Acta Biomaterialia 6 (2010) 1055–1060

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Particle seeding enhances interconnectivity in polymeric scaffolds foamed using supercritical CO_2

Niki J. Collins, Rachel H. Bridson, Gary A. Leeke, Liam M. Grover *

Centre for Formulation Engineering, School of Chemical Engineering, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

ARTICLE INFO

Article history:
Received 27 April 2009
Received in revised form 13 July 2009
Accepted 7 August 2009
Available online 9 August 2009

Keywords:
Tissue engineering
Supercritical
Scaffolds
X-ray microtomography
Interconnectivity

ABSTRACT

Foaming using supercritical CO_2 is a well-known process for the production of polymeric scaffolds for tissue engineering. However, this method typically leads to scaffolds with low pore interconnectivity, resulting in insufficient mass transport and a heterogeneous distribution of cells. In this study, microparticulate silica was added to the polymer during processing and the effects of this particulate seeding on the interconnectivity of the pore structure and pore size distribution were investigated. Scaffolds comprising polylactide and a range of silica contents (0–50 wt.%) were produced by foaming with supercritical CO_2. Scaffold structure, pore size distributions and interconnectivity were assessed using X-ray computed microtomography. Interconnectivity was also determined through physical measurements. It was found that incorporation of increasing quantities of silica particles increased the interconnectivity of the scaffold pore structure. The pore size distribution was also reduced through the addition of silica, while total porosity was found to be largely independent of silica content. Physical measurements and those derived from X-ray computed microtomography were comparable. The conclusion drawn was that the architecture of foamed polymeric scaffolds can be advantageously manipulated through the incorporation of silica microparticles. The findings of this study further establish supercritical fluid foaming as an important tool in scaffold production and show how a previous limitation can be overcome.

1. Introduction

There is an increasing demand for the development of new approaches to replace hard tissues that have been removed from the human body during surgery due to damage caused by injury, disease or the aging process. One possibility being widely investigated is the use of tissue-engineered implants, in which the desired cells are grown onto a biodegradable three-dimensional (3-D) structure (a scaffold) prior to implantation. The necessity of finding biodegradable polymer materials suitable for use as tissue implant scaffolds is well documented, as is the body of work investigating the application of the aliphatic polyesters in this field (such as polylactide, PLA) [1–14].

In order for the scaffold to be fully functional, the material of construction must have physical and mechanical properties similar to those of the tissue it replaces and it should degrade at the same rate as the replacement tissue grows. In addition, it must exhibit a surface chemistry suitable for cellular growth and attachment [1,2], enabling cells to grow at a rate and density and in a spatial arrangement similar to that found in a naturally occurring physiological system [3]. The internal scaffold microstructure is also of the utmost importance for it to be integrated successfully into a host system [3–5], since highly interconnected pores allow infiltration, attachment of and colonization by the cells, transport of nutrients to the cells and transport of metabolites away from them. Non-porous scaffolds have been found to lead to tissue necrosis, implant failure and rejection [6]. In addition, pore morphology and size must be tailored to the type of tissue being regenerated since different tissues have specific requirements [7,8].

Many different techniques can be used in the scaffold fabrication process but most require the use of large volumes of potentially harmful organic solvents [4,8,9]. Supercritical fluid gas foaming has been proposed as an alternative to these techniques since the amount of organic solvent used can be reduced, as in some cases can the processing temperature [1,2,10–13]. One major problem that has been identified using this method was that the pores had a closed structure, meaning that mass transport and cellular infiltration to the centre of the scaffold would be limited. One group attempted to address this problem by incorporating sodium chloride crystals into the polymer matrix during processing [13]. After processing, the particles were leached from the structure in water, yielding a structure that was proposed to be interconnected. The leaching step, however, added a further stage to the process and the interconnectivity reported in the study was evaluated only qualitatively using scanning electron microscopy. More recently

* Corresponding author. Tel.: +44 121 414 3887; fax: +44 121 414 5324.
E-mail address: l.m.grover@bham.ac.uk (L.M. Grover).

1742-7061/$ - see front matter © 2009 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2009.08.013

[14], we have seeded a PLA polymer structure with silica particles (125–250 μm diameter). The presence of silica in the scaffolds allowed the pore size distribution to be controlled independently of overall porosity. It has been proposed that the silica particles acted as nucleation sites for gas bubble formation. In addition to enabling some control over pore size distribution, the particle-seeded samples were also thought to exhibit a more highly interconnected pore structure. As with the earlier study, however, this was evaluated only qualitatively.

In order to assess the interconnectivity of the pores within a scaffold structure in a more quantitative manner, non-invasive techniques such as X-ray computed microtomography (microCT) allow non-destructive visualization, reconstruction and quantification in three dimensions with minimal sample preparation. Much of the work involving microCT has been carried out on bone as an improvement upon histological methods in assessing bone degeneration and disease, with the emphasis being on either analysing the morphology of the trabeculae [15–21] or the interconnectivity of the trabecular network [22–25], thereby focusing on the solid components of the bone rather than the marrow spaces. Due to the specific structural requirements of tissue-engineering scaffolds, the emphasis is as much on the structure of the pores as it is on the solid, and the use of microCT as an analytical tool is becoming more widespread [26–29]. In this study, microCT was used to develop a greater understanding of the connectivity between and within both the polymer–silica matrix (the solid) and the pores, seeking to establish the true nature of the 3-D pore networks within the scaffolds rather than estimating it from two dimensions with scanning electron microscopy. The work also further establishes supercritical fluid foaming of a polymer–particle system as a viable technique for producing scaffolds containing interconnected pores.

2. Materials and methods

A solvent casting method was used to produce the starting material that was to be used in supercritical foaming experiments as previously described [14]. Briefly, polymer–silica films were solvent cast with silica concentrations between 0 and 50 wt.%. The silica particles were 125–250 μm in diameter. Once the solvent had evaporated, the films were cut into small pieces (approximate dimensions 4 mm × 2 mm × 1 mm). In order to fabricate the scaffolds, 200–250 mg of the PLA–silica film was placed into each of four stainless steel cylindrical moulds (10 × 20 mm), which in turn were placed inside a small capacity pressure vessel and exposed to supercritical CO_2 at a temperature of 160 °C and a pressure of 160 bar. These conditions were maintained for 2 h to allow complete saturation of the CO_2 within the melted polymer, before the vessel was depressurized at a rate of 2.25 l min^{-1} to ambient pressure. 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.

The total porosity of the scaffolds was calculated from geometrical measurements, with the fraction of interconnected pores being determined through application of the Archimedes principle (described in context within Section 3). Assessment of the pore size distribution, porosity, and distribution of silica within the scaffold was undertaken using microCT (Skyscan model 1072; Skyscan, Belgium), operating with no filter at 50 kV and 98 μA. Two–dimensional cross-section images were reconstructed from the X-ray attenuation data (a measure of density linked to X-ray absorption) using NRecon software (version 1.4.3) and from these images the area required for analysis (the region of interest, ROI) was drawn.

The 2-D images were then converted to 3-D images using CTan software (version 1.7.0) by collating the ROIs to form the volume of interest (VOI), including as much of the height and width of the scaffold as possible in order to give a true representation of the internal structure of the scaffolds. Thresholding (setting of light/darkness levels according to X-ray attenuation) was carried out in order to highlight and select either the solid structure of the polymer matrix (threshold values of 25–255), or the open structure of the pores (threshold values of 0–24) before measurements of the internal structure were made on the VOI. MicroCT measurements included the per cent object volume (the per cent of total VOI occupied by the object, interpreted as per cent porosity), the fragmentation index (the number of connections between thresholded structures, interpreted as interconnectivity) and the structure thickness (the pore wall thickness, interpreted as inter-pore separation). A full description of the microCT parameters and how they are calculated can be found at www.skyscan.be/next/CTAn03.pdf. (All software was provided by Skyscan, Belgium.)

3. Results

All of the scaffolds were porous as demonstrated by representative cross-sections (mid-height) and longitudinal sections (mid-width) through scaffolds containing 0, 10 and 50 wt.% silica (Fig. 1). The incorporation of silica particles had a significant

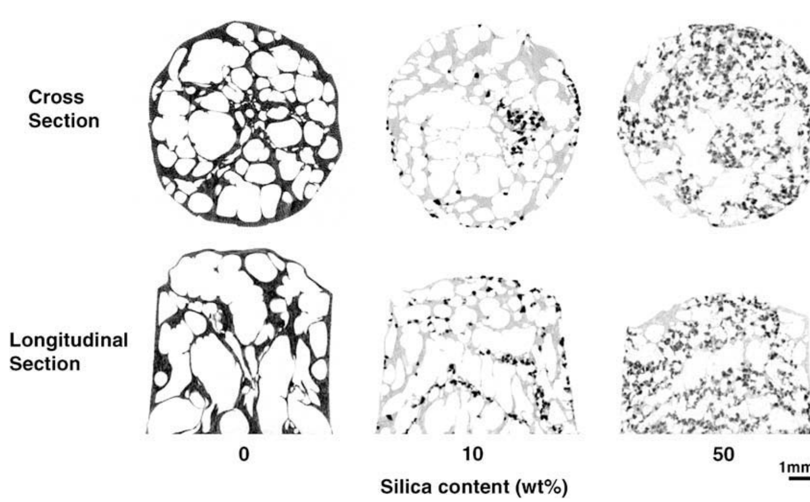

Fig. 1. Representative sections through polylactide scaffolds foamed using supercritical CO_2 in the absence and presence of silica particles. The incorporation of silica into the scaffold during processing had a significant influence on the pore structure of the resulting material and resulted in a reduction in scaffold height.