

A Novel Biomanufacturing System to Produce Multi-Material Scaffolds for Tissue Engineering: Concept and Preliminary Results

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Keywords: Tissue engineering, Biomanufacturing, Bioprinting, Micro-extrusion, Heterogenous scaffolds.

Abstract. This research work aims to validate a new system that enables the fabrication of multi-material 3D structures using poly(ϵ -caprolactone) and sodium alginate for potential use in Tissue Engineering applications. To produce multi-material scaffolds for Tissue Engineering, accurate techniques are needed to obtain three-dimensional constructs with clinically appropriate size and structural integrity. This paper presents a novel biomanufacturing system which can fabricate 3D scaffolds with precise shape and porosity, through the control of all fabrication modules by an integrated computational platform. The incorporation of a clean flow unit and a camera makes it possible to produce scaffolds in a clean environment and provides a monitoring tool to analyse constructs during the production, respectively.

Introduction

The emergence of novel and advanced tailored regenerative approaches to improve human life expectancy and well-being has been receiving tremendous attention. Tissue Engineering (TE) is the major technique in regenerative medicine that aims toward the development of biological substitutes that mimic anatomical and functional features of native tissues, mitigating the critical shortage of donor tissues and organs. Their strategies can be divided into two major categories: 1) cell-based and 2) scaffold-based approaches. The classic tissue engineering approach attempts to develop a structure support (scaffold) that replicates the natural three-dimensional (3D) environment, i.e., the extracellular matrix (ECM) for cells adhesion, proliferation and differentiation to obtain the tissues or organs that can maintain their specialized configurations and their morphologies [1–3]. The scaffolds overall goal is to directly influence cells, support cell signaling and degrade in a controllable, non-toxic manner. It is fundamental that they present high porosity and pore interconnectivity and their pore size should match the ones of the target tissues in order to provide enough space for cells to migrate and to promote proper tissue vascularization [4–6]. A broad variety of biomaterials and techniques have been investigated and tested to develop 3D scaffolds. Recent studies have demonstrated that scaffold development is significantly dependent on diversified biomanufacturing systems and technologies [5,7,8].

A challenge for TE is to produce 3D, vascularized cellular constructs of clinically relevant size, shape, surface morphology and structural integrity. In this context 3D printing technology has been revealing massive and promising advancements for creating complex tissue constructs. 3D bioprinting has been demonstrating major potential since it enables layer-by-layer precise positioning of biological materials, biomolecules and living cells, with spatial control of the functional components placement. This technique offers plenty of opportunities for product and process innovation, and is often touted to ‘revolutionize’ today’s manufacturing operations and its associated supply chains structures [9–13].

In the last few years, a huge development has been achieved in the field of TE, particularly in the branch of 3D biomanufacturing technologies. The incessant demand for mimicking the complexity of human tissues led to the development of overwhelming amount of TE strategies and consequently of alternative fabrication approaches. Recent progress in scaffold fabrication has propelled the field of TE toward higher goals. Motivated by the evolution of technology, continuous innovations have been carried out and as a result, new and different 3D (bio)printers have emerged [14,15]. One main feature of an optimal system, would be to combine micro and nano filaments, with laden-cells hydrogel [16]. This would promote a significant enhancement for mimicking the structures of human native tissues.

BioMaTE is a brand new biomanufacturing system that encompasses with this scenario adding some value to this growing area. As a follow-up of our previously described system [17], it is composed by three different fabrication modules (thermoplastic micro-extrusion, multi-head deposition of hydrogels and electrospinning) allowing the combination of these different techniques for the construction of functionally graded scaffolds with well-defined architectures. Apart from the fabrication modules that enables the production of multi-material constructs, BioMaTE equipment will also integrate 1) a monitoring module which allows the supervise of manufacturing process in real time, 2) a photopolymerization module composed by one LEDs array, and 3) a laminar flow module to provide a clean environment on fabrication area (Fig. 1).

The objective of this work was to evaluate the BioMaTE biomanufacturing system for fabrication of heterogeneous 3D scaffolds through the combination of thermoplastic micro-extrusion system and multi-head dispensing of hydrogels. Poly(ϵ -caprolactone) (PCL) and sodium alginate (SA) were selected to obtain 3D multi-material constructs and morphological and mechanical tests were performed.

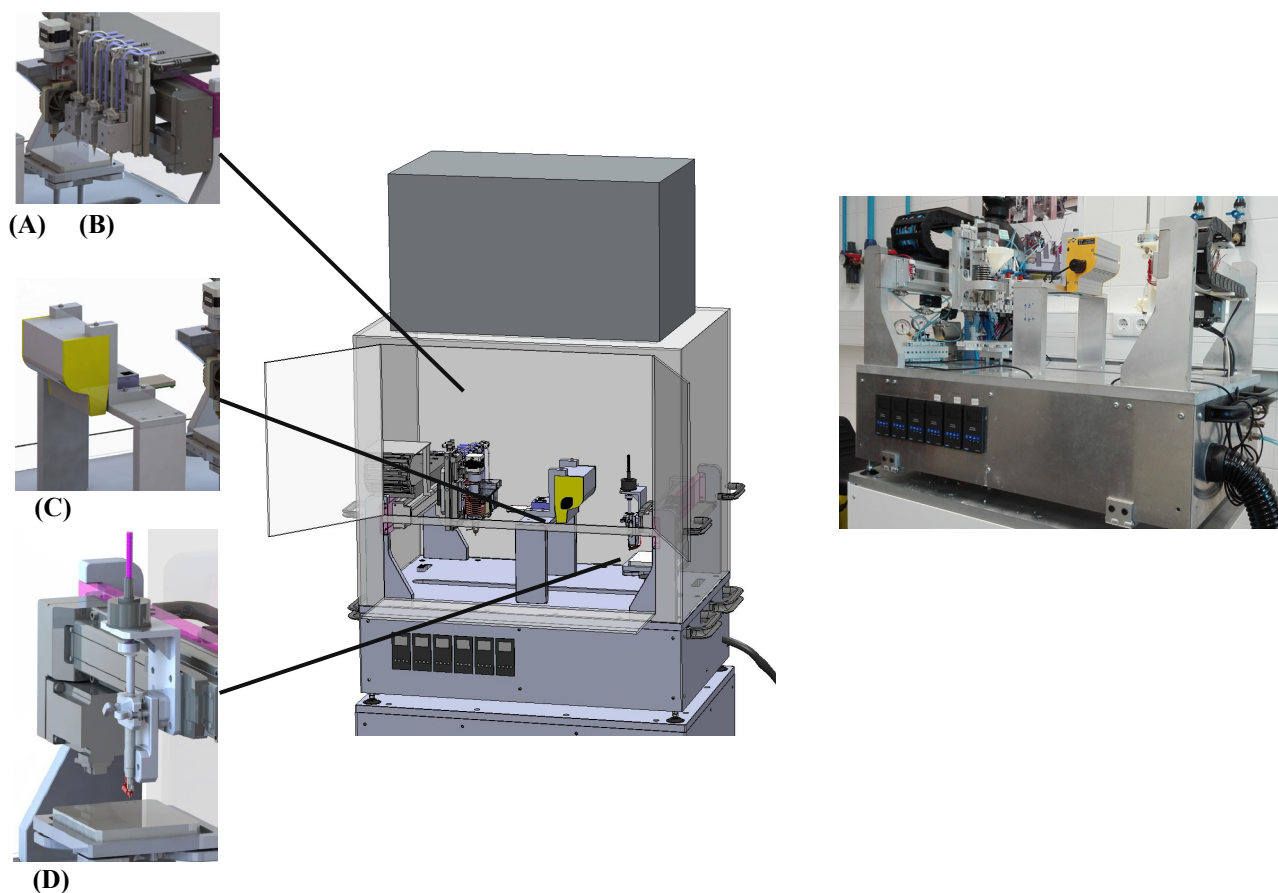


Figure 1 The BioMaTE equipment consists in the following units: A) micro-extrusion system B) multi-head dispensing module, C) Monitoring and photopolymerization modules, D) electrospinning system.

Materials and Methods

Materials. PCL polymer (CAPA® 6500, Mw: 50,000 Da) was purchased from Perstorp Caprolactones (Cheshire, UK). SA solutions were prepared by dissolving 6% and 7% w/v SA (PROLABO BDH: 27660.296) in deionized water, and crosslinking solution was prepared by dissolving calcium chloride di-hydrate ($\text{CaCl}_2 \cdot \text{H}_2\text{O}$) (Honeywell Fluka; Mw 147.01 g/mol) at a concentration of 0.6 M in deionized water to obtain a hydrogel.

Scaffolds: design and fabrication. Multi-material constructs with dimensions of 15 mm x 15 mm were produced combining PCL with SA hydrogel. The adopted parameters to obtain the multi-material scaffolds, switching between the steps of synthetic polymer deposition and hydrogel printing are indicated in Table 1.

Table 1 Process parameters used to produce PCL/SA scaffolds.

Process parameters	PCL	SA
Deposition velocity	500 mm/min	
Screw rotation velocity	11 rpm	-
Liquefier temperature	90°C	Room temperature
Nozzle diameter	400 μm	
Reservoir pressure	5 bars	3 and 5 bars
Filament distance	1300 μm	
Lay-down pattern	0/90°	

After the production of scaffolds with 2 and 8 layers, the SA was crosslinked with CaCl_2 (0.6M).

Morphological Analysis. The surface morphology of the scaffolds was examined by optical microscopy (Daffodil MCX100, Micros Austria) at a magnification of 40x. Additionally, micro-computed tomography (Micro-CT) scans of the scaffolds were performed using a SkyScan microtomograph model 1174 by Brucker Company (Brussels, Belgium). The CT system was operated with a rotation step of 0.7 degrees, voltage of 50 kV, exposure time of 3300ms, and a current of 800 μA with a nominal resolution of 19.61 $\mu\text{m}/\text{pixel}$. The micro-CT analysis allowed the visualization of the internal and external morphologies of the samples. The reconstructed set of slices was viewed in SkyScan CTvox program.

Mechanical Analysis. Compression tests were performed to evaluate the mechanical properties of the heterogeneous scaffolds. The tests were conducted according to ASTM standards, using a ZWICK Z100, with a cross-head displacement speed of 1 mm/min and a maximum deformation to 2 mm. Mechanical testing was carried out using scaffolds samples in the dry state, with a length of 15 mm, a width of 15 mm, and a height of 4 mm. Stress-strain data were computed from load displacement measurements and the compressive modulus (E) was determined from the elastic region of the obtained curves.

Results and Discussion

Morphological Analysis of multi-layer heterogeneous scaffolds. Our preliminary studies with the new 3D printing system were with the micro-extrusion and multi-head dispensing systems. 3D constructs of two and eight layers were produced and after printing, droplets of CaCl_2 solution were deposited on all the samples to crosslink the SA.

Optical micrographs were obtained to analyse the surface of the 3D constructs. Fig. 2 and 3 demonstrate that scaffolds with two layers present good control over the geometric parameters of the filaments and pores (Fig. 2 A and C), as well as scaffolds with eight layers and SA 6%. On the other hand, structures with higher SA concentration have significant difficulty to maintain geometric precision (Fig. 2 D), this behaviour can be a consequence of ionic crosslinking and reduced control of Ca^{2+} along the filaments.

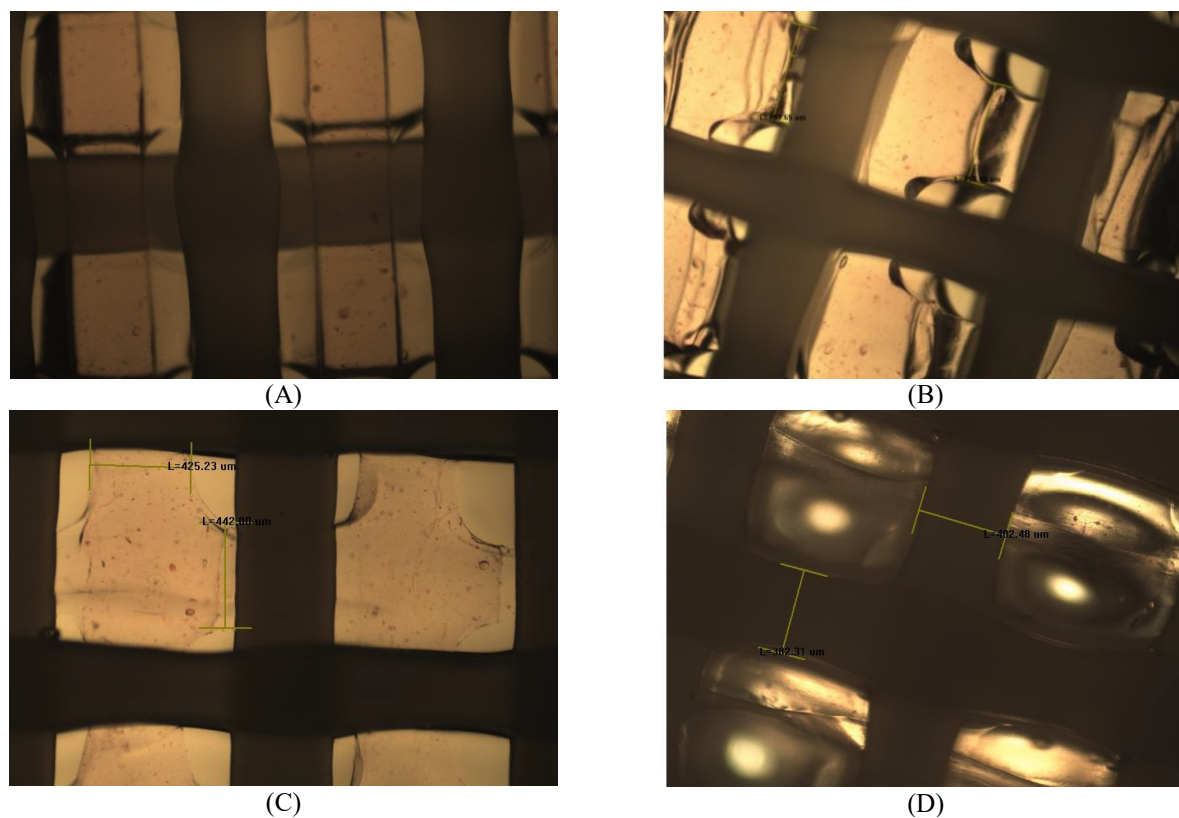


Figure 2 Micrographs of PCL/SA scaffolds with: 2 layers with SA hydrogel at concentration of (A) 6%(w/v); (C) 7%(w/v); and with 8 layers with SA hydrogel at concentration of (B) 6%(w/v); (D) 7%(w/v). Adapted from [17], with permission from Elsevier.

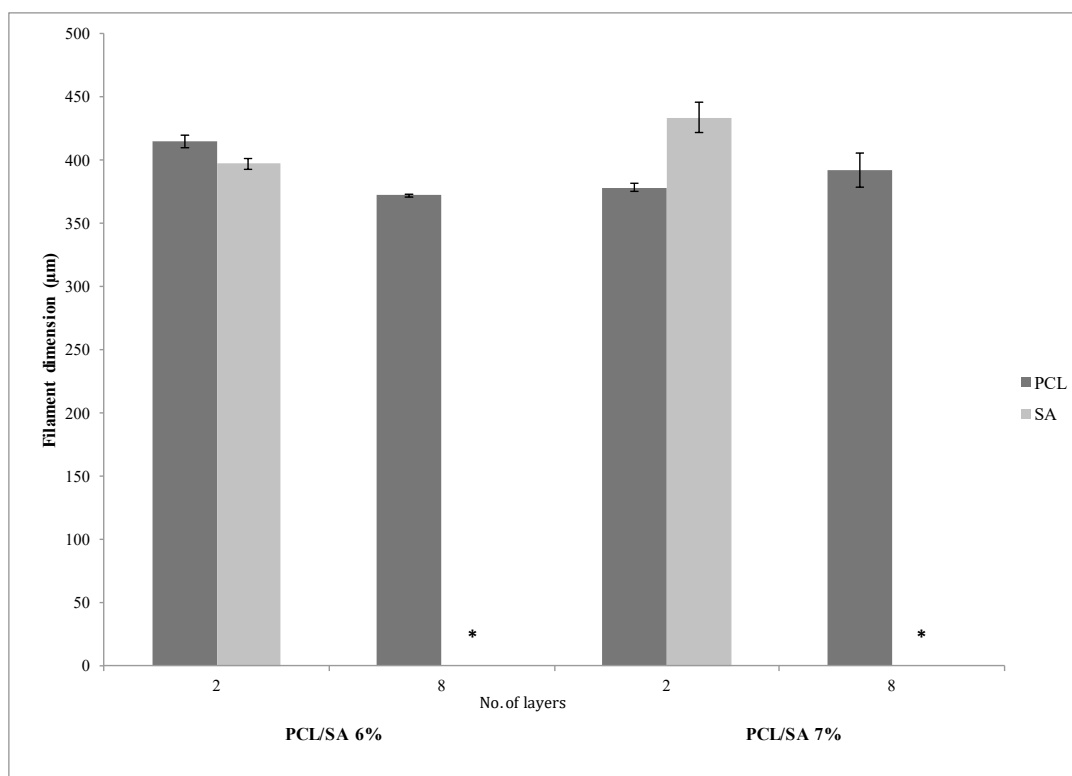


Figure 3 Filament dimensions of the heterogeneous scaffolds. * In samples with 8 layers, the SA filaments are not possible to measure. Adapted from [17], with permission from Elsevier.

Through the micro-CT analysis (Fig. 4), it is possible to distinguish the thermoplastic and the hydrogel along the 3D construct, and verify the alignment of the filaments along the scaffold. PCL filaments present good geometric accuracy. However, alginate aligned between PCL strands, show heterogeneous filaments and this behaviour is more evident with the increase of the hydrogel solution concentration.

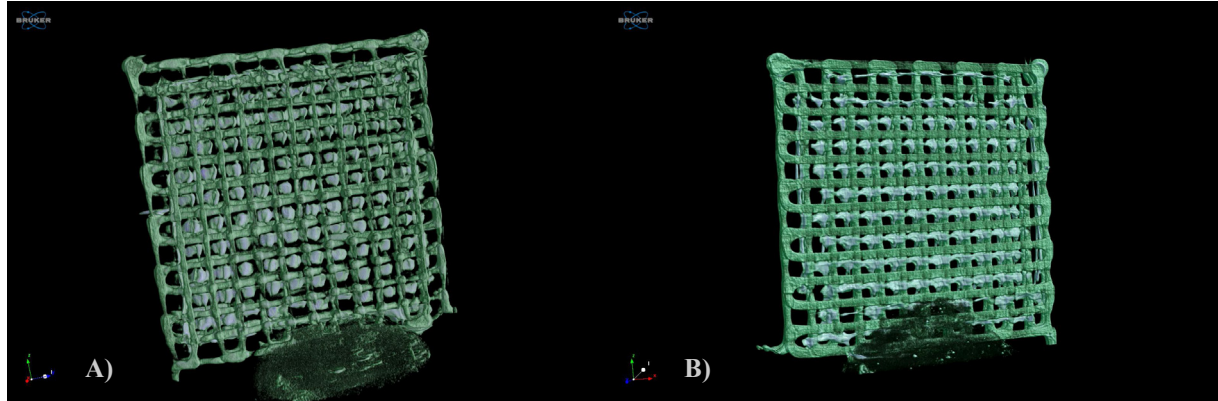


Figure 4 3D reconstructions obtained from Micro-CT analysis of: A) PCL/SA 6% and B) PCL/SA 7% scaffolds.

Mechanical Analysis. The mechanical properties of scaffolds have an important role during the regeneration of neo-tissues. They affect various cellular activities, proliferation and the cytoskeleton [18,19]. Fig. 5 demonstrates the obtained values of compressive modulus for PCL, PCL/SA 6% and PCL/SA 7%. No significant differences were observed, with the addition of SA slightly increasing the mechanical properties of the 3D matrices. The thermoplastic reinforcement with hydrogel filaments reduces the porosity of the scaffolds and consequently slight improve the performance of the structures under compressive loads.

As expected, the PCL scaffold showed a compressive modulus of 52.90 ± 2.23 MPa [20] and PCL/SA (6% and 7% SA) scaffolds a higher compressive modulus, in this case of 53.15 ± 3.24 MPa and 53.65 ± 1.38 MPa, respectively.

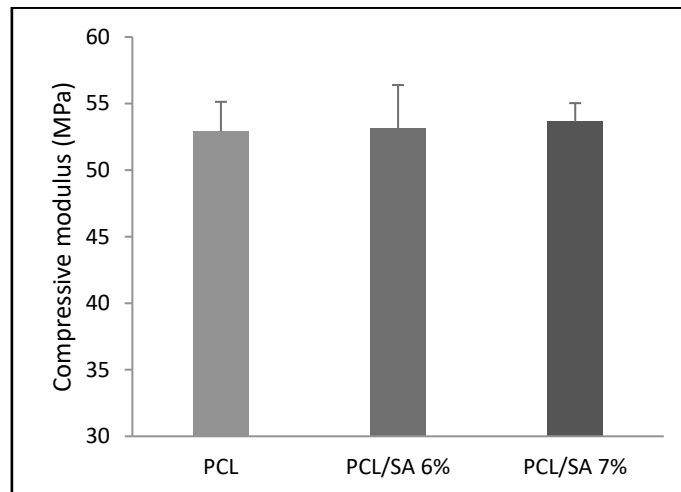


Figure 5 Mechanical properties of produced scaffolds.

A slight increase of hydrogel concentration promotes the compressive mechanical properties improvement, allows the increase of filaments size during crosslinking and consequently the decrease of scaffolds porosity. The mechanical properties of heterogeneous constructs (PCL/SA) are strongly dependent on the PCL scaffolds properties. The overall mechanical stiffness can easily be tailored by changing filaments spacing, orientation and/or thickness, i.e. by changing the porosity and the geometry of the scaffolds.

Conclusions

In this research work it was shown that BioMaTE can generate 3D structures with different biomaterials and provides a good control over the structural characteristics of the scaffolds through the manipulation of several processing parameters. Morphological analysis reveals that hydrogels with high water concentration can be printed with well-defined filaments. However, the mechanical support of the thermoplastic material is extremely important to maintain all structural stability of the multi-material scaffold. The addition of hydrogel filaments in the 3D constructs do not change significantly the mechanical properties comparing to PCL scaffolds and all scaffolds reveals potential to be used for bone regeneration. Nevertheless, more validation tests must be performed to confirm the possibility of these multi-material scaffolds being used in Tissue Engineering applications.

Acknowledgements

This work is supported by the Fundação para a Ciência e a Tecnologia (FCT) and Centro2020 through the Project references: BioMaTE - POCI-01-0145-FEDER-016800, UID/Multi/04044/2013 and PAMI - ROTEIRO/0328/2013 (Nº 022158). In addition, the authors also acknowledge the funding from the project insitu.Biomass (project reference: POCI-01-0247-FEDER-017771) from the Portuguese National Innovation Agency.

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