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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 \textit{BioCell Printing}. PCL/PLA blends were prepared using melt blend and solvent casting techniques. Scaffolds with 0/90º 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 \textit{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.

Keywords: Biomanufacturing, Polymer blends, Scaffolds, Tissue Engineering

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 \textit{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 (≈60°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 (≈160°C) and glass transition temperature (≈60°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 produced 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 homogenously 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 μm and slice thickness of 280 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 de deposition velocity was 17 mm/s.
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 × 5 mm × 3.36 mm) were seeded with MG-63 cells, using a density of \(70\times10^3\) 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 \(70\times10^3\) 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.](image)

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

|                      | Theoretical values [μm] | PCL scaffold [μm] | PCL/PLA scaffold - blends prepared by melt blending [μm] | PCL/PLA scaffold - blends prepared by solvent casting [μm] |
|----------------------|-------------------------|-------------------|--------------------------------------------------------|----------------------------------------------------------|
| PS                   | 350                     | 354.4 ± 3.9       | 311.6 ± 17                                             | 294 ± 12                                                 |
| FW                   | 300                     | 310 ± 9.5         | 414.8 ± 12                                             | 434 ± 27                                                 |
| ST                   | 280                     | 263 ± 13.7        | 285 ± 14.8                                             | 367 ± 27                                                 |
| FD                   | 650                     | 647.2 ± 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).](image)

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 osteosarcoma 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.

| Scaffold                  | % Reduction Alamar Blue™ |
|---------------------------|--------------------------|
| PCL scaffold              | 8.08 ± 1.06              |
| PCL/PLA scaffold - blends prepared by melt blending | 10.18 ± 1.10 |
| PCL/PLA scaffold - blends prepared by solvent casting | 14.34 ± 1.08 |
| PCL/PLA scaffold - blends prepared by melt blending | 10.18 ± 1.10 |
| PCL/PLA scaffold - blends prepared by solvent casting | 14.34 ± 1.08 |

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.

| Scaffold                  | ALP Activity (%) [ng ALP/ng DNA] |
|---------------------------|----------------------------------|
| PCL scaffold              | 1.61 ± 0.53                      |
| PCL/PLA scaffold - blends prepared by melt blending | 1.91 ± 0.66 |
| PCL/PLA scaffold - blends prepared by solvent casting | 1.87 ± 0.56 |

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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References

[1] Lebourg, M., Suay Antón, J., Ribelles, J., 2012. Hybrid structure in PCL-HAp scaffold resulting from biomimetic apatite growth, Journal of Materials Science 21, p. 33.
[2] Melchels, F.P.W., Domingos, M.A.N., Klein, T.J., Malda, J., Bártolo, P.J., Hutmacher, D.W., 2012. Additive Manufacturing of Tissues and Organs, Progress in Polymer Science 37, p. 1079.
[3] Bártolo, P.J., Kruth, J.P., Silva, J., Levy, G., Malhe, A., Rajurkar, K., Mitsuishi, M., Curana, J., Leu, M., 2012. Biomedical production of implants by additive electro-chemical and physical processes, CIRP Annals – Manufacturing Technology 61, p. 635.
[4] Bártolo, P.J., Chua, C.K., Almeida, H.A., Chou, S.M., Lim, A.S.C., 2009. Biomonitoring for tissue engineering: present and future trends, Virtual and Physical Prototyping 4, p. 203.
[5] Domingos, M., Chiellini, F., Gloria, A., Ambrosio, L., Bártolo, P., Chiellini, E., 2012. Effect of process parameters on the morphological and mechanical properties of 3D Bioextruded poly(e-caprolactone) scaffolds, Rapid Prototyping Journal 18, p. 56.
[6] Santis, R., Gloria, A., Russo, T., D’Amora, U., Zeppetelli, S., Tampieri, A., Herrmannsdorfer, T., Ambrosio, L., 2011. A route toward the development of 3D magnetic scaffolds with tailored mechanical and morphological properties for hard tissue regeneration: preliminary study, Virtual and Physical Prototyping 6, p. 189.
[7] Liao, H.T., Chang, K.H., Jiang, Y., Chen, J.P., Lee, M.Y., 2011. Fabrication of tissue engineered PCL scaffold by selective laser-sintered machine for osteogenesis of adipose-derived stem cells, Virtual and Physical Prototyping 6, p. 57.
[8] Tan, J.Y., Chua, C.K., Leong, K.F., 2010. Indirect fabrication of gelatin scaffolds using rapid prototyping technology, Virtual and Physical Prototyping 5, p. 45.
[9] Domingos, M., Chiellini, F., Cometa, S., Giglio, E., Grillo-Fernandes, E., Bartolo, P.J., Chiellini, E., 2010. Evaluation of in vitro degradation of PCL scaffolds fabricated via Bioextrusion. Part 1: Influence of the degradation environment, Virtual and Physical Prototyping 5, p. 65.
[10] Domingos, M., Chiellini, F., Cometa, S., Giglio, E., Grillo-Fernandes, E., Bartolo, P.J., Chiellini, E., 2011. Evaluation of in vitro degradation of PCL scaffolds fabricated via Bioextrusion. Part 2: Influence of pore size and geometry, Virtual and Physical Prototyping 6, p. 157.
[11] Chen, F., Yoo, J., Atala, A., 1999. Acellular collagen matrix as a possible off the shelf biomaterial for urethral repair, Urology 54, p. 407.
[12] Bártolo, P., Domingos, M., Gloria, A., Gay, J., 2011. BioCell Printing: Integrated Automated Assembly System for Tissue Engineering Constructs, CIRP Annals-Manufacturing Technology 60, p. 271.
[13] Woodruff, M. A., Hutmacher, D. W., 2010. The return of a forgotten polymer - Polycaprolactone in the 21st century, Progress in Polymer Science 35, pp. 1217-1256.
[14] Garlotta, D., 2001. A literature review of Poly(Lactic Acid), Journal of Polymers and the Environment 9, p. 63.
[15] Hamid, R., Rotstein, R., Rabadi, L., Parikh, R., Bullock, P., 2004. Comparison of alamar blue and MTT assays for high through-put screening, Toxicology in Vitro 18, p. 703.
[16] O’Brien, J., Wilson, I., Orton, T., Pogran, F., 2000. Investigation of the alamar blue (resazurin) fluorescent dye for the assessment of mammalian cell toxicity, European Journal of Biochemistry 267, p. 5421.