Journal of the Mechanical Behavior of Biomedical Materials 84 (2018) 151–160

Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

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

Synthesis, microstructure, and mechanical behaviour of a unique porous PHBV scaffold manufactured using selective laser sintering

Sven H. Diermann[a], Mingyuan Lu[a], Yitian Zhao[a], Luigi-Jules Vandi[b], Matthew Dargusch[c], Han Huang[a,*]

[a] School of Mechanical and Mining Engineering, The University of Queensland, St Lucia, QLD 4072, Australia
[b] School of Chemical Engineering, The University of Queensland, QLD 4072, Australia
[c] Centre for Advanced Materials Processing and Manufacturing (AMPAM), School of Mechanical and Mining Engineering, The University of Queensland, QLD 4072, Australia

ARTICLE INFO

Keywords:
Selective laser sintering
Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV)
Mechanical properties
Scaffold
Bone tissue engineering

ABSTRACT

Selective Laser Sintering (SLS) is a promising technique for manufacturing bio-polymer scaffolds used in bone tissue engineering applications. Conventional scaffolds made using SLS have complex engineered architectures to introduce adequate porosity and pore interconnectivity. This study presents an alternative approach to manufacture scaffolds via SLS without using pre-designed architectures. In this work, a SLS process was developed for fabricating interconnected porous biodegradable poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) scaffolds with large surface areas and relative porosities of up to 80%. These characteristics provide great potential to enhance cell attachment inside the scaffolds. The scaffold microstructure was dependent on the laser energy density (LED) during the SLS process. An increase in LED led to scaffolds with higher relative densities, stronger inter-layer connections, and a reduced quantity of residual powder trapped inside the pores. An increase in relative density from 20.3% to 41.1% resulted in a higher maximum compressive modulus and strength of 36.4 MPa and 6.7 MPa, respectively.

1. Introduction

Repair of complex bone fractures, including scenarios involving large bone loss, continues to pose great challenges for clinical management. Autologous and allogeneic bone grafting are commonly applied surgical procedures which come with inherent limitations and risks, including graft availability, harvest site pain, morbidity, rejection reactions, and risk of disease transmission (Dimitriou et al., 2011). Scaffold-assisted tissue engineering (TE) is regarded as a promising bone regeneration technique to replace the current bone grafting methods (Ramay and Zhang, 2004; Dean et al., 2003; Chua et al., 2004; Wu et al., 2013). In scaffold-assisted bone TE, a porous scaffold is implanted to provide support for cell migration, colonization, growth, and differentiation to promote tissue generation at the fracture site. Therefore, scaffolds are usually made of biocompatible and biodegradable materials and have an interconnected porous network (Cao and Hench, 1996; O'Brien, 2011; Yang et al., 2011; Hutmacher et al., 2014; Dutta et al., 2017).

The fabrication of porous TE scaffolds have employed a variety of manufacturing techniques, such as thermally induced phase separation (TIPS) (Nam and Park, 1999; Jack et al., 2009), solvent casting and particulate leaching (Thomson et al., 1996), electrospinning (Sombatmankhong et al., 2007), and sintering (Wu et al., 2007). While these methods have their unique benefits, existing technical issues including manual intervention, inconsistency and inflexibility in processing, and exclusion of toxic solvents or porogen still need to be addressed. Most significantly, customised scaffold geometries often cannot be achieved due to the inherent restrictions of the conventional manufacturing processes. Over the last decade, additive manufacturing (AM) has emerged as a promising method for the synthesis of TE scaffolds and has opened a new field of intriguing bioengineering possibilities. AM techniques are ideally suited for biomedical applications due to their ability to make customised parts with complex shapes (Leong et al., 2003; Mazzoli, 2013; Chia and Wu, 2015; Chua et al., 2017; Caulfield et al., 2007). Among various AM techniques, selective laser sintering (SLS) was extensively used to make bone scaffolds due to its capability to make porous structures using a large variety of materials in powder form. SLS does not require support structures during the fabrication process and the existing porosity between particles can be preserved (Chia and Wu, 2015). In previous studies, SLS manufactured

* Corresponding author.
E-mail address: han.huang@uq.edu.au (H. Huang).

https://doi.org/10.1016/j.jmbbm.2018.05.007
Received 13 March 2018; Received in revised form 21 April 2018; Accepted 2 May 2018

scaffolds typically used a computer designed three-dimensional (3D) architecture to achieve the desired porosity and interconnectivity (Hutmacher et al., 2014; Duan et al., 2010; Eosoly et al., 2010; Shirazi et al., 2014; Du et al., 2017). However, the use of a pore architecture is potentially accompanied with drawbacks. First, the manufacture of a scaffold structure with small features via SLS has limited resolution due to relatively large laser spot sizes (Lohfeld et al., 2010). Second, scaffolds made using SLS with a pre-designed pore architecture have lower specific surface areas than scaffolds manufactured using alternative techniques such as e.g. TIPS, (Nam and Park, 1999, Jack et al., 2009), solvent casting and particulate leaching (Thomson et al., 1996), or electrospinning (Sombatmankhong et al., 2007).

Requirements on biocompatibility, bioactivity and biodegradability have limited the choice of materials for scaffolds used in bone TE. Till now, ceramics (Shuai et al., 2017; No et al., 2017) and biodegradable polymers are frequently used for TE strategies in AM. For example, poly (ε)caprolactone (PCL) (Williams et al., 2005; Partee et al., 2005; Lohfeld et al., 2010), poly-L-lactide (PLLA) (Duan et al., 2010), poly-D-lactide (PDLA) (Bukharova et al., 2010), polyhydroxybutyrate (PHB) (Pereira et al., 2012), and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) (Duan et al., 2010) are representative polymers that were used to manufacture scaffolds via SLS. Among these materials, PHBV offers favourable surface chemistry for cell attachment and proliferation (Kumarasuriyar et al., 2005), while its degradation by-products are biocompatible and can be metabolized. Previous in vitro and in vivo biocompatibility investigations of PHBV in TE applications have confirmed its inert nature (Sultana and Wang, 2007; Sultana and Khan, 2012). PHBV elicits minimal inflammatory responses as it degrades into products, normally found as constituents of human blood (Sultana and Wang, 2007; Sultana and Khan, 2012). In addition, PHBV degrades over a long period of time which allows the polymeric scaffold to maintain its mechanical integrity until an adequate portion of new bone grows into the scaffold (Sultana and Khan, 2012; Gogolewski et al., 1993).

The objective of this study is to develop a SLS process to manufacture PHBV scaffolds with interconnected porous networks without engineered architectures, addressing the issues of process resolution and scaffold surface area. Using the developed SLS process, the manufactured scaffolds would have much smaller strut sizes and larger surface areas than those with pre-designed architectures, which is in favour of enhancing cell attachment inside the scaffold (O'Brien et al., 2005). In this study, the effect of SLS processing parameters on the microstructural and mechanical properties was systematically investigated, with a particular emphasis placed on the scaffolds' compressive deformation behaviour in both the normal and lateral direction.

2. Experimental details

2.1. PHBV powder

Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) powder (ENMAT^{TM}, Y1000) with a 3-hydroxyvalerate (HV) content of 2–5% and average molecular weight of 1,187,000 g/mol, was used (supplied by TianAn Biologic Materials Ltd, Ningbo, China). The as-received powder consists of fine spherical particles that agglomerate to form large irregular clusters. The powder was sieved to below 250 μm using a vibratory sieve shaker (Retsch, Germany). The particle size distribution (PSD) of the powder was measured by means of laser diffraction analysis of a water-based dispersion using a Mastersizer Hydro (Malvern Instruments, England). The cumulative particle size distribution values D_{10}, D_{50}, D_{90}, based on a volume distribution, were 11.3 μm, 73.7 μm, and 188 μm, respectively. Differential Scanning Calorimetry (DSC) analysis on the as-received PHBV powder revealed a melting temperature T_m and crystallisation temperature T_c of 172 °C and 114 °C respectively. The onset temperature of T_m and T_c of 161 °C and 120 °C was measured respectively, resulting in a SLS processing window of 41 °C, defined as the metastable area between two phases.

Table 1
SLS processing conditions.

Test	Laser power /P (W)	Scan spacing/S_S (mm)	Laser energy density/ LED (J/mm^3)
T1	3	0.2	0.030
T2	3	0.15	0.040
T3	3	0.1	0.060
T4	4	0.2	0.040
T5	4	0.15	0.053
T6	4	0.1	0.080
T7	5	0.2	0.050
T8	5	0.15	0.067
T9	5	0.1	0.100
T10	6	0.2	0.060
T11	6	0.15	0.080

2.2. Manufacture of scaffolds

Porous scaffolds were fabricated using a SLS system (DTM Sinterstation 2500 Plus, 3D Systems, USA) equipped with a CO_2 laser with an inherent wavelength of 10.6 μm and a spot size of 420 μm. To minimize the usage of powder in building small-scale specimens, a powder feeding system was designed and adapted into the SLS system. Both the volume of the powder feeding cartridges and the build area were substantially reduced. The area of the feed bed and build area were reduced to 125 × 105 mm^2 and 85 × 85 mm^2 respectively. Cubes ($L \times W \times H = 10 \times 10 \times 10$ mm^3) and cylinders ($D = 6$ mm, $H = 10$ mm) were manufactured in eleven tests, using different processing conditions, as shown in Table 1. The laser power (P) and scan spacing (s_s) varied from 3 to 7 W and 0.1–0.2 mm respectively. The laser scan speed was fixed at 5000 mm/s. The powder was spread by a roller system with a traverse speed of 177.8 mm/s. The layer thickness of each powder layer was 0.1 mm. The SLS chamber was filled with nitrogen gas to maintain an inert environment with an oxygen content below 5%. The processing temperature was 125 °C. Loose powder particles were removed from the manufactured scaffolds after the SLS process using a fine brush.

The altered SLS processing parameters were characterized using laser energy density (LED). The LED (J/mm^3) describes the intensity of the laser irradiation and is the quotient of P divided by s_s, scan speed (v), and layer thickness (l) (Shahzad et al., 2013; Hagedorn, 2013):

$$LED = \frac{P}{s_s v l} \quad (1)$$

The LED used in each test was calculated using Eq. (1) and is also shown in Table 1.

2.3. Characterization

2.3.1. Microstructural characterization

The microstructure and pore architecture of the fabricated scaffolds were characterized using scanning electron microscopy (SEM, FEI, Orgegon, USA) and 3D measuring laser microscope (OM, Olympus LEXT OLS4100, Japan). For SEM observation, the scaffolds were cross-sectioned in different directions using a scalpel, and then coated with a 20 nm conductive film. For OM observation, the PHBV scaffolds were mounted in epoxy resin, sectioned, ground, and polished. The entire area of the polished scaffold was imaged to provide a holistic view of the sectioned plane. A minimum of 67 OM images were taken for each specimen and then stitched together to form a large area image mosaic.

The stitched images of cross-sections along the x-y (normal) and y-z (lateral) plane were used to quantify the areal size of the skeleton (material, l_m) and pores (l_p) using a line inter-section method, which is detailed in the supplementary material S1.