BIO-DEGRADABLE SINTERED COMPOSITES OF PLA AND HA IN SCAFFOLDS

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ABSTRACT

Porous poly-L-lactide acid (PLA) scaffolds are prepared using polymer sintering and porogen leaching method. Different weight fractions of the Hydroxyapatite (HA) are added to the PLA to control the acidity and degradation rate. The three dimensional morphology and surface porosity are tested using micro CT, optical microscopy and scanning electron microscopy (SEM). Results indicate that the surface porosity does not change by addition of HA. The micro Ct examinations show slight decrease in the pore size and increase in wall thickness accompanied with reduced anisotropy for the scaffolds containing HA. SEM micrographs show detectable interconnected pores for the scaffold with pure PLA. Addition of the HA results in agglomeration of the HA which blocks some of the pores. Compression tests of the scaffold identify three stages in the stress-strain curve. The addition of HA adversely affects the modulus of the scaffold at the first stage, but this was reversed for the second and third stages of the compression. The results of these tests are compared with the cellular material model. The manufactured scaffold have acceptable properties for a scaffold, however improvement to the mixing of the phases of PLA and HA is required to achieve better integrity of the composite scaffolds.

INTRODUCTION

Bioresorbable scaffolds of polylactic acid (PLA) offer several benefits. They require only a single surgery and leave native tissue behind. They gradually transfer load to tissue during the degradation period, allowing for bone remodelling and reducing the risk of re-fracture. They also can be used to deliver drugs, growth factors or other substances conducive to the healing of bone locally to the implant site.1

Several manufacturing processes are tried and tested for producing scaffolds of biodegradable polymers. In this research we manufacture scaffolds of PLA with HA as reinforcement phase through sintering solvent free process. The strength and structure of the final scaffolds are sensitive to the sintering time in the novel sintering process. The time must be sufficient to allow bonding between polymer particles but not too long as to allow the polymer to fully surround the porogen particles and cause a closed pore structure. The use of pressure and heating in the process creates a dense structure and hence care must be taken in ensuring compete removal of the porogen. The removal of the salt particles is normally achieved by leaching the composites in distilled water. The advantages of the method used are simplicity, lack of presence of solvent and high porosity and strength. The produced scaffolds are characterised for their morphology and mechanical response under compressive load and their degradation rate. The degradation results are not reported here to keep the article within the limit set. The initial evaluation proves that scaffolds manufactured through the proposed process show desirable characteristics expected of scaffolds.

MATERIALS AND METHOD

Materials and scaffold fabrication: Porous poly-L-lactide / hydroxyapatite scaffolds have been prepared using a polymer sintering and porogen leaching method similar to that reported by Jung et al [1]. Poly-L-lactide (PLA; Lakeshore Biomaterials, USA) with an inherent viscosity of 1.6 dl/g is milled at liquid nitrogen temperatures to produce polymer particles. Particles below 125 µm are separated via sieving and stored in a vacuum desiccator prior to use. Hydroxyapatite particles (HA; Plasma Biotal ltd., UK) from 0 – 5 µm are used as received. Sodium chloride particles (NaCl; Fischer Scientific, UK) are sieved to yield particles from 250 – 500 µm. PLA, HA and NaCl are mixed by shaking before being transferred to a stainless steel pellet die (diameter, 20mm) and compressed at 130Mpa for 3 minutes. The consolidated pellets are subsequently heated at 210 ºC for 30 minutes; inverting the pellets every 7 minutes 30 seconds. After cooling, the pellets are placed in an excess of distilled water for 48 hours with slow stirring, the water is changed after 24 hours. Finished scaffolds are stored in a desiccator until characterisation.

Micro-CT tests: The 3D morphology of scaffolds is examined using Micro-CT (Skyscan 1172). The following scanning parameters are used: Rotation step: 0.7, Frame averaging: 4, Pixel size: 4.1µm. PLA scaffolds and 10%HA scaffolds are scanned with a low energy X-Ray filter due to the low X-Ray absorbance of the scaffolds. 30%HA and 50%HA scaffolds are scanned using a 0.5mm aluminium filter. Analysis is carried out on an arbitrary $3mm^3$ cube from the centre of samples. Images are first thresholded to clearly define pore and pore wall volumes. Image noise was removed using a de-speckling plugin set to remove areas under 70 pixels in 2D space. Sample 2D images and their processed version are shown in figure 1a and 1b. The micro-CT tests on the 3D structures provided more detail and accurate information regarding the porous structure.

Microstructure study: Scanning electron microscopy (SEM, Mk 2, Camscan, UK) at 6 kV is used to study the internal microstructure of the manufactured scaffolds. The scaffolds are cut using razor blade to look in to the internal surfaces of the scaffold. Different magnifications are employed to consider the pore shape and connectivity and the microstructure of the polymer/HA phase.

Compression tests: One of the major problems for mechanical characterization of porous ceramic scaffolds is the difficulty in machining and gripping specimen; hence the conventional methods of mechanical characterization such as tensile, biaxial and impact testing are usually inapplicable to porous materials [2]. Instead, the compression test has been widely accepted and used successfully for characterization of cancellous bone and porous HA [3-4]. Scaffolds are tested in compression (Hounsfield, UK) with a crosshead speed of 0.5 mm/min. Scaffold stiffness is calculated initially, at 5% (stage 1) , 20% (stage 2) and 70 – 75% strain (stage 3). Ten samples are tested for each composition.

RESULTS AND DISCUSSION

Micro-CT results: The micro-CT tests on the 3D structures provided more detail and accurate information regarding the porous structure (table 1) The analysis of the microstructure using the micro-CT software indicates that addition of the HA to PLA results in slight reduction in mean pore size shown in figure 1c. The decrease in the average pore size could be attributed to the smaller pores appearing in the structure as a result of lack of adhesion and integrity of the polymer and ceramic phase. This is observed in the SEM micrographs as well. Lack of perfect leaching of the porogen particle can as well results in the reduction of the average pore size. Micro-CT analysis indicates increase of the wall thickness of the pores with addition of HA shown in figure 1d. Once again lack of complete removal of the porogen could result in the extra thickness of the walls. Relative pore surface area shows some increase in the samples containing HA which might be mainly due to reduced pore size and increased pore numbers. Slight reduction in degree of anisotropy in the composite scaffolds is observed which is shown in figure 1d.

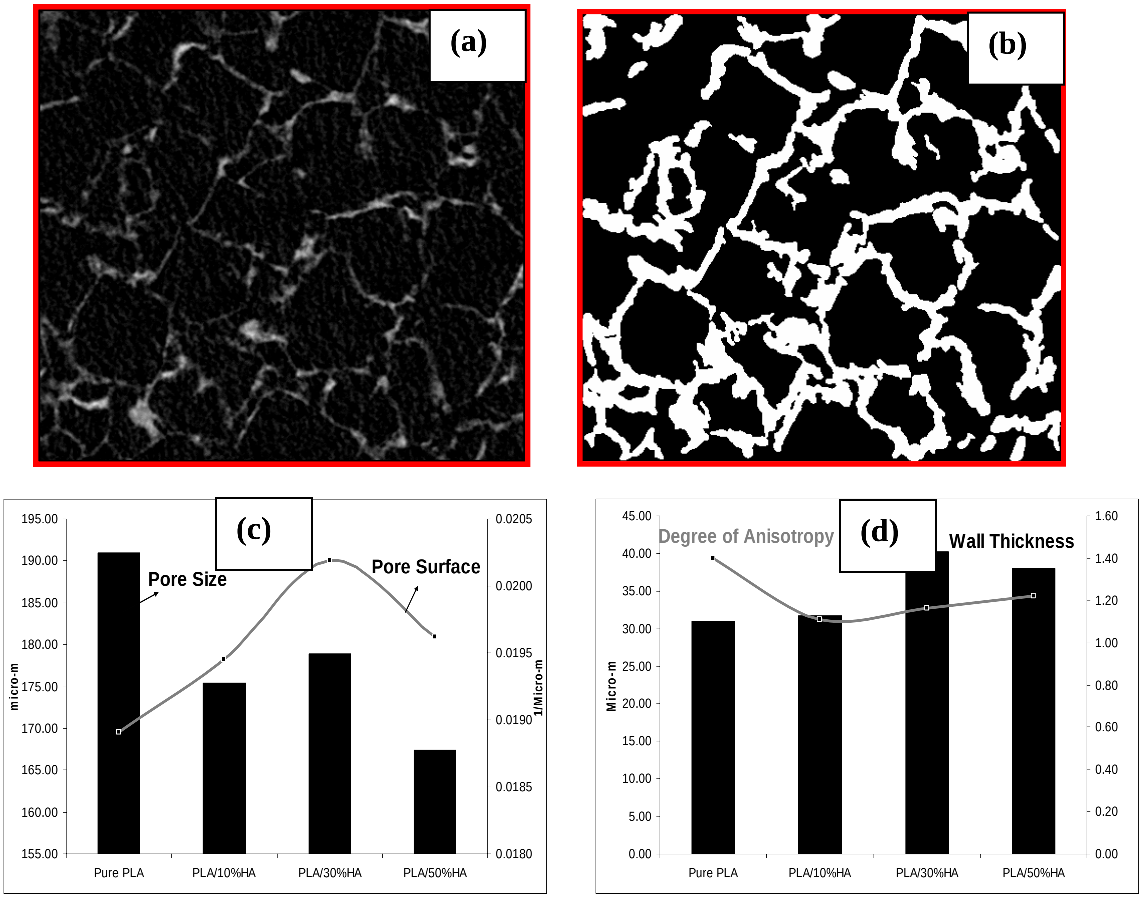

Figure 1: (a) 2D images of the structure produced by micro-CT, (b) processed image of micro-Ct clarifying the pores and its borders, (c) mean pore size and relative pore surface (d) wall thickness and degree of anisotropy estimated by the micro-CT software.

This could be due to more irregular and random shape of the pores observed in these scaffolds compared to neat PLA scaffolds. The SEM micrographs confirm the same conclusion which is increased irregularities in the structure of the composite scaffolds.

Scanning Eelectron Microscopy of pore wall texture: The SEM study indicates that in the neat PLA (figure 2a) regular porous network of square plates providing high surface area for cell attachment is observed. The shapes of the pores are defined by the porogen. The structure seems to be uniform. By increasing the magnification to about hundred times higher, we are able to examine the structure of the walls for neat PLA (figure 2b). The pore walls seem to be made of a continuous and homogenous phase of PLA. PLA/50% HA scaffolds also exhibited a porous network of square plates, although pore edges were rougher and less defined (figure 2c). Discontinuity of PLA matrix due to HA particle agglomeration is observed for the walls when higher magnification is used (figure 2d). The pore structure becomes more irregular with the addition of the HA which results in lower degree anisotropy in the composite scaffold. This is proved by the micro-CT results where the degree of anisotropy is measured through 3D analysis of the micrographs.

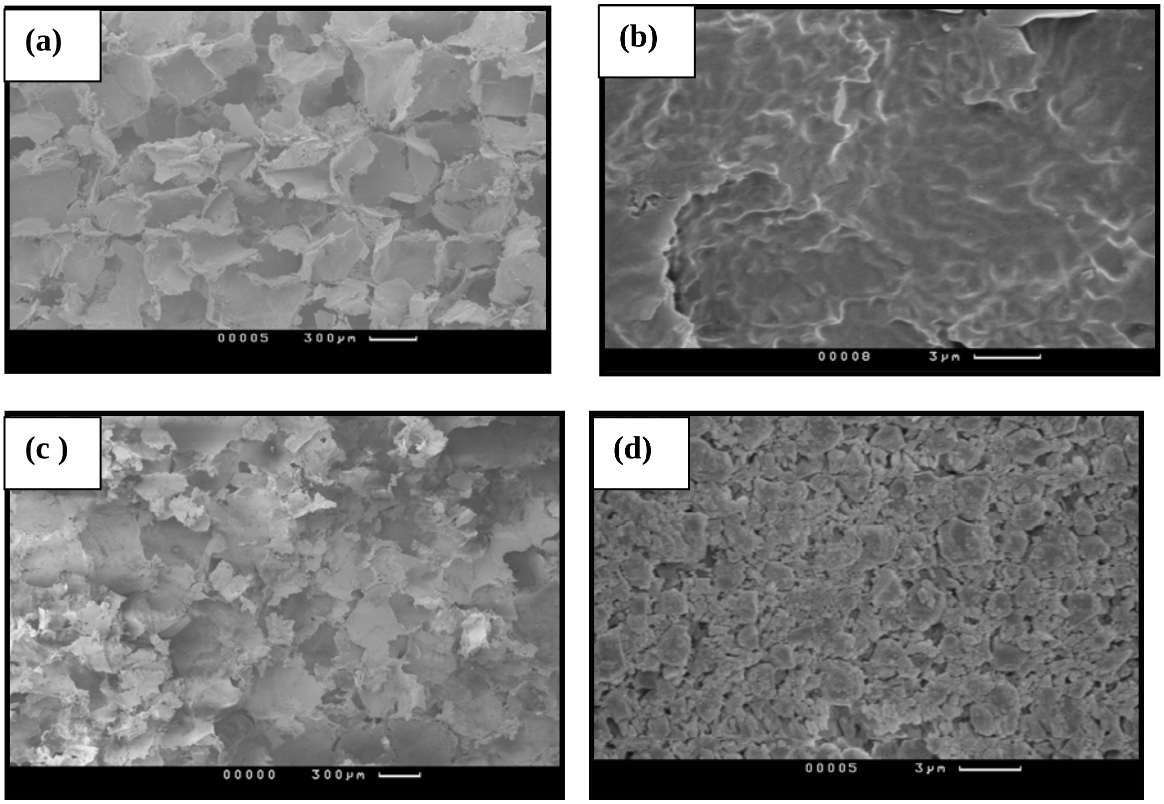

Figure 2: SEM micrograph of (a) neat PLA scaffold pore network morphology, (b) neat PLA scaffold wall texture, (c) PLA/50% HA scaffold pore network morphology, (d) PLA/50% scaffold wall texture.

Zhang et al [4] have made similar observations for the composite scaffold. They have used solid-liquid phase separation with subsequent sublimation of the solvent for

manufacturing of scaffold of PLA with different percentage of HA. In their study, SEM observation similarly prove more isotropic scaffold in composite case compared to pure PLA scaffolds.
Despite the irregularity introduced by the HA in the polymer phase, still there is interconnectivity between pores to keep the characteristics required for a scaffold.

Roether et al [5] have used the EPD method for manufacturing the PLA and bioglass scaffolds and they notice that the original interconnected porous structure of the foam become ‘‘sealed’’ by the Bioglasss particles, and therefore, this technique is found to be inadequate for the purpose of fabricating porous composite scaffolds,

The manufacturing technique used here does not seem to cause similar problem as the interconnectivity of the pores are maintained. However improving mixing of the HA and PLA in the solid state could improve the homogeneity of the composite. Similarly Improvement of adhesion of the HA and PLA seems to be required.

Mechanical Properties: During the compression tests three stages are observed as follows: The moduli of scaffolds of 85% and 90% porosity of different composition for the first stage are reported in figure 3a. In first stage the pore walls contribute to the resistance to the compressive load which results in elastic response to the load. Addition of HA to PLA results in decreased modulus. This is attributed to the agglomeration of the HA phase and lack of uniform distribution of the HA particles and increased level of micro-porosity as observed in the SEM images. Significant reduction in the modulus as a result of the loose HA particles, is observed by other researchers [5]. The moduli of scaffolds of 85% and 90% porosity of different composition for the second stage are shown in figure 3b. In the second stage the failure and collapse of pores initiates and continues. The PLA scaffolds show increased stress in this stage as a result of resistance of pore walls to fracture. For PLA scaffolds increased modulus with addition of HA is observed at this stage. Significant change in dimension of the samples occurs as a result of densification of the sample with disappearance of the pores.
The moduli of scaffolds of 85% and 90% porosity of different composition for the third stage are shown in figure 3c. The third stage indicates large increase in the modulus as a result of densification of the scaffold. At this stage as well addition of the HA would result in increased modulus.
The behaviour observed in our scaffolds are very similar to the observations made for cellular materials in the past. The cellular structure of materials gives rise to similarities in their mechanical behaviour. Their compressive stress-strain curves for instance have the same characteristic stages: an initial linear elastic regime is followed by a stress plateau which continues to large strains and is terminated by the final sharp increase in stress. Each regime of behaviour corresponds to a particular mechanism of cell deformation: linear elasticity to cell wall bending, the stress plateau to cell collapse by elastic buckling, plastic yielding or brittle crushing. Depending on the nature of the cell wall material and the final sharp increase in stress to densification of the material once the cell has most completely collapsed [6]. In order to assess the performance of our scaffold, it is best to first review the properties registered for the components in our scaffold which are PLA and HA in different form and the properties of some of the scaffolds of similar compositions. Amorphous PLA exhibits the glass transition

temperature (Tg) in the range of 50-60 C. Below the temperature PLA is rigid and brittle having the elastic modulus of about 1.2-3.8 GPa and the low ability to plastic deformation. Amorphous PLA yields at the deformation about 5% and the stress of 47 MPa and exhibits some ability to plastic flow. The average elongation and stress at break are around 18% and 28-50 MPa [7]. The mechanical properties of HA is highly dependent on the manufacturing process for densification. If sintering is used the temperature and grain size would be crucial in the properties of the resultant HA [8]. Several other densification techniques is used for the ceramics including the dry processes or the wet processes such as sol-gel technique, solid free form fabrication, tape casting, direct casting methods, reticulated ceramics and colloid processing are suggested. Each results in different microstructure of the ceramic phase and different pore size, shape and distribution [9].

Scaffolds made of HA with 86-90% porosity which is similar to our scaffolds have shown the modulus and compression strength in the range of 0.83-1.6 MPa and 0.21-0.41 MPa respectively [10] . This is whilst the dense and sintered HA has the modulus and compressive strength in the order of 100 GPa and 400 MPa respectively [10]

The PLLA/HAP/composite foams are prepared by solid–liquid phase separation and subsequent sublimation of solvent by previous researchers [11]. The moduli of these scaffolds are reported to be about 6.5 MPa for PLA foams and 10 MPa for PLA/50%HA. The strength of PLA foam is reported to be about 0.25 MPa and the strength of the PLA/50% HA is estimated as about 0.4 MPa. Our scaffold shows different properties, with modulus around 17.5 MPa for the neat PLA scaffold and yield stress of 0.7 MPa. Our Scaffold of PLA with 50% of HA show modulus of 6.3 MPa and the yield stress of 0.35. This indicates increased modulus and failure stress compared to the phase separation method for the neat PLA. Whereas in our scaffolds addition of the HA to the polymer cause reduction of the modulus and failure stress for the first stage. In order to achieve improved mechanical properties with addition of HA, modification of the manufacturing process needs to be considered. Improvement of the mixing of the phases and densification of the HA phase should be provisioned in the next phase of the research.

Man-made cellular materials, such as honeycombs and foams, can be successfully modeled by identifying and analyzing the mechanisms by which the cells deform and fail. Gibson et al [12] have modelled the mechanical behaviour of the honeycomb structures in the plane of the hexagonal cells. The modulus of the honey comb under compression is related to the density of the foam and the density of the cell wall and the modulus of the cell wall through the following equation: $E_1^* = C E_s \left[\frac{\rho^*}{\rho_s}\right]$. In this equation E^*_1 is the modulus of the cellular structure and E_s is the modulus of the wall and ρ^* and ρ are the density of the wall and the porous structure respectively. C is a coefficient which depends on the geometry of unit cell and its arrangement. I

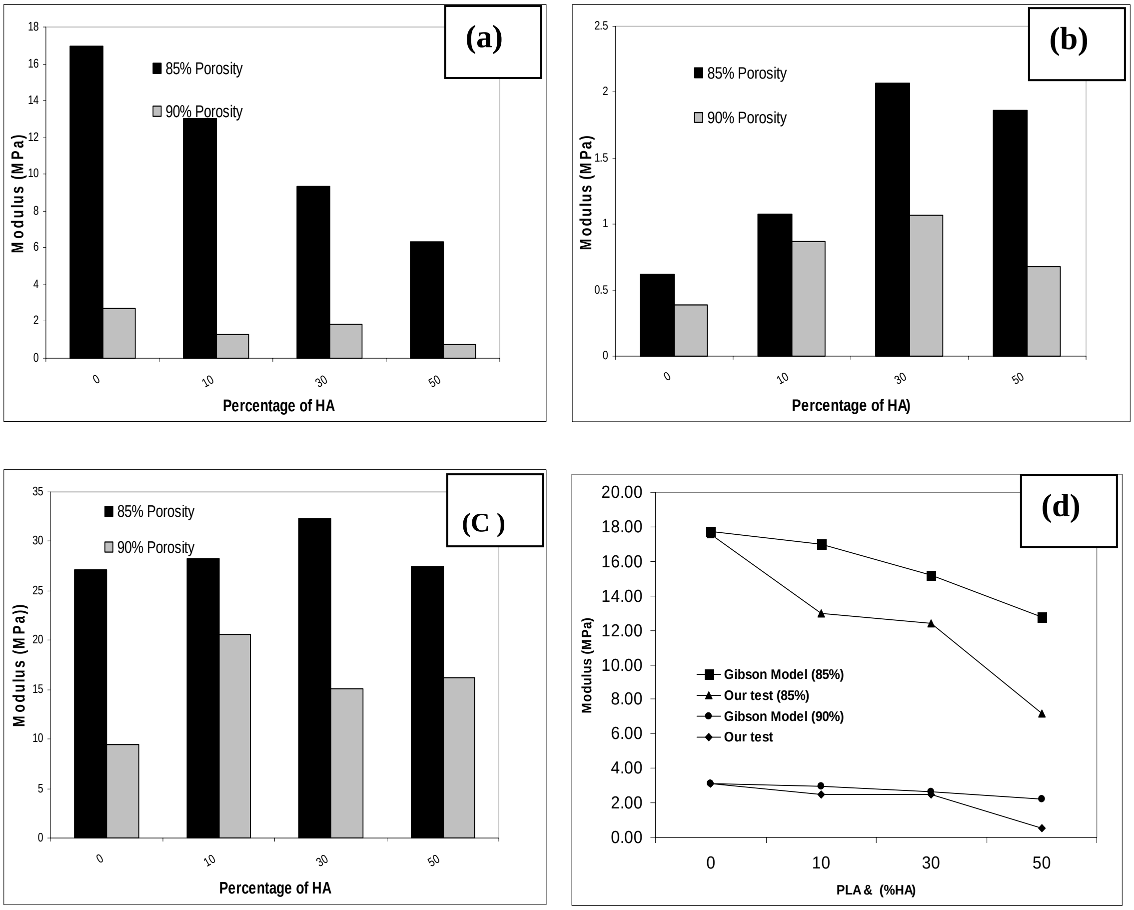

Figure 3: (a) modulus of the first stage of compression in elastic region (b) modulus of the second stage of compression in collapse of the wall (c) modulus of the third stage of the compression in densification for different scaffolds with 85% and 90% porosity. (d) Comparison of the modulus of the scaffolds measured with the Gibson model predictions.

In the current work we have concluded that C=1.5 gives a better data fit for the scaffolds with 85% porosity whereas for the scaffolds of the 90% porosity C=1.0 fits the data better. This could be explained by the increased irregularity in the scaffolds with 90% porosity. The modulus of the first stage of compression for 85% and 90% porosity and different compositions are estimated using the above model. The results of this estimation along with the compression test results are compared in figure 3d. Gibson model predictions match our test results very closely. Though with addition of the HA the model continuously overestimates the modulus of the composite scaffolds. The low modulus of the scaffolds as a result of addition of HA is related to the lack of integration of the phases, micropores and agglomeration of HA particles.

CONCLUSIONS

► The proposed sintering process is able to produce bio-resorbable scaffold which offers properties comparable to the commercial ceramic scaffolds.

► The produced scaffold has pore size distribution in range of 300-500 μm at the surface and around 200 μm internal pore size which is suitable for tissue growth.
► The produced scaffold has a network morphology with interconnected pores which assist the tissue interaction.
► Addition of the HA results in rough edge micro-pores. The effect on the tissue growth needs to be examined.
► HA results in increased compressive stiffness during pore collapse and densification.
► Addition of the HA to PLA results in slight reduction in pore size, increase of the wall thickness of the pores and pore surface area and reduction in anisotropy in the structure.

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