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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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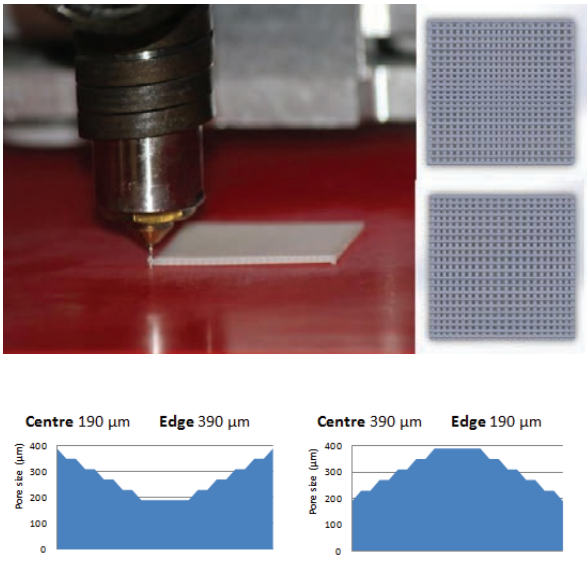
Using this technique we are able to create scaffolds with different configurations, varying the pore sizes which influence the porosity of the matrices produced. So, scaffolds with a gradient of pore sizes were produced with the two materials. They were assessed for mechanical properties, such as stiffness, 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].

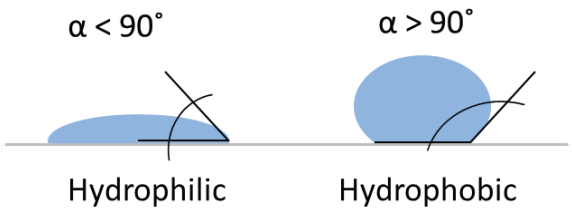


Fig. 2 Contact Angles formed by sessile drops on a solid surface.

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