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Mechanical and Biological Behaviour of PCL and PCL/PLA *Scaffolds* for Tissue Engineering Applications

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A new biomanufacturing system allowing to produce three-dimensional matrices (*scaffolds*) with well defined internal geometries, uniform pore distribution and good adhesion among different adjacent layers. Polymers selected are Poly ε-caprolactone (PCL) and Poly Lactic Acid (PLA), both these polymers are used in medical applications. These two polymers are interesting biomaterials because they are complementary on their physical properties and biodegradability. This work aims to assess the temperature evaluation during the extrusion process and the influences of the temperatures on the PCL and PCL/PLA *scaffolds* with lay down pattern 90º and pore size 350µm. The results demonstrated that extrusion process not modified the thermal properties of the *scaffolds* and these structures are able to sustain MG-63cells.

1. Introduction

The tissue engineering is an interesting multidisciplinary field, which allows to constructs 3D structures with different materials, for regenerate, restore or maintain the tissue or a whole organ function (Chen, *et al*. 1999; Shi, *et al.* 2010). In tissue engineering used a 3D structures called, *scaffolds*, that promote the regeneration of the tissue, because it have interconnective pores, that is important for guided cell proliferation and controls the shape of the bioartificial device (Kellomaki, *et al.* 2003).
The *scaffold* material can be varying between natural material (example chitosan, collagen) and synthetic material (example Poly ε-caprolactone (PCL), Poly Lactic Acid (PLA), Poly Glycolic Acid (PGA)) (Dell'Erba, *et al.* 2001).
In this research work was used two different polyester aliphatic polymers, PCL and PLA. The use of these polymers is an advantage in terms of material reproducibility, good processability and the information (Chen, *et al.* 2012), that there are of the material behaviour in the human body (Liu, *et al.* 2009). These two polymers have attracted increasing attention in pharmaceutical and medical fields such as sutures, artificial skins, bone fracture internal fixation devices, tissue engineering *scaffolds* and drug delivery system (Liu, *et al.* 2009).
The main aim of this research work is study the extrusion process and the influence of the temperature in the polymers properties through thermal, morphological, mechanical and biological properties.

2. Materials and Methods

2.1 Materials

The melt blending process was used for produce PCL/PLA blend. The PCL used in this research work was Capa 6500 (Perstorp, UK), and PLA was PLA 2002D (Cargill Down, USA).

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2.2 Physical blending

The blending process of PCL and PLA (50/50 wt%) was performed using melt blending, the blend was prepared using 55g of each material and it was carried out using a Plastograph® EC mixer (Brabender® GmBH & Co. KG, Germany) at 160 ºC and 40 rpm, during 5 min.

2.3 Methods

2.2.1 *Scaffold* Fabrication

A novel biomanufacturing process, *BioCell Printing* (Figure 1), was used for fabrication of 3D polymeric *scaffolds*, with the blends, being developed by the Centre for Rapid and Sustainable Product Development of the Polytechnic Institute of Leiria (Portugal) (Bártolo, *et al.* 2011).

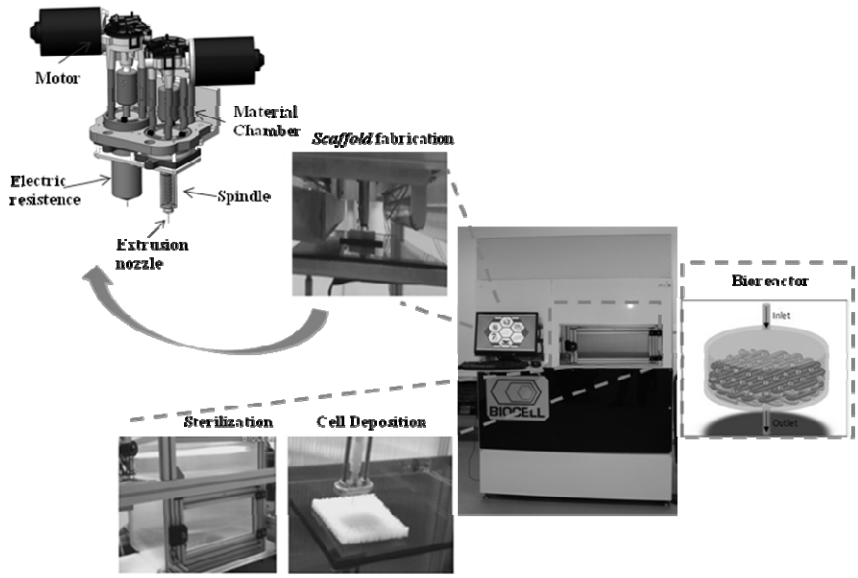

Figure 1: Biocell Printing System and different integration zones; Zone 1 - scaffold fabrication; Zone 2 - sterilisation; Zone 3 - cell deposition; Zone 4 - bioreactor.

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 (Bártolo, *et al.* 2011). 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.
In the Table 1 is possible observe the design and process parameters for produce PCL and PCL/PLA *scaffolds*.

Table 1: Design and Process Parameters.

Materials	**Design Parameters**	**Process Parameters**		
		Temperature	**Screw Rotation velocity**	**Air Pressure**
PCL **PCL/PLA (blends prepared by melt blending)**	Lay-Down Pattern = 0/90º Pore size = 350 µm Deposition velocity = 17mm/s	80ºC 180ºC	45rpm 26,25rpm	4 bar

2.2.2 Thermography characterisation of the extrusion process

Thermography is an excellent way to objectively diagnosis the variation of temperature during the extrusion process. And also is important for analyses if the temperatures input in the extrusion process are

the same when the extruder fabricates the scaffolds. The equipment used in this characterisation called FLIR T335 (FLIR Systems, Inc., UK).

2.2.3 Thermal characterisation

The thermal stability of both processed and non-processed material was investigated using a thermogravimetric analyzer, TA Instruments Q500 (TA Instruments, USA), with a thermobalance sensitivity of 0.1 µg. The temperature calibration was performed in the range of 25-1000ºC by measuring the Curie point of nickel standard, using open platinum crucibles, a dry nitrogen purge flow of 100mL min^{-1}, and a heating rate of 5ºC min^{-1}. At least two runs were performed for each sample (sample weights of ca. 8 mg) in order to check the repeatability of measurements.

2.2.4 Morphological characterisation

The morphological analysis allows to observe the *scaffold* surface through Scanning Electron Microscopy (SEM). 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. SEM images allow observing the *scaffold* surface morphology and measuring the pore size (PS), filament distance (FD), filament width (FW) and slice thickness (ST). All dimensions were measured using the software Image J. The average and standard deviation obtained from 10 measurements were reported for each sample group, as suggested by Zein and co-authors (2002).

2.2.5 Mechanical characterisation

Compression tests were performed on both PCL and PCL/PLA *scaffolds* to evaluate the effect of both the addition of PLA and the preparation method used to produce the PCL/PLA blends. All tests were carried out using *scaffolds* (4x4x8mm) in dry state, at a rate of 1 mm/min up to a strain value of 0.5 mm/mm, through an INSTRON 5566 testing system equipped with a 1 kN load cell. The "apparent" stress was evaluated as a force ***F*** measured through the load cell divided by the total area ***A*** of the apparent cross section of the *scaffold*. The strain ***ε*** was defined as the ratio between the *scaffold* height variation ***Δh*** (the vertical displacement equal to the crosshead displacement) and the *scaffold* initial height $\boldsymbol{h_0}$.

2.2.6 Biological Characterisation

Quantitative evaluation of cell viability and proliferation was evaluated using Alamar Blue™ Assay. The 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 x 5 mm x 3.36 mm) were seeded with MG-63 cells, using a density of $17x10^3$ cells/sample. The number of viable cells can be correlated with the magnitude of dye reduction and is expressed as a percentage of Alamar Blue™ reduction.

3. Results and Discussion

3.1.1 Thermography characterisation of the extrusion process

The thermography allows to observe the variation of temperature during the extrusion process (80ºC for PCL and 180ºC for PCL/PLA). The Figures 2a and 2b demonstrate that in the two situations do not occurs significant temperature differences between different extruder zones. This result are important because is possible to know that the materials didn't suffer temperature differences and also when were produce scaffolds for tissue engineering applications, the materials must be the original properties.

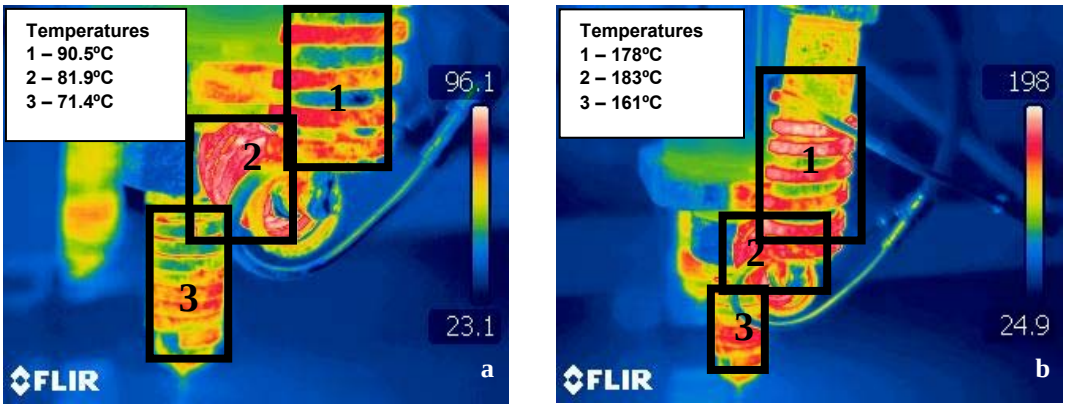

Figure 2: Thermography of the extrusion process during the PCL scaffold (a) and PCL/PLA scaffold fabrication (b).

3.1.2 Thermal characterisation

The thermal stability of the blends was analysed by thermogravimetry. This study is extremely relevant considering that the polymers are subjected to high temperatures, during the *scaffold* fabrication process. It is important to avoid any degradation phenomena, during the *scaffolds* fabrication due to the processing temperature. The degradation effects of temperature for both non-processed and processed materials are presented in Table 2.

Results show that the degradation temperature (T_d) varies between 300 ºC and 400 ºC, showing that no degradation events occur during the *scaffold* fabrication, since the maximum temperature was 180º C. The results show a similar behaviour between raw materials and the blends, suggesting that no chemical transformations occur during the mixing process. The degradation profiles of PCL/PLA blend show two degradation stages, corresponding to the PCL degradation (≈ 380 ºC) and PLA degradation (≈ 320 ºC). Thermogravimetry is also important to evaluate possible material characteristic changes due to the extrusion process. In the case of melt blending materials, PCL and PLA are exposed to a double heating process, the melt blending mechanism and the heat extrusion process. From these results, it is possible to conclude that the extrusion process does not influence the chemical properties of the samples.

Table 2: Degradation temperatures of non-processed and processed materials.

Samples	Degradation Temperatures (ºC)
Non processed PCL	375,48
Non processed PLA	329,60
Processed PCL	365,95
Non-processed PCL/PLA (blends prepared by melt blending)	PLA - 332,72 PCL - 383,37
Processed PCL/PLA (blends prepared by melt blending)	PLA - 309,32 PCL - 384,82

3.1.3 Morphological characterisation

Figure 3 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 3. 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* (180ºC).

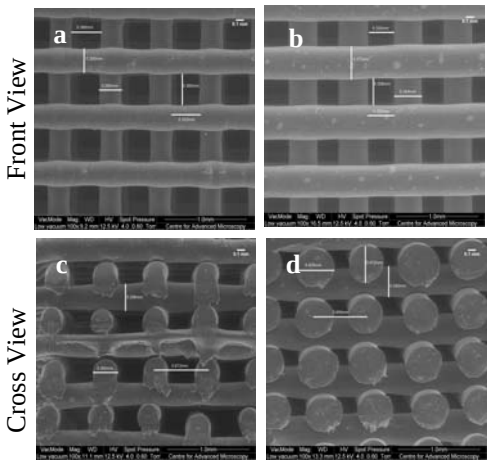

Figure 3: SEM micrographs. Top section views: a) PCL scaffold; b) PCL/PLA scaffolds (blends prepared by melt blending); Cross-section views: d) PCL scaffold; e) PCL/PLA scaffolds (blends prepared by melt blending).

Table 3: Design parameters (average of 10 measurements with ± standard deviation).

	Theoretical values [µm]	**PCL *scaffold*** [µm]	**PCL/PLA *scaffold* - blends prepared by melt blending [µm]**
PS	**350**	354,4 ± 3,9	311,6 ± 17
FW	**300**	310 ± 9,5	414,8 ± 12
ST	**280**	263 ± 13,7	288 ± 14,8
FD	**650**	647,2 ± 27	678 ± 37

3.1.4 Mechanical characterisation

Compression tests highlight that the mechanical behaviour of both PCL and PCL/PLA *scaffolds* and flexible foams are similar (Kyriakidou *et al.*, 2008). Compressive modulus and maximum stress values are reported in Table 4 for both PCL and PCL/PLA *scaffolds*. The addition of PLA strongly enhances the performance of PCL *scaffolds* under compressed loads, as expected due to the high mechanical properties of PLA.

Table 4: Mechanical properties of the scaffolds.

Scaffolds	Compressive Modulus E (MPa)	Maximum Stress σ_{max} (MPa)
PCL	18.7 ± 3.0	4.4 ± 0.4
PCL/PLA (blends prepared by melt blending)	146.5 ± 14.0	18.5 ± 1.3

3.1.5 Biological characterisation

A preliminary biological evaluation of PCL and PCL/PLA *scaffolds* was carried out using osteosarcome MG-63, to sustain cell adhesion and proliferation. A quantitative evaluation of cells was performed after 7 and 14 days of static cell culture, using the Alamar Blue™. Results are reported as mean value ± standard deviation in Figure 4 as a percentage of Alamar Blue™ reduction.

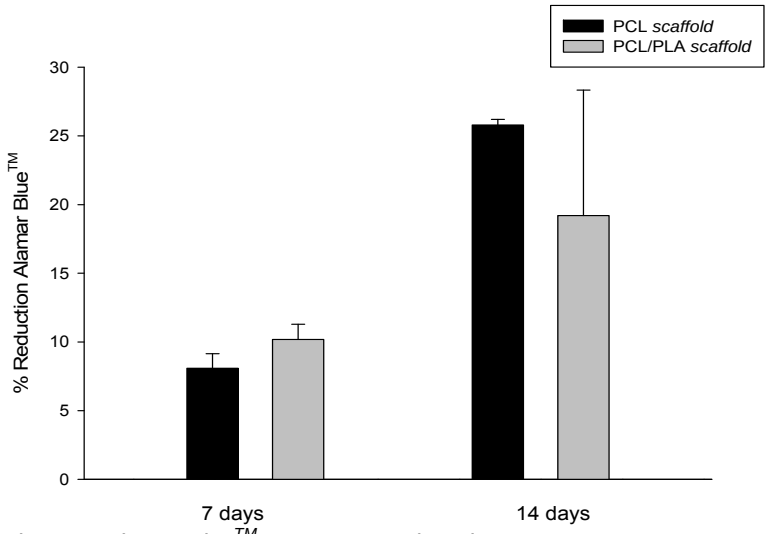

Figure 4: Alamar Blue™ assay at 7 and 14 days.

These results, allow us to conclude that, regardless of the material, all *scaffolds* are able to sustain cell adhesion and proliferation. It is also possible to observe an increase 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 hydrophilic characteristics of PLA. PLA is more hydrophilic than PCL (Dash and Konkimalla, 2012; Ahmed and Discher, 2004), so a more uniform distribution of PLA induces a more uniform degree of hydrophilicity, which enhances cell attachment and proliferation.

4. Conclusion

This research work investigates the PCL and PCL/PLA *scaffolds* produced by Biocell Printing. The thermography characterisation reveals that the temperatures into the extruder didn't vary significantly, this is an important result when produce scaffolds for tissue engineering applications. In terms of thermal characterisation of the scaffolds it is possible observe that the extrusion process not influence the thermal properties.

Results also show that PCL/PLA *scaffolds* blend prepared by melt blending present a better mechanical and better biological behaviour, compared with PCL *scaffolds*.

Acknowledgements

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