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The influence of silica on pore diameter and distribution in PLA scaffolds produced using supercritical CO_2

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Abstract Macroporous polylactide (PLA) scaffolds were fabricated using a supercritical CO_2 foaming process. The addition of silica particles to the polymer matrix resulted in a significant modification in the pore size distribution exhibited by the scaffold. In the absence of silica, the scaffolds contained pores between 88 μm and 980 μm in diameter as determined using X-ray computed microtomography. The addition of silica at only 2 wt% resulted in the elimination of pores of >620 μm, with no significant influence on the total porosity of the material. This effect was attributed to the silica nucleating the formation of gas bubbles in the polymeric material. Although the addition of further silica to the scaffold resulted in a further reduction in modal pore diameter, when more than 20 wt% was added to the matrix little additional effect was noted. In addition to enabling some control over pore diameter, mineral deposition was shown to occur considerably more rapidly on the silica-modified scaffolds than on those containing no silica.

1 Introduction

At present, the surgical 'gold-standard' in bone replacement is autograft tissue, which is typically harvested from the iliac crest or ribs. The harvesting procedure, however, often necessitates a secondary surgical site and has been shown to carry a complication rate of up to 49% [1]. Reported complications include chronic pain, arterial injury, nerve injury, cosmetic deformity, fracture, infection, and gait disturbance. In addition, autograft tissue is usually available in short supply (21 ml in the iliac crest) [2], is of unpredictable quality and yields little or no mechanical integrity. With these problems in mind other sources of replacement tissue are being sought, and one of these alternatives is an engineered tissue implant.

As bone exhibits a complex architecture, to produce functional tissue it is necessary to culture osteogenic cells on to a scaffold material that bears a structural resemblance to it [3]. To facilitate the formation of new tissue in a scaffold, the scaffold structure should: have a highly interconnected pore network to allow cell growth and mass transport; degrade to be replaced by nascent tissue; and exhibit a surface chemistry suitable for cell attachment, proliferation and differentiation [4]. A considerable body of work has been dedicated to the development of scaffolds from aliphatic polyesters such as polylactide (PLA) which degrade within the body [5]. There are many ways in which polymers can be formed into scaffolds [6] but most involve the use of organic solvents or other chemicals and residual amounts of these chemicals may have a toxic effect on cells in a physiological system. Such methods include solvent casting-particle leaching, computer aided design related technologies (such as 3D printing), emulsion freeze drying and liquid–liquid phase separation [4]. An alternative to these methods is to use a supercritical fluid (SCF) [3], which may help to reduce the volume of organic solvent used.

An SCF dissolved in a liquefied polymer under pressure is thermodynamically stable but when this system is depressurised a thermodynamic instability is created, which results in nucleation of the gas molecules thereby creating

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pores as the polymer resolidifies. The degree of foaming of the polymer and the extent of its porosity can hence be controlled by the amount of gas incorporated, and also the rate at which it is released from the polymer; rapid release gives smaller pores (due to the limited opportunity for nucleated bubbles to grow) whilst slow release gives large pores as reported by Barry et al. [3]. In this study, we have attempted to control the pore size distribution of a PLA scaffold material produced using a supercritical foaming process by incorporating silica particles into the polymer matrix. The total porosity of the material was determined from geometrical measurements and pore structure was characterised using X-ray computed microtomography (microCT).

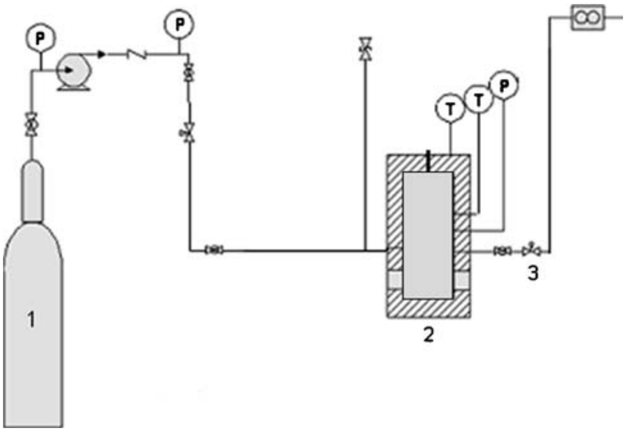

Fig. 1 A schematic diagram showing the rig used to process the scaffold materials. (1) CO_2 cylinder; (2) Pressure vessel; (3) Release valve

2 Methods and materials

A solvent casting method was used to produce the scaffold material that was used in the supercritical foaming experiments. Poly($_{DL}$)lactide ($P_{DL}LA$ 3051D, L:D ratio 95:5, $M_w = 1.8 \times 10^5$; Natureworks LLC, Minnetonka, MN, USA) was dissolved in dichloromethane (99.8% purity, HPLC grade; Riedel de Haën, Sigma, Poole, UK) at 10% w/v. As complete dissolution of the polymer took a minimum of 3 h, sealed glass bottles were used to avoid unnecessary evaporation. Once the polymer had dissolved, silica (particle diameter 125–250 μm; BDH chemicals, Poole, UK) was added at 2, 5, 10, 20, 30, 40, and 50 wt% content with respect to the polymer. The solution was manually agitated in a circular motion to obtain homogeneity and was then poured into a shallow glass dish and left overnight to allow the solvent to evaporate. The resulting film was removed from the glass and cut into small pieces (2 mm × 4 mm × 1 mm) using a scalpel. These pieces were stored in labelled sealed glass bottles in the dark until required for use. The entire process was carried out in a fume hood working at ambient temperature and pressure.

In order to fabricate the scaffolds, 200–250 mg of the PLA/silica film was placed into each of four stainless steel cylindrical moulds (internal diameter = 10 mm, height = 20 mm) lined with PTFE tape, which in turn were placed inside a small capacity pressure vessel of internal volume 25 cm^3 (Parr Instruments; Illinois, USA) and exposed to supercritical CO_2 (99.8% purity; BOC, UK) using a temperature of 160°C and pressure of 160 bar. The rig used for producing the porous scaffolds is shown in Fig. 1. These conditions were maintained for 2 h to allow complete saturation of the CO_2 within the melted polymer, before the vessel was depressurised to ambient pressure. The rate of depressurisation was varied between 0.25 and 2.25 l/min. Once the internal vessel temperature had cooled sufficiently for it to be manipulated (~60°C) the vessel was opened and the moulds and scaffolds were removed for further testing.

Relative porosity was calculated using geometrical measurements and scaffold mass. Theoretical density was determined by using the polymer density of 1.24 g/cm^3 and silica density of 2.6 g/cm^3. Assessment of the pore size distribution, porosity, and homogeneity of silica within the scaffold was undertaken using X-ray computed microtomography (microCT) (Skyscan1072; Skyscan, Belgium), operating with no filter at 50 kV and 98 μA. Scanned images were reconstructed using N Recon software, and CTan software was used to assess the internal structure using the American Society for Bone and Mineral Research (ASBMR) parameters for trabecular bone. Three-dimensional reconstructions based upon the CTan calculations were made using ANT.exe software (all software by Skyscan, Belgium). Internal scaffold structure was visualised using a scanning electron microscope, (JSM 840A; Jeol, Tokyo, Japan) set in low vacuum mode with a secondary electron detector operating at an accelerating voltage of 20 kV. Sections through the midpoint of each scaffold (~0.5 mm in thickness) were cut using a scalpel, carbon mounted on stubs and sputter coated with a 15 nm layer of gold prior to scanning (k550x; Emitech, Ashford, Kent). The elemental composition of any deposits formed on the surface of uncoated scaffolds was determined using energy dispersive X-ray spectroscopy (EDS) (Oxford Inca 300; Oxford Instruments, Abingdon, Oxford).

To examine the ability of the scaffolds to nucleate calcium salts, they were immersed in simulated body fluid (SBF) for 28 days at 37°C, with mass increase being monitored every 7 days. Continuous circulation of the SBF from a reservoir to the sample chamber was achieved by the use of peristaltic pumps. The scaffolds were analysed by microCT at 14 and 28 days to examine the homogeneity of the mineral formation throughout the scaffold. The presence of bone-like mineral in the deposited material was also ascertained by von Kossa's staining technique.

3 Results

Depressurisation of the supercritical reactor containing the melted polymer resulted in the production of a foamed PLA scaffold material (Fig. 2a). It was found that varying the rate at which depressurisation occurred allowed some control over the total porosity of pure PLA scaffolds. Depressurising the vessel at 0.2 l/min resulted in scaffolds with an average porosity of 83.4 ± 1.2%, whereas a more rapid rate of depressurisation (2.25 l/min) resulted in the formation of PLA scaffolds containing an average porosity of 64.6 ± 2.0% (Table 1).

Table 1 The influence of vessel depressurisation rate on the overall porosity of PLA scaffolds not modified with silica ($n = 4$)

Depressurisation rate (l/min)	Porosity (%)
0.2	83.4 ± 1.2
0.5	77.9 ± 1.7
1	74.9 ± 0.6
1.5	64.4 ± 2.7
2	66.4 ± 1.6
2.25	64.6 ± 2.0

Statistical analysis (one-way ANOVA and *post hoc* Dunnett test, $n = 8$) suggested that the presence of silica within the polymer matrices at a controlled depressurisation rate (2.25 l/min) had no significant influence on the total porosity of the scaffolds (Table 2) up to a silica loading level of 40 wt%. At greater silica concentrations, a small, but statistically significant increase in porosity was observed when compared with PLA foamed in the absence of silica (levels of significance: $P = 0.05$ for 40 wt% and 0.01 for 50 wt%). Examination of sectioned samples using scanning electron microscopy demonstrated that silica addition had a marked influence on the diameter of the pores present in the scaffold (Fig. 2a, b). In order to visually assess pore size distribution, models of the scaffolds were built using microCT data; the diameters of the pores present on the reconstructions were consistent with the scanning electron micrographs (Fig. 2c, d). Image analysis of the microCT data showed that the structure of the scaffold manufactured from PLA alone contained pores with a wide range of diameters (88–980 μm) with no single largest contributor to percentage volume (Fig. 3). By adding 2 wt% silica to the polymer, the pore size distribution was considerably narrowed and the majority of the pore volume was contributed to by pores of diameter 380–420 μm. Further addition of silica to the polymer to a loading level of 50 wt% resulted in a further narrowing in

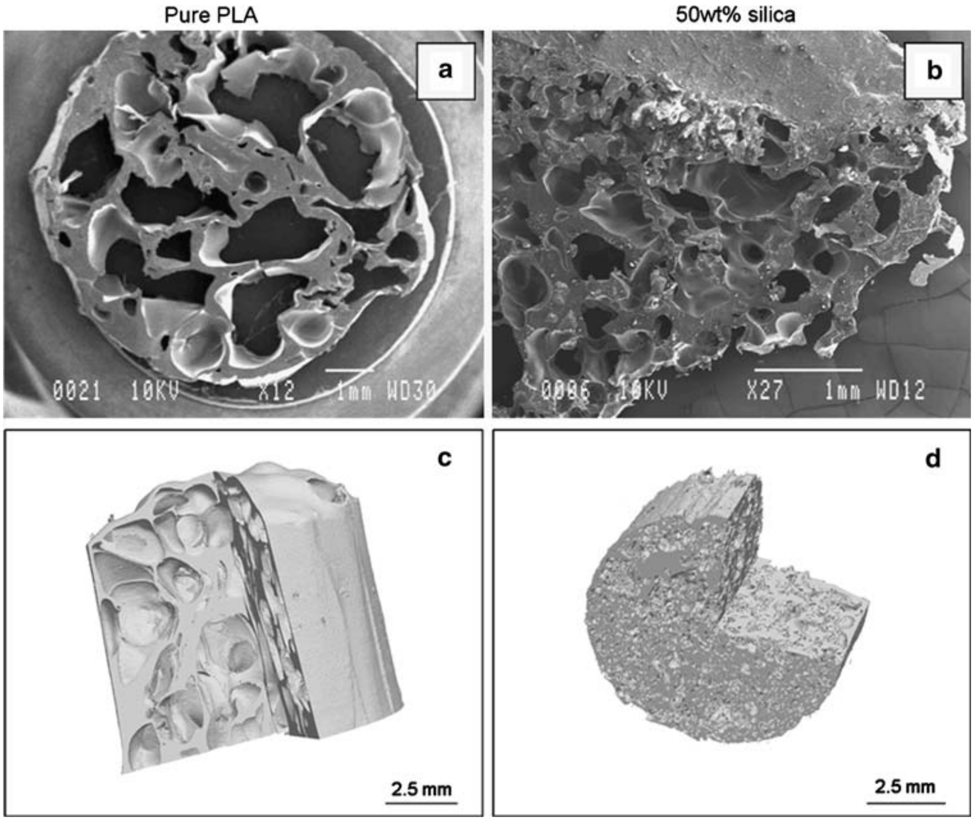

Fig. 2 The structure of the PLA scaffolds produced using a supercritical foaming method. Scanning electron micrographs demonstrating the pore diameters of scaffolds formed using (**a**) Pure PLA and (**b**) PLA containing 50 wt% silica. X-ray computed microtomography was used to characterise the pore structure of each scaffold (**c**, **d**)

Table 2 The influence of silica upon the overall porosity of the PLA scaffolds ($n = 8$)

Silica content (w/w% of polymer)	Porosity (%)
0	66.7 ± 2.7
2	68.4 ± 3.5
5	65.8 ± 3.5
10	68.2 ± 2.3
20	67.3 ± 2.5
30	68.0 ± 2.2
40	70.2 ± 1.3
50	73.0 ± 1.5

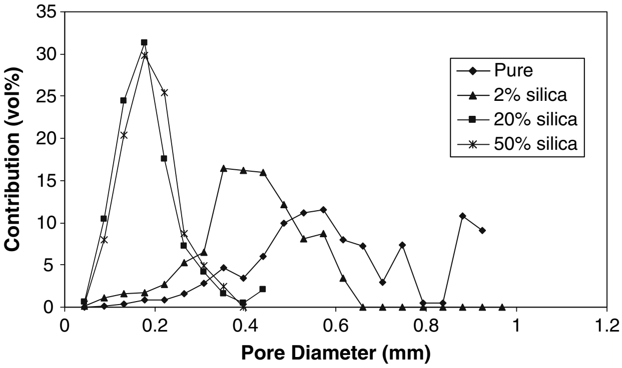

Fig. 3 Pore size distributions of foamed PLA scaffolds with and without silica addition

pore size distribution with the largest proportion of pore volume contributed by pores of diameter approximately 130 μm.

The silica and non silica-modified scaffolds were immersed in SBF for periods of up to 28 days, in order to monitor the deposition of mineral on the surface of the scaffold. Over a period of 7 days ageing there was a marked increase in the mass of the silica-modified scaffolds (35%), with little further increase for the remainder of the study (Fig. 4). In comparison, there was an increase of 22% during the first 7 days of ageing with the non silica-modified PLA scaffolds, and the total increase in mass was only 27% after 28 days. The deposition of a mineral phase on the surface of the scaffold materials was

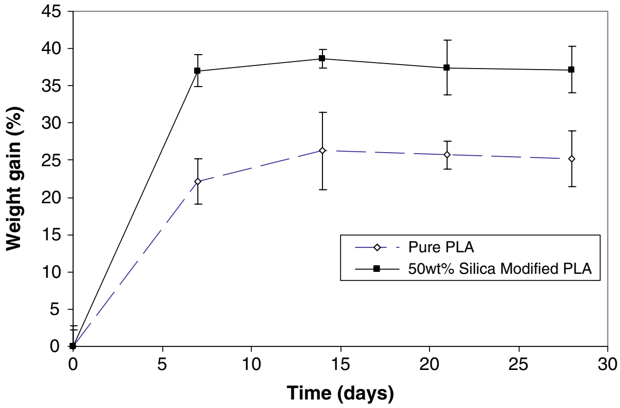

Fig. 4 Mass gains of the scaffolds produced by using Pure PLA or with 50 wt% silica addition during ageing in SBF (±standard deviation, $n = 4$)

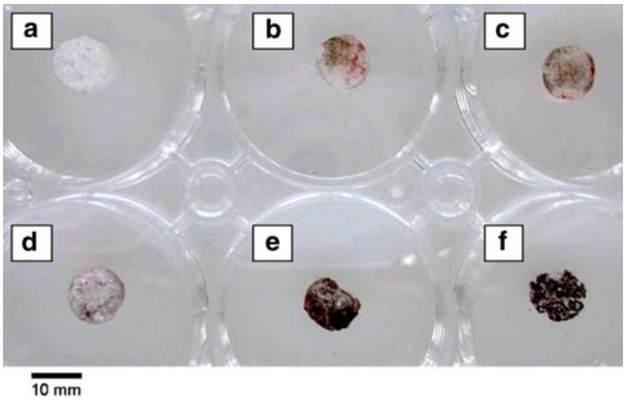

Fig. 5 Foamed PLA scaffolds stained using the von Kossa method to show the presence of mineral deposits. (**a**, **b**, **c**) Pure PLA, PLA containing 20 wt% and 50 wt% silica respectively prior to ageing. (**d**, **e**, **f**) Pure PLA, PLA containing 20 wt% and 50 wt% silica respectively following ageing in SBF for 28 days

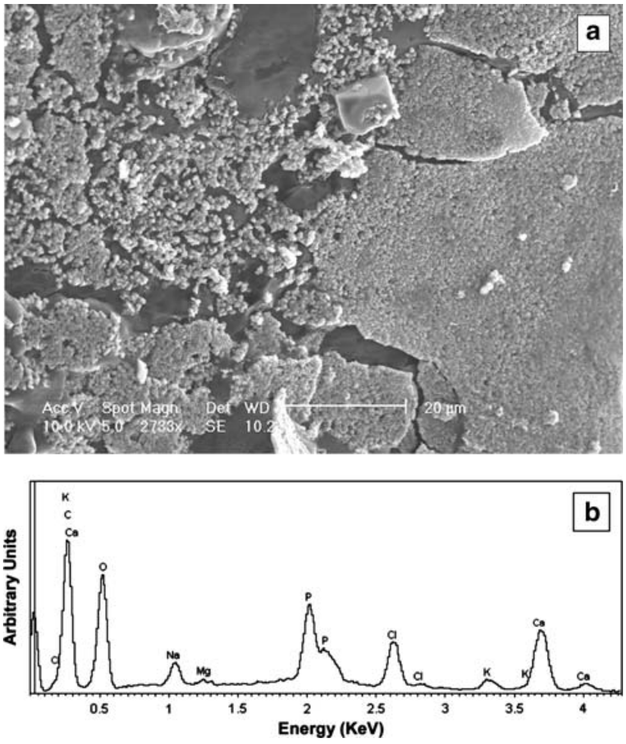

Fig. 6 (**a**) A scanning electron micrograph of the mineral deposit identified on the surface of a polymeric scaffold containing silica (50 wt%). (**b**) EDS demonstrated that the deposit was predominantly calcium phosphate based

confirmed using von Kossa staining. At time zero (Fig. 5a–c), little or no staining was apparent on the surface of the scaffolds. Following 28 days of ageing in SBF all scaffolds stained indicating the presence of a calcium salt (Fig. 5d–f). The intensity of the staining on the surface of the scaffolds modified with silica was considerably higher than on the surface of the non-modified material, indicating more extensive mineral deposition. Elemental analysis of the mineral deposited on the surface revealed that the precipitate was a calcium phosphate compound (Fig. 6a, b)

4 Discussion

A variety of ceramic [7], polymer [8] and even metallic materials [9] have been developed for use as tissue engineering scaffolds. Any material that is used must support cell growth, attachment and proliferation, in addition to not inducing an unfavourable tissue reaction if the construct is subsequently implanted. One of the most important requirements of a tissue engineering scaffold is that it contains an open pore network to enable mass transport into and out of the scaffold to maintain tissue viability. Consequently, a large body of research has been dedicated to processing materials to produce such a structure. Processing methods have included salt leaching, the use of ice as a porogen [10], emulsion methods and a number of foaming methodologies [6]. It is generally accepted that pores of diameter of ~200 μm are required to allow the migration of cells into the scaffold material and to support prolonged cell growth [11]. In the case of the non-silica modified material, when foamed using the supercritical CO_2 the scaffold contained a wide range of pore sizes from diameter 88–980 μm. While these pores are large enough to facilitate tissue in-growth, it is possible that the pore size would be too large to allow the formation of a complex tissue and may effectively compromise the mechanical properties exhibited by the scaffold. One previously reported method of controlling pore diameter was by increasing depressurisation rate of the vessel as has previously been shown by Barry et al. [3]. In this study we have demonstrated that depressurisation rate influenced not only the pore size distribution of the material, but also that it had a marked influence on the total porosity of the scaffold. By incorporating silica into the polymeric matrix, it was possible to exhibit some control over the pore size distribution of the foamed structure. When foamed in the absence of the silica particles, the nucleation of gas bubbles occurred randomly throughout the molten polymer in an uncontrolled manner. The presence of silica provided sites of nucleation on which gas bubbles formed, as previously reported by Whang et al. [12], who foamed PLA polymer containing sodium chloride crystals. The initiation of a larger number of sites for bubble growth with the polymer containing the same quantity of CO_2 meant that individual gas bubbles had less opportunity for growth, resulting in a net reduction in the modal pore size of the material. Statistical analysis showed that the incorporation of silica in amounts less than 40 wt% did not have a statistically significant effect on the total porosity of the scaffold, demonstrating that it is possible to tailor the pore size distribution of the scaffold without influencing total porosity.

The bioactivity of bone tissue engineering scaffold materials is frequently assessed by monitoring the deposition of calcium salts on the surface of the scaffold in simulated body fluid. In this study, a larger mass increase was noted for the scaffolds modified with silica than the non silica-modified material during the first 7 days of immersion. A reduction in the pore size distribution of the scaffold could have increased the surface area available for the formation of the calcium salt. Furthermore, it is possible that the silica particles provided nucleation sites for the formation of the calcium salt on the surface of the material. Silica-containing biomaterials such as Bioglass have been shown to facilitate hydroxyapatite formation on their surface [13] and silica has also been proposed to play a role in mineral aggregation within organisms such as coral and diatoms [14].

5 Conclusion

Here we have demonstrated that it is possible to exhibit some control over the pore size distribution of a macroporous PLA scaffold produced using supercritical CO_2 by incorporating silica particles into the molten polymer. In addition to affecting pore size distribution, the presence of the silica particles enhanced the rate of mineral formation on the surface of the scaffold material when compared with pure PLA alone. Further work will seek to determine the influence of silica incorporation on the mechanical properties exhibited by the scaffold, and to refine the gas foaming process.

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