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The effect of processing variables on morphological and mechanical properties of supercritical CO_2 foamed scaffolds for tissue engineering

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ABSTRACT

The porous structure of a scaffold determines the ability of bone to regenerate within this environment. In situations where the scaffold is required to provide mechanical function, balance must be achieved between optimizing porosity and maximizing mechanical strength. Supercritical CO_2 foaming can produce open-cell, interconnected structures in a low-temperature, solvent-free process. In this work, we report on foams of varying structural and mechanical properties fabricated from different molecular weights of poly(DL-lactic acid) $P_{DL}LA$ (57, 25 and 15 kDa) and by varying the depressurization rate. Rapid depressurization rates produced scaffolds with homogeneous pore distributions and some closed pores. Decreasing the depressurization rate produced scaffolds with wider pore size distributions and larger, more interconnected pores. In compressive testing, scaffolds produced from 57 kDa $P_{DL}LA$ exhibited typical stress–strain curves for elastomeric open-cell foams whereas scaffolds fabricated from 25 and 15 kDa $P_{DL}LA$ behaved as brittle foams. The structural and mechanical properties of scaffolds produced from 57 kDa $P_{DL}LA$ by $scCO_2$ ensure that these scaffolds are suitable for potential applications in bone tissue engineering.

1. Introduction

The treatment of critically sized bone defects currently involves autograft or allograft transplantation or implantation procedures. The shortage of organ donors coupled with the risk of rejection and disease and the difficulties inherent with artificial implants have led to a great demand for tissue engineered strategies [1,2]. These strategies often use scaffolds, in combination with cells and/or bioactive compounds, to generate new tissue [3]. Considerations for scaffold design are naturally complex and involve not only mechanical and structural constraints but also material composition, degradation properties and products, and surface properties of the scaffold. Additionally, the processing technique must produce scaffolds that can match irregular shapes and sizes of bone defects. The scaffold must promote cell adhesion and growth, and degrade over time into non-toxic components.

Synthetic biodegradable polymers such as poly(lactic acid) (PLA) and poly(lactic acid-co-glycolic acid) (PLGA) copolymers are commonly used in scaffold fabrication as they are approved for certain clinical applications by the US Food and Drug Administration (FDA), degrade in vivo and the degradation products are processed by normal metabolic pathways [4–6]. Scaffolds may be produced from these polymers by a variety of methods, including solvent casting/salt leaching [7–9], phase separation [10,11] and rapid prototyping/solid free-form fabrication [12–15]. These conventional methods, however, generally employ elevated processing temperatures or the use of solvents, which prohibit the incorporation of bioactive molecules in the scaffolds. To overcome these limitations, carbon dioxide (CO_2) has been used as a plasticizer in gas foaming to produce three-dimensional (3-D) polymer constructs [16–20].

CO_2 is a non-toxic, non-flammable, inexpensive reagent that is available in high purity. At a temperature and pressure above its critical point (T_c = 31.1 °C and P_c = 73.8 bar) carbon dioxide is a supercritical fluid, with properties of both gaseous and liquid states [21,22]; the liquid-like density provides much increased solvent power, whilst the gas-like viscosity leads to high rates of diffusion [23]. The addition of supercritical CO_2 ($scCO_2$) to amorphous polymers can produce dramatic changes in the glass transition temperature (T_g), viscosity, interfacial tension and permeability of the polymer [24], and result in the production of foamed materials. Supercritical carbon dioxide ($scCO_2$) foaming is a well-documented process [20,25–33], with two key stages: (i) a soak stage and (ii) a depressurization stage [34]. During the soak, the glassy polymer is saturated with $scCO_2$ at elevated pressures. This acts as a plasticizer, lowering the polymer T_g, and consequently the polymer state becomes rubbery. In the depressurization stage, with temperature constant, the pressure drop from the equilibrium solution state

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induces bubble nucleation; these nuclei grow becoming pores. As the pressure is decreased, the concentration of the plasticizer is also decreased. The polymer T_g increases and vitrification occurs with the porous structure fixed in the glassy state.

Open-cell, interconnected foamed structures are produced by this solvent-free, low-temperature process [35–38]. Drug molecules and proteins can be encapsulated within these constructs as protein structure and activity are retained during processing. Successful applications of this technique include the controlled release of proteins [39,40], promotion of bone formation in vitro and in vivo [41,42] and the induction of angiogenesis in vitro [43].

Scaffold structure is a vital concern in bone tissue engineering. Careful balance must be maintained between optimizing porosity and maximizing mechanical properties in situations where the scaffold may be required to substitute the mechanical function of the tissue that it aims to repair [44]. Whilst highly porous scaffolds (>90%) are needed to ensure cell delivery and tissue ingrowth [45,46], porosities not exceeding 80% are recommended for polymeric scaffolds for implantation into orthopaedic defects [47]. A pore size greater than 100 μm is the minimum recommended for vascularization [48], although more recent in vitro and in vivo studies have suggested that pore sizes and pore interconnections >200 μm may be required [49]. The permeability and interconnectivity of the scaffold are also crucial in determining cell infiltration and successful tissue ingrowth [15].

Recent work on a series of PLGA polymers has shown that modifying polymer composition, molecular weight and foaming process parameters can produce scaffolds with tailored porosities and pore sizes [24]. In this paper, a series of different molecular weights of poly(DL-lactic acid) ($P_{DL}LA$) polymers were employed for a detailed investigation of the effect of the depressurization rate and molecular weight upon scaffold characteristics. This study sought to elucidate the effects of the processing parameters on the porosity, pore size, interconnectivity and mechanical properties of foamed scaffolds as potential devices for bone tissue engineering.

2. Experimental

2.1. Materials

In this study a series of amorphous $P_{DL}LA$ polymers with different inherent viscosity were purchased from Purac (Gorinchem, Netherlands) and Boehringer Ingelheim (Resomer® product) (Ingelheim, Germany), and used as received (Table 1). The weight-average molecular weights (M_w) and polydispersity (*PDI*) of the polymers were determined using gel permeation chromatography (GPC) (PL-120, Polymer Labs) with a refractive index (RI) detector, as described in Ref. [24]. The T_g of the polymers was determined with a TA2920 differential scanning calorimeter. A heating rate of 10 °C min^{-1} was used with a test range of −10 to 120 °C. Food grade CO_2 was supplied by Cryoservice (Worcester, UK) and used without purification.

2.2. Scaffold fabrication

To each well of a Teflon mould was add 130 ± 3 mg of polymer; the mould contained 12 wells, each with a diameter and height of 10 mm (a similar mould is shown in Ref. [35]). The moulds, which were made in-house, had no lid and had a detachable base to facilitate easy removal of scaffolds post-fabrication.

The mould was then placed inside a 60 ml clamp sealed stainless steel high-pressure autoclave (made in-house), which was equipped with a pressure transducer to monitor pressure and a heating jacket with a CAL 3300 temperature controller (CAL Controls, Brighton, UK). HiP (High Pressure Equipment Company, Pennsylvania, USA) high-pressure valves and Swagelok (Ohio, USA) tubing and fittings were used to connect the system. The CO_2 was compressed using a high pressure PM101 pump (New Ways of Analytics, Lörrach, Germany).

The high-pressure vessel was heated to the desired temperature (T) prior to the introduction of CO_2. During the fill time, CO_2 was introduced until the desired pressure (P) was reached. This pressure was maintained during the soak time; the vessel was then depressurized (at a constant rate) to ambient pressure throughout the vent time. The pressure of the vessel in each of the three stages of scaffold fabrication was controlled by a back-pressure regulator (Bronkhorst, Ruurlo, Netherlands) and associated computer software. In this work the desired temperature and pressure were 35 °C and 232 bar, respectively. The porous scaffolds fabricated had diameters of approximately 10 mm and were 5–10 mm in height; a non-porous skin surrounded each scaffold.

2.3. Scaffold characterization

Scaffolds were characterized by micro-X-ray computed tomography (μCT; Skyscan 1174, Skyscan, Aartselaar, Belgium). The μCT was originally designed for non-destructive analysis of unprocessed surgical bone biopsies, but has been adapted for the analysis of polymeric scaffolds [50]. Prior to scanning, the non-porous skin on the scaffolds was removed and scaffolds were cut into uniform cubes, with width, length and height of 5 ± 0.5 mm. The cubic scaffolds were then mounted on a stage at a height of 3 mm within the imaging system and scanned. Measurements were obtained at a voltage of 40 kV, a current of 800 μA and a voxel resolution of 8.9 μm. The transmission images were reconstructed using Skyscan supplied software (NRecon); the resulting 16 bit, 2-D images were saved in tagged image file format (tiff). Quantitative analysis of porosity and pore architecture was obtained using direct morphometry calculations in the Skyscan CTAn software package. The mean pore diameter was calculated by filling maximal spheres into the pores with a distance transformation, as described by Hildebrand and Rüegsegger [51].

In this study, interconnectivity was quantified as the fraction of the pore volume in a scaffold that was accessible from the outside through openings of a certain minimum size; quantitative analysis and a 2-D representation of the process are provided in Ref. [52]. A three-dimensional "shrink wrap" was performed using the Skyscan analysis software to shrink the outside boundary of the volume of interest (VOI) in a scaffold through any openings whose size was equal to or larger than the connection diameter chosen. Connection diameters of 2, 4, 8, 12, 16 and 20 times the voxel size were used in

Table 1
Polymer characteristics.

Polymer	Resource	Form	M_w (kDa)	Inherent viscosity (dl g^{-1})	*PDI*	T_g (°C)
$P_{DL}LA$ (57 kDa)	Purac	Granular	57	0.5	1.87	46.9
$P_{DL}LA$ (25 kDa)	Resomer®	Powder	25.7	0.25–0.35	1.70	47.2
$P_{DL}LA$ (15 kDa)	Resomer®	Powder	15	0.16–0.25	2.34	41.8