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Comparison of three-dimensional extruded poly (ε-caprolactone) and polylactic acid scaffolds with pore size variation

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Abstract

Additive manufacturing (AM) has become a prominent approach among the scientific community for the production of three-dimensional (3D) matrices able to support tissue engineering approaches, promoting cell adhesion, proliferation and organization aiming to repair different tissues, such as bone or cartilage. In this study we used an extrusion-based technique for the production of poly (ε-caprolactone) (PCL) and polylactic acid (PLA) scaffolds and performed a side-by-side scaffold characteristics comparison. Using this technique we were able to create fully 3D interconnected porous scaffolds with pore size variations ranging from 190 µm to 390 µm with both materials. These scaffolds were assessed for stiffness, wettability and cell adhesion using mesenchymal stem/stromal cells (MSC). Comparisons between these two materials were made. The compressive modulus obtained is on the same order of magnitude for both materials. However, PCL presents a statistically significant higher compressive modulus. Results confirmed that PCL is a more hydrophobic material, so it presents a lower wettability when compared to PLA. Interestingly cell adhesion is similar for PLA and PCL, therefore selection between these two materials for the use of this versatile platform can be defined according with biodegradability aimed.

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Keywords: Mesenchymal stem/stromal cells; Poly (ε-caprolactone); Polylactic acid; Scaffolds

Nomenclature

3D	Three-dimensional
AM	Additive Manufacturing
MSC	Mesenchymal Stem Cells
PCL	Poly (ε-caprolactone)
PLA	Polylactic Acid
TE	Tissue Engineering

1. Introduction

Materials used as support matrices for Tissue Engineering (TE) should fulfill some biological and mechanical requirements [1]. As mechanical requirements the matrices should present high porosity and interconnectivity, present adequate mechanical properties and superficial finishing and biological requirements should include material biocompatibility, biodegradability, be able to provide biochemical recognition elements and present an adequate environment for cell adhesion and proliferation [2-4].

This work involve the comparison of two different materials, poly(ε-caprolactone) (PCL) and polylactic acid, scaffolds produced by an Additive Manufacturing (AM) process named extrusion [5,6]. These two materials have different hydrophobicity and biodegradability. This technique is a layer-by-layer technique which involves the heating of the material until it reaches the melting temperature and liquefies and then, it is extruded by a nozzle in a form of fibre [7].

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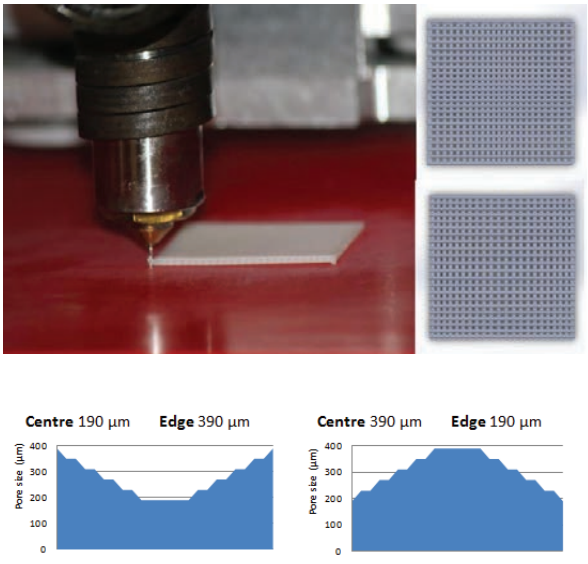
Using this technique we are able to create scaffolds with different configurations, varying the pore sizes which influence the porosity of the matrices produced. So, scaffolds with a gradient of pore sizes were produced with the two materials. They were assessed for mechanical properties, such as stiffness, contact angle and, also, for biological properties, cell adhesion was performed using bone marrow (BM) human mesenchymal stem/stromal cells (MSC). MSC are adult tissue-derived multipotent cells with the capacity to differentiate into osteogenic, chondrogenic, adipogenic, and myogenic lineages [8].

2. Materials and Methods

2.1. Production of scaffolds

Scaffolds of Poly (ε-caprolactone) (PCL), Mw 50,000 Da, and Polylactic Acid (PLA) (MakerBot) were produced by extrusion, an AM process. In this process, the material is heated until the melting temperature and then in extruded by a nozzle following a layer-by-layer approach (Fig. 1).

(a)



(b)

Fig. 1 Extrusion process: (a) production of the scaffolds; (b) squematic representation of the two configurations adpoted.

In order to produce the scaffolds, the following properties of the machine were adjusted to obtain an effective extrusion: deposition velocity 20 mm/s; slice thickness 280 µm; and nozzle diameter 300 µm. To extrude PCL a temperature of 80˚C were used and to extrude PLA we used 230˚C.
Different samples were produced and tested for stiffness, hydrophobicity/hydrophilicity and cell adhesion. Samples produced for these assessments presented pore size variations, between 190 and 390 µm, following two different configurations: (a) pore size increases from the centre to the edge and (b) pore size decreases from the centre to the edge, as in a previous work [9].

2.2. Mechanical testing

Mechanical compression tests to the produced scaffolds were performed using a universal testing machine from Instron (model 5544) equipped with a load cell of 2 kN and the extension rate of 1 mm/min. The results of the tests were processed with the use of Bluehill® 3 software. Compressive stress was defined as the compressive load per unit area of minimum original cross section carried by the test scaffold at any given moment, being the compressive strength defined as the maximum compressive stress carried by a test specimen. The compressive modulus of elasticity was calculated by the slope of the initial linear portion of the stress-strain curve, being the compressive strain defined as the change in length per unit of original length along the longitudinal axis.

2.3. Wettability

Wettability is measured by the contact angle, which is defined as the angle formed by the intersection of the liquid-solid interface (Fig. 2). When the angle (α) is below 90˚C the material is considered hydrophilic, above 90˚C is hydrophobic. If the angle is greater than 150˚C is considered super-hydrophobic [10].

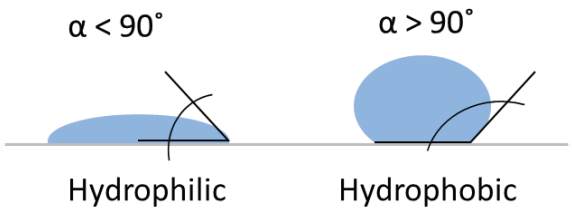


Fig. 2 Contact Angles formed by sessile drops on a solid surface.

To perform the contact angle for each configuration a DSA25B goniometer (Krüss) was used. A sessile drop was added to the top of the scaffolds in the 190 µm and 390 µm pore size and it was analysed by Drop Shape Analysis 4 (version 2.1) software at 0 sec and every 5sec until 30 sec. These measurements were done in triplicates for both PCL and PLA scaffolds.

2.4. Cell Adhesion

Human BM-derived MSC were recovered from cryopreservation [11] and cultured in culture medium consisting of low-glucose Dulbecco's modified Eagle's medium (DMEM, Gibco®), 10% fetal bovine serum (FBS, Hyclone®), and 1% penicillin/streptomycin and fungizone (PS, Gibco®). Culture medium was replaced every 3 days.

PCL and PLA scaffolds were sterilized with 70% ethanol (v/v) (Merck) and UV light overnight and placed on an ultra-low attachment 6-well plate (VWR).

To understand the influence of the material, PCL and PLA, in cell adhesion as well as the size of pores, 8.0 x 10^4 cells/scaffold were placed on the top centre of the scaffold for

both configurations, ensuring that the cells were seeded on a region with the same pore size. Cells were left to incubate for 1 hour in order to allow cell adhesion; then culture medium was added to immerse the scaffold. Experiments were performed under static conditions in an incubator under controlled atmospheric conditions (37ºC, 5% CO_2 and 20% O_2).

Cell adhesion was performed after 24h of incubation and estimated using an indirect method, Alamar-Blue™ (AB) (Invitrogen), where Resazurin is reduced to the bright red–fluorescent Resorufin form by metabolic active cells. AB solution was diluted in DMEM supplemented with 10% FBS solution (1:10) and cells were incubated in this solution for 2 hours. Fluorescence of the samples was measured using an Infinite 200 PRO (TECAN) multiplate fluorometer with 560 nm of excitation wavelengths and 590 nm of emission wavelengths. Three readings for each sample were taken. Equivalent cells number was estimated through a calibration curve that correlates cell counts against AB estimations. Three scaffolds of each configuration were seeded in parallel.

2.5. Statistical analysis

Statistical analysis was performed using the statistical analysis features of Microsoft Excel and GraphPad Prism. Triplicates were used for each assay, except for the mechanical testing in which duplicates were used.

3. Results and discussion

3.1. Production of scaffolds

Samples produced present 15 x 15 x 3 mm with different pore sizes (Fig. 3): (a) increase from the centre to the edge and (b) and decrease from the centre to the edge. The basis (in x and y axis) of the pore ranges from squares with sides of 190 µm to 390 µm, with increments of about 40 µm every two pores in each side of the scaffold. This increment scheme is valid for all scaffold regions except for its centre, which comprises a region of 2 mm with fibres equally spaced [9].

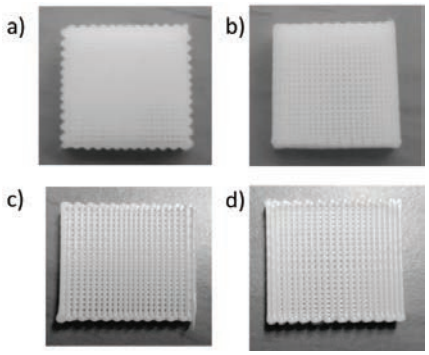


Fig. 3 Produced scaffolds: a) PCL scaffolds with smaller pore size in the centre (390 – 190 – 390 µm); b) PCL scaffolds with higher pore size in the centre (190 – 390 – 190 µm); c) PLA scaffolds with smaller pore size in the centre (390 – 190 – 390 µm); PLA scaffolds with higher pore size in the centre (190 – 390 – 190 µm).

As shown in Fig. 3, using the extrusion process it is possible to produce 3D highly interconnected porous scaffolds of PCL and PLA materials with pores as small as 190 µm.

3.2. Mechanical testing

The resultant PCL and PLA compressive stress-strain curves are present in Fig. 4. Both scaffolds present a clear linear region, corresponding to the elastic behaviour. To observe a plastic region, a higher load should be used in these tests. While for higher stresses PCL seems to tend to the plastic region, PLA clearly stays in the elastic region within the range of values tested. The compressive modulus (E) values obtained for the different scaffolds are of the same order of magnitude, (Table 1), but the value for PCL is statistically significant higher than the value for PLA ($p<0.06$).

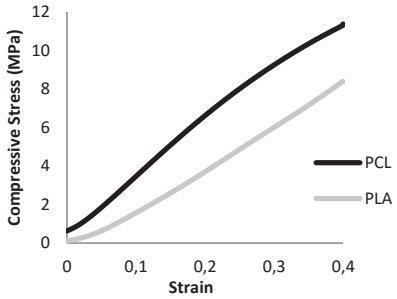


Fig. 4 Stress-strain curve of the PCL and PLA scaffolds.

Table 1. Compressive mechanical properties of PCL and PLA scaffolds.

Material	Compressive Modulus E (MPa)	Compressive Stress (MPa)
PCL	31.88 ± 1.03	11.4 ± 0.1
PLA	26.27 ± 1.09	8.4 ± 0.4

3.3. Wettability

Measuring the contact angle of the materials it is possible to understand the hydrophilicity/hydrophobicity of the PCL and PLA material. We hypothesized that the differences in hydrophilicity for these two materials, in combination with the use of different pore sizes, could impact on cell behaviour. Results obtained are summarized in Fig. 5. According to those results it is possible to see that PCL scaffolds present higher contact angle, when compared to PLA scaffolds (approximately 24-28˚ more in the 190 µm and 16-21˚ more in the 390 µm). It is also possible to see that in the case of PCL, contact angle (t=0) are slightly lower than contact angle (t=30). This is true for both pore sizes, and in the case of PLA the opposite situation is observed. Interestingly, scaffolds with higher pore size result in higher contact angle for both materials. The measured contact angles were definitely affected by surface configuration. Nevertheless, the results

obtained confirm PCL as a more hydrophobic material than PLA. Therefore, PCL present a lower wettability potential.

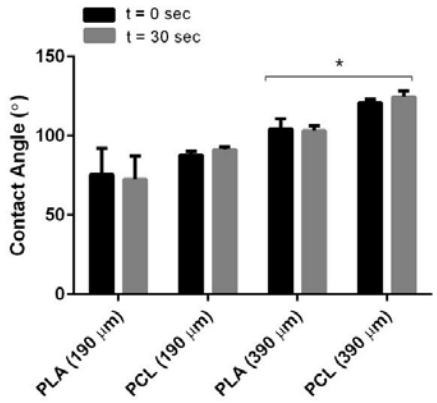


Fig. 5. Contact Angle measurements to PCL and PLA scaffolds.

3.4. Cell Adhesion

Results obtained for cell adhesion are summarized in Table 2, with cell numbers estimated indirectly as described in the previous section in biological triplicates (from the same donor). PLA scaffolds promoted a slightly higher cell adhesion when compared to PCL scaffolds. Cell adhesion seems to be similar for the two PLA configurations. The lowest cell adhesion was observed for PCL with cell seeded at the centre with lower pore size, which can be a result of a combination of low pore size and high hydrophobicity at the cell seeding site.

Table 2. Cell Adhesion to scaffolds (average ± SE).

Material	Configuration	Cell Adhesion (x10^4 cells)
PCL	190-390-190	1.70 ± 0.20
	390-190-390	1.55 ± 0.40
PLA	190-390-190	1.68 ± 0.03
	390-190-390	1.77 ± 0.04

Comparing the results obtained for the contact angle with cell adhesion to the scaffolds, it was expected that PLA present a better cell adhesion compared to PCL, since this last one present a more hydrophobic nature. However, the results obtained are equal in terms of cell adhesion obtained for the same materials (PCL vs. PLA 190-390-190), when cells are seeded at a centre with higher pore size, and only slightly higher for the PLA scaffolds when cells are seeded in the smaller pore region. Therefore, we can conclude that, within the range of pores used, cell adhesion efficiencies are fairly similar for both materials.

4. Conclusions

In this work highly interconnected porous PCL and PLA scaffolds with a spacing gradient were successfully produced by a layer-by-layer technique called extrusion. These scaffolds were then tested for stiffness, hydrophilicity/hydrophobicity and cell adhesion of BM MSC. Whereas compressive modulus is on the same order of magnitude for both materials, PCL presents a statistically significant higher compressive modulus. PCL appears as a more hydrophobic material when compared to PLA.
This work shows successful adhesion of BM MSC to/in the developed scaffolds. Comparative results show similar levels of adhesion both configurations being the cell adhesion to PCL slightly lower when compared to PLA when cells are seeded in the region with smaller pores. This corresponds to the expected results, since PCL present a more hydrophobic nature.

Acknowledgements

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