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High-resolution PLA-based composite scaffolds via 3-D printing technology

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ABSTRACT

Fabrication of new biodegradable scaffolds that guide and stimulate tissue regeneration is still a major issue in tissue engineering approaches. Scaffolds that possess adequate biodegradability, pore size, interconnectivity, bioactivity and mechanical properties in accordance with the injured tissue are required. This work aimed to develop and characterize three-dimensional (3-D) scaffolds that fulfill the aforementioned requirements. For this, a nozzle-based rapid prototyping system was used to combine polylactic acid and a bioactive CaP glass to fabricate 3-D biodegradable scaffolds with two patterns (orthogonal and displaced double layer). Scanning electron microscopy and micro-computer tomography showed that 3-D scaffolds had completely interconnected porosity, uniform distribution of the glass particles, and a controlled and repetitive architecture. Surface properties were also assessed, showing that the incorporation of glass particles increased both the roughness and the hydrophilicity of the scaffolds. Mechanical tests indicated that compression strength is dependent on the scaffold geometry and the presence of glass. Preliminary cell response was studied with primary mesenchymal stem cells (MSC) and revealed that CaP glass improved cell adhesion. Overall, the results showed the suitability of the technique/materials combination to develop 3-D porous scaffolds and their initial biocompatibility, both being valuable characteristics for tissue engineering applications.

1. Introduction

Rapid prototyping (RP), also known as additive manufacturing (AM), has emerged in the biomaterials field as a new tool for the fabrication of scaffolds with well-defined and reproducible architectures. RP techniques open the possibility of building custom-made scaffolds based on patient-specific tissue defects. These techniques combine computer design together with automated printing technology. In addition, temporary, tailor-made scaffolds fabricated by RP provide an excellent in vitro platform for the study of the effect of geometry/architecture on cell response, and for computer modeling of the scaffold's behavior. It also allows three-dimensional (3-D) structures with improved mechanical performance to be obtained. In fact, RP structures show mechanical properties significantly higher than those of structures fabricated by other well-known techniques such as solvent-casting and particle leaching, thermal-induced phase separation and gas foaming, among others [1–4].

Several RP techniques have been developed in recent decades. The elaboration of different polymer and ceramic scaffolds with different geometries has been reported [1–8]. Of remarkable interest are the nozzle-deposition-based techniques, particularly the approach consisting in a dispensing system integrated with pumping technology and a CAD/CAM tool. This is a versatile technique that allows the building of 3-D structures and complex geometry models with precise control and reproducibility, using a large variety of materials [5].

Reviewing the literature on RP fabricated scaffolds reveals that numerous degradable polymers such as polycaprolactone, polylactic acid (PLA), polyglycolic acid, chitosan and their copolymers have been used to fabricate 3-D scaffolds [2,6,8–11]. In particular, PLA is a currently used biodegradable polymer that has been approved by the FDA for various biomedical applications. Though this polymer has been extensively studied, its use in the fabrication of RP scaffolds and specifically those elaborated through nozzle-based systems has been limited and scarcely reported. At present, most of the reported PLA-based scaffolds fabricated by RP require the molecular modification of the PLA matrix, the use of temperature during printing or further processing of the structure by freeze-drying [12,13]. The RP tool used in the present study allows the fabrication of PLA 3-D structures without modifying the polymer structure with specific chemical groups, without melting the

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polymer and without using any subsequent process to remove the solvent from the final structure.

One of the strategies to improve the bioactivity and mechanical integrity of polymer scaffolds is the incorporation of an inorganic phase such as calcium phosphate (CaP) particles [14]. Indeed, several studies combining biodegradable polymers with different CaP ceramics have been reported [15–17]. In this area, CaP-based glasses are an interesting option, given their controlled biodegradability and bioactive potential [18]. In particular, CaP glasses in the system P_2O_5–CaO–Na_2O–TiO_2 have shown excellent biocompatibility both in vitro and in vivo [19,20].

This work describes the fabrication of PLA-based 3-D scaffolds by RP. Both polyethylene glycol (PEG) and G5 glass particles were combined with the PLA matrix to obtain 3-D fully biodegradable porous composite structures with superior mechanical and bioactive properties. The structures obtained were characterized in terms of their processing effect, final architecture, mechanical behavior, surface properties and biological response.

2. Materials and methods

2.1. Material

Poly(95L/5DL) lactic acid (PURAC) and PEG (M_w = 400 Da; Sigma Aldrich) were dissolved in chloroform (5% w/v) and combined to obtain a homogeneous polymer blend solution. Regarding the composite material preparation, a polymer blend (2.5% w/v in chloroform) was prepared to mitigate the increase in viscosity due to the presence of glass particles. PEG was used as a plasticizer to facilitate scaffold processing. A titania-stabilized, completely degradable CaP glass with molar composition $44.5P_2O_5$–$44.5Ca_2$-O–$6Na_2O$–$5TiO_2$ coded G5, was used in the form of particles (<40 μm) and added to the solution [18]. Materials were combined according to the compositions shown in Table 1.

2.2. Scaffolds design and fabrication

A nozzle-deposition system also known as a direct-print tool (Tissue Engineering 3-Dn-300, Sciperio/nScrypt Inc. Orlando, FL, available in the Rapid Prototyping service of the Biomedical Networking Center, CIBER-BBN and IBEC www.ibecbarcelona.eu/biomaterials) was used to fabricate the 3-D scaffolds. The machine consists of a dispensing system integrated with pumping technology to conformably deposit various types of materials. It uses a computer-aided-design/computer-aided-manufacturing (CAD/CAM) approach to build 3-D structures. The dispensing process is controlled by the motion control software and the CAD program, allowing flexible alteration of parameters such as speed of deposition, air pressure in the pneumatically actuated pump, dispensing height and 3-D geometry of the deposition pathways. The tool provides accuracy and reproducibility of the *XYZ* positioning of the dispensing nozzle with a resolution within few microns [21]. In order to study the influence of pore size and pore distribution in the axial and transversal direction, two different architectures were designed and fabricated (see Fig. 1): (a) an orthogonal layer configuration (ORTH) with distance between struts axes (d_1) of 500 μm and diameter of the struts (Ø) ~200 μm, and (b) a displaced

Table 1
Composition of the studied materials.

Material	Polymer matrix (w/w%)		G5 particles (w/w%)
	PLA	PEG	
PLA/PEG	95	5	–
PLA/PEG/G5	95	5	50

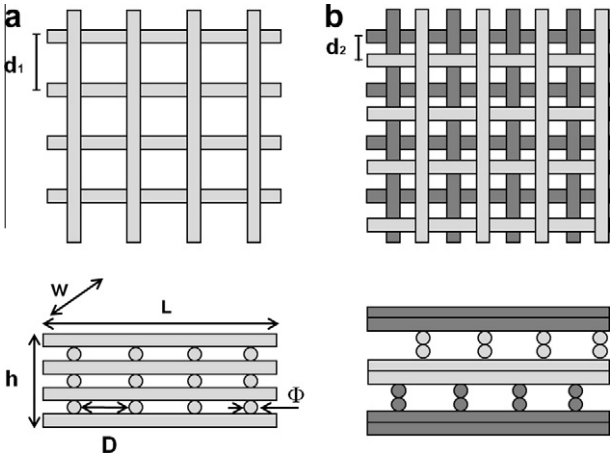

Fig. 1. Axial and cross-section view of the theoretical 3-D structures of (a) ORTH and (b) DISPL scaffolds: d_1 = 500 μm; d_2 = 250 μm; Ø = 200 μm.

double-layer design (DISPL) [22] with distance between struts $d_2 = d_1/2$ dispensing a double layer in each direction.

Three-dimensional structures were built accordingly to the created designs by means of the layer-by-layer deposition of the material using the pumping equipment. A printing pressure in a range between 40 and 80 psi and a motor speed of 3 mm s^{-1} were used to enable the material flow through a G27 (200 μm) nozzle. The syringe temperature was set at 40 ± 5 °C using a heating jacket, and room temperature was kept at 25 ± 2 °C.

2.3. Scaffold characterization

2.3.1. Differential scanning calorimetry

Differential scanning calorimetry (DSC; DSC-2910, TA Instruments) was used to determine the effect of PEG in the thermal properties of the polymer blend, and the thermal properties of the material pre- and post-processing. Samples (5–10 mg) of the PLA/PEG 3-D-deposited scaffolds, were first heated from 10 °C to 200 °C, then cooled to −25 °C and heated up to 200 °C at a heating rate of 10 °C min^{-1} in aluminum pans, with nitrogen as a purge gas. The resulting DSC curves were analyzed to determine the glass transition (T_g) temperature, and the crystallinity (X_c) of the polymer. T_g values were taken from the thermograms corresponding to the second heating cycle, whereas for the X_c calculation, enthalpy values were taken from the first cycle.

2.3.2. Morphological scanning electron microscopy study

Morphological analysis of the 3-D structures was carried out by scanning electron microscopy (SEM; JEOL JSM 6400, Tokyo, Japan) to visualize and evaluate the architecture of the 3-D scaffolds, surface morphology and structural stability of the deposited struts and layers. SEM observation allowed qualitative evaluation of the differences between the theoretically defined pore geometry and size and those obtained after processing. Image J software was used to calculate the diameter of the struts and pores obtained. A one-way analysis of variance (ANOVA) test was performed to determine the statistical significance ($p < 0.05$) of the differences in the experimental values obtained. It also allowed verification of the distribution of glass particles within the polymer matrix and the final 3-D scaffold.

2.3.3. Porosity

The theoretical volume porosity percentage (%$Vol_{theoretical}$) was calculated for each scaffold using the initially designed geometries based on a unit cube (Fig. 1), whereby the strut diameter and