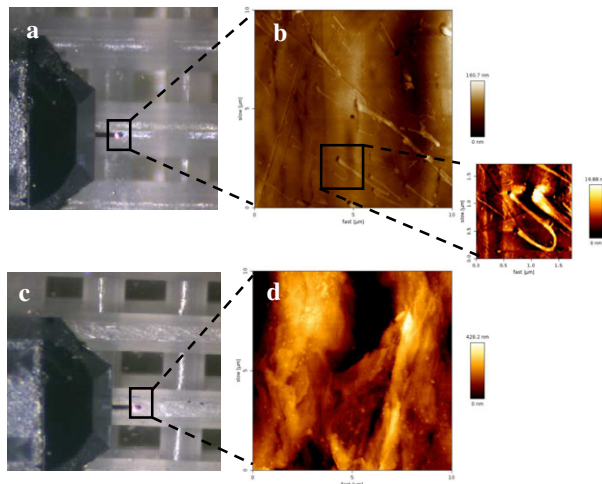


Fig. 5. Surface characteristics PCL/PLA *scaffolds*. a) Optical pictures of both the silicon cantilever and the PCL/PLA *scaffold* (blend prepared by melt blending); b) AFM micrograph of the PCL/PLA *scaffold* (blend prepared by melt blending); c) Optical pictures of both the silicon cantilever and the PCL/PLA *scaffold* (blend prepared by solvent casting); d) AFM micrograph of the PCL/PLA *scaffold* (blend prepared by solvent casting).

3.2 Biological Characterisation

Alamar Blue Assay

A preliminary biological evaluation of PCL and PCL/PLA *scaffolds* to sustain cell adhesion and proliferation was carried out using osteosarcome MG-63. Quantitative evaluation of cells was performed after 7 and 14 days of static cell culture by means of Alamar Blue. The percentage of Alamar Blue reduction is reported in Table 2.

Table 2. Results obtained from Alamar Blue™ assay at 7 and 14 days.

Scaffold	% Reduction Alamar Blue™	
	7 days	14 days
PCL <i>scaffold</i>	8.08 ± 1.06	25.80 ± 0.40
PCL/PLA <i>scaffold</i> - blends prepared by melt blending	10.18 ± 1.10	19.20 ± 9.14
PCL/PLA <i>scaffold</i> - blends prepared by solvent casting	14.34 ± 1.08	46.80 ± 3.51

These results allow us to conclude that, independently of the material, all *scaffolds* are able to sustain cell adhesion and proliferation. Additionally, it is possible to observe an increment in terms of % reduction

of Alamar Blue from day 7 to day 14, which corresponds to an increment of cell viability.

The addition of PLA into the PCL matrix clearly enhances cell adhesion and proliferation, possible due to the higher hydrophilic characteristics of PLA.

From a biological point of view, it is also interesting to note that the PCL/PLA blend preparation method has a strong influence on the adhesion and proliferation of MG-63 cells. Higher cell proliferation was observed on PCL/PLA *scaffolds* prepared by solvent casting. The low biological performance of PCL/PLA *scaffold* blend prepared by melt blending is probably due to the smooth surface of the *scaffolds* that reduces cell attachment and the heterogeneous distribution of PLA within the PCL matrix.

Alkaline Phosphatase

The osteogenic differentiation of the MG-63 cells was assessed by ALP activity (%) and is indicated in Table 3.

The significant increase of the ALP activity, from day 7 to 14, indicates cell differentiation during this period. From day 14 to 21, the osteogenic differentiation process decreases, probably due to cell colonization. These results highlight the ability of the produced *scaffolds* to allow the osteogenic differentiation of MG-63 cells, independently of the material used.

Table 3. Alkaline phosphatase (ALP) activity (%) at 7, 14 and 21 days.

Scaffold	ALP Activity (%) [ng ALP/ng DNA]		
	7 days	14 days	21 days
PCL <i>scaffold</i>	1.61 ± 0.53	9.38 ± 1.86	3.01 ± 0.99
PCL/PLA <i>scaffold</i> blend prepared by melt blending	1.91 ± 0.66	9.49 ± 1.89	6.55 ± 1.97
PCL/PLA <i>scaffold</i> blend prepared by solvent casting	1.87 ± 0.56	9.54 ± 1.91	3.45 ± 1.04

4. Conclusion

This research work investigates the use of PCL and PCL/PLA *scaffolds* produced with mixtures prepared by solvent casting and melt blending. Results show that PCL/PLA *scaffolds* present a better biological behaviour. Additionally, it was possible to observe that the blending method strongly determines the *scaffold* surface roughness and cell adhesion and proliferation. PCL/PLA *scaffolds* produced from blends prepared by solvent casting, present a reduced pore size, which also enhances cell proliferation.

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