

This article was downloaded by:

On: 15 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Experimental Nanoscience

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t716100757>

PHBV/PLLA-based composite scaffolds containing nano-sized hydroxyapatite particles for bone tissue engineering

N. Sultana^a; M. Wang^a

^a Department of Mechanical Engineering, The University of Hong Kong, Hong Kong

To cite this Article Sultana, N. and Wang, M.(2008) 'PHBV/PLLA-based composite scaffolds containing nano-sized hydroxyapatite particles for bone tissue engineering', Journal of Experimental Nanoscience, 3: 2, 121 – 132

To link to this Article: DOI: 10.1080/17458080701867429

URL: <http://dx.doi.org/10.1080/17458080701867429>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

PHBV/PLLA-based composite scaffolds containing nano-sized hydroxyapatite particles for bone tissue engineering

N. Sultana and M. Wang*

Department of Mechanical Engineering, The University of Hong Kong, Hong Kong

(Received 26 July 2007; final version received 13 December 2007)

Composite scaffolds made of PHBV/PLLA blends and nano-sized hydroxyapatite (HA) particles can be useful for bone tissue engineering as different blends of PHBV with PLLA should have higher degradation rates than PHBV itself and scaffolds containing bioactive HA particles will provide desired osteoconductivity. In this investigation, composite scaffolds were produced via an emulsion freezing/freeze-drying process and subsequently characterised using several techniques. Highly porous and interconnected scaffolds with porosity greater than 70% could be made. It was found that the crystallinity of PHBV in the scaffolds was decreased due to blending with the PLLA polymer, which is beneficial for the faster degradation of the scaffolds *in vitro* and *in vivo*. It was shown that the incorporation of HA nanoparticles enhanced compressive mechanical properties of scaffolds.

Keywords: poly(hydroxybutyrate-co-hydroxyvalerate); poly (L-lactic acid); polymer blend; scaffold; freeze drying; porosity; crystallinity; degradation

1. Introduction

Tissue engineering (TE) seeks to provide a new solution to human tissue loss [1]. Biodegradable scaffolds with appropriate porous microstructures are commonly used in TE. The use of temporary scaffold is envisaged in the situation where the natural body tissue has been weakened by disease, injury or surgery and for this reason requires artificial support [2]. Scaffolds should have high porosity, high interconnectivity and proper pore sizes in order to facilitate cell adhesion, tissue in-growth and mass transportation. The appropriate pore characteristics of scaffolds are vital in TE, especially considering the late stage of implantation when cells need to migrate deep into the scaffold [3–5]. The requirements for TE scaffolds have already been well documented in the literature [3–5]. Commonly used biodegradable polymers for TE scaffolds include poly (glycolic acid) (PGA), poly (lactic acid) (PLA) and their copolymers poly (lactic acid-co-glycolic acid) (PLGA). Polyhydroxybutyrate (PHB) and poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) are natural polymers which can be used in tissue engineering. PHB and PHBV polymers have longer degradation times than PLA and PLGA polymers, which is

*Corresponding author. Email: memwang@hku.hk

useful for tissue engineering scaffolds as this allows scaffolds to maintain mechanical integrity until there is sufficient tissue formation in the constructs. Compared to PHB, PHBV copolymers are less brittle and are easier to be thermo-mechanically processed. PHBV copolymers have also been found to cause minimal inflammatory reactions in long-term studies of implants in mice and rats because their degradation product is a normal constituent of human blood [6].

Blending of biodegradable polymers can reduce the overall cost of materials and modify both mechanical properties and degradation rates of materials (i.e., polymer blends) [7]. The driving force for studies in this area is the control of the kinetics and properties during degradation, together with the requirement of specific properties for specific applications. PHBV polymers are hydrophobic and their abiotic hydrolysis is relatively slow [8]. It has been reported that the surface area and thickness of a film had little effect on the rate of biodegradation of PHB or PHBV. The major factor controlling the microbial hydrolysis rate of the PHB or PHBV is the degree of crystallinity [9]. On the other hand, poly (L-lactide) (PLLA) degrades much faster than PHBV via hydrolysis in the presence of water and is hence a resorbable, non-toxic material for surgical uses [2,10]. The miscibility, crystallisation and melting behaviour of blends of PHB and PLLA were studied and the results indicated that low molecular weight PLLA ($M_n=1,759$) was miscible in the melt over the whole composition range, while a blend of high molecular weight PLLA ($M_n=159,400$) with PHB showed biphasic separation [11]. It was found from another study that PHB/PLA blends prepared through solvent casting were immiscible over the range of composition studied, while the melt-blended samples prepared at a high temperature showed some evidence of miscibility and the crystallisation of PHB in the blends was affected by the level of addition of PLA [12]. It was also reported that the thermal history caused a depression of the melting point and a decrease in the crystallinity of PHB in the blends. It was demonstrated that compared with PHB, the polymer blends exhibited improvements in mechanical properties. Films of PLLA/PHBV blends were also found to be immiscible and it was reported that PLLA in the blends began its degradation in a few weeks (around 2 weeks) whereas the eventual degradation of PHBV was observed up to 53 weeks [13]. It is likely that the degradation behaviour of TE scaffolds can be controlled by varying the composition of PHBV/PLLA blends. However, there have been rarely, if any, reports on scaffolds made of PHBV/PLLA blends.

Hydroxyapatite (HA), which is similar to the mineral component of natural bone and possesses osteoconductivity (i.e., capability to promote bone formation), has been extensively studied and now used for bone tissue repair [14]. For bone tissue engineering, biodegradable composite scaffolds containing osteoconductive HA particles are being investigated [15]. The incorporation of nano-sized HA particles in PHBV/PLLA blend scaffolds should render these scaffolds osteoconductive and thus potentially useful in bone tissue engineering.

A number of techniques have been developed for the fabrication of polymer-based tissue engineering scaffolds [10]. In this investigation, the emulsion freezing/freeze-drying technique, which has been successfully used to produce PHB and PHBV scaffolds [16] and HA/PHBV composite scaffolds [17], was employed for scaffold fabrication. The pore structure and morphology, crystallinity, and compressive mechanical properties of scaffolds were subsequently investigated.

2. Materials and methods

2.1. Materials

Both PHBV and PLLA used in this investigation were commercially available. The PHBV contained 2.9% of 3-hydroxyvalerate and had an average molecular weight of 310,000. The PLLA had an inherent viscosity of 1.6dL/g. These polymers were used in their as-received state without further purification. All chemicals were of analytical grade and were also used in the as-received state. The nano-sized HA used for composite scaffolds was produced in-house through a nanoemulsion process [18]. Figure 1 shows the nano-sized HA together with a selective area diffraction (SAD) pattern of the particles. (The SAD pattern was obtained using a transmission electron microscope.) As with the previous study [18], the particle size of the HA powders was found to be in the range of 20–30 nm. The nanoparticles were basically HA crystallites precipitated from the chemical reaction in the nanoemulsion process.

2.2. Methods

2.2.1. Fabrication of scaffolds

The PHBV/PLLA and HA-containing PHBV/PLLA composite scaffolds were produced using an emulsion freezing/freeze-drying technique [16, 17]. The scaffolds were made using various polymer blend ratios (PHBV/PLLA: 100/0, 75/25, 50/50, 25/75, 0/100) and different polymer solution concentrations (5%, 7.5%, 10% (w/v)). Various amounts of nano-sized HA (5%, 10%, 15% and 20% by weight) were incorporated into composite scaffolds. The production of composite scaffolds consisted of making an emulsion by

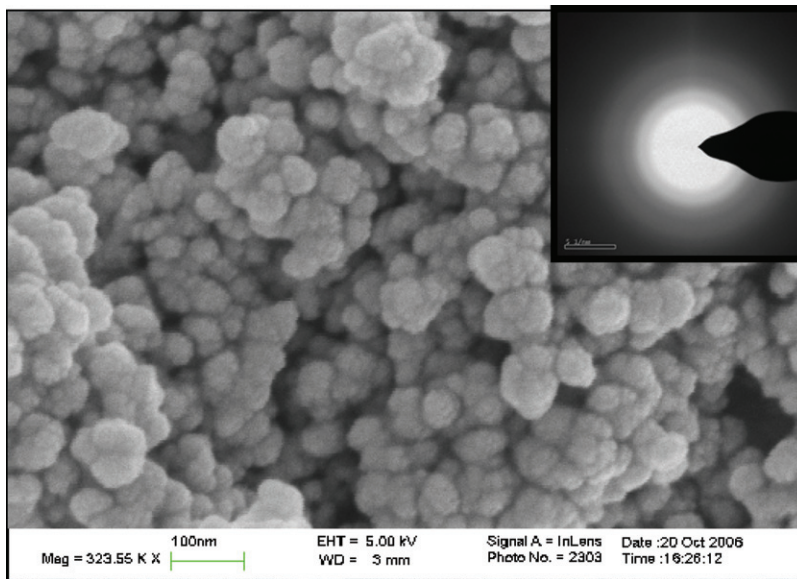


Figure 1. An SEM micrograph of nano-sized HA particles synthesised in-house together with an SAD pattern of the particles.

homogenisation of PHBV/PLLA-chloroform solution, addition of nano-sized HA powder and acetic acid, freezing at a pre-set temperature to induce solid-liquid phase separation, and freeze-drying with a freeze-drying vessel (LABCONCO-Freeze dry system, USA) to sublimate the solvent and water phase crystals.

2.2.2. Characterisation

The emulsion viscosity was measured using a Brookfield-type viscometer. The pore structure and morphology of scaffolds were studied using scanning electron microscopy (SEM; Stereoscan 440, UK). The thermal properties of scaffolds were investigated using differential scanning calorimetry (DSC, Pyris 6, Perkin-Elmer, USA). The density and porosity of scaffolds were determined using the liquid displacement method [16]. Energy dispersive X-ray spectrometry (EDX, INCA 300, UK) was performed in order to determine the presence and distribution of HA nanoparticles in composite scaffolds. The degree of crystallinity, X_c , of scaffold samples was calculated according to the equation given by Iannace et al. [19] and with the use of DSC melting curves of scaffolds.

2.2.3. Mechanical testing

Compressive mechanical properties of scaffolds were determined using a mechanical testing machine (Instron 5848, USA) at a crosshead speed of 0.5 mm/min. From each scaffold sample, cubic specimens of 5 mm × 5 mm × 5 mm were prepared for compression tests. The compressive modulus of a scaffold specimen was calculated from the initial linear region of the stress–strain curve. At least three scaffold specimens were tested for each type of scaffolds.

3. Results and discussion

3.1. Emulsion viscosity

When using the emulsion freezing/freeze-drying technique to produce TE scaffolds, the quality of the emulsion prepared can affect the scaffold quality and also pore characteristics. Furthermore, too viscous an emulsion will hinder the dispersion and uniform distribution of nano-sized HA particles in the emulsion and consequently in the resultant scaffolds. Table 1 shows the emulsion viscosity of the PHBV/PLLA polymer blend of different compositions. The viscosity increased with the increase in PLLA component.

Table 1. Emulsion viscosity, melting enthalpy, and crystallinity of PHBV/PLLA scaffolds.

PHBV/PLLA ratio (10% w/v polymer solution)	Emulsion viscosity (cp)	ΔH_m (J/g)	Crystallinity (%)
100/0	326.7	83.13	61
75/25	330.0	64.94	53
50/50	333.5	43.19	45
25/75	359.4	38.20	38
0/100	388.3	31.89	31

It has been well established that polymer emulsions present large interfacial areas and any reduction in interfacial free energy can reduce the driving force towards emulsion coalescence. The presence of an emulsifier reduces the interfacial free energy and thereby stabilises the emulsion. It was reported that at certain emulsion viscosity, the polymers themselves acted as emulsifier [20]. Therefore, the stability of the emulsion can be set by varying the polymer solution concentration. In the emulsions for making composite scaffolds, nano-sized HA particles were present. The presence of these nanoparticles may have also promoted emulsion stability.

3.2. Microstructure of scaffolds

Freeze-drying of the polymer/solvent/HA/water phase emulsions had produced hard and tough scaffolds with interconnected but anisotropic pores having a good handling quality. The PHBV/PLLA polymer scaffolds had pore sizes ranging from several microns to more than three hundred microns. The average pore diameter of 50/50 PHBV/PLLA scaffolds made from 10% w/v polymer solution was found to be around 270 μm . The porosity of these scaffolds was greater than 70%. Figure 2 shows the morphology of PHBV/PLLA scaffolds and PHBV/PLLA composite scaffolds containing nano-sized HA particles of different amounts (5, 10, 15 and 20%). An anisotropic pore morphology (elongated pores) was present. Internal ladder-like microstructures were observed in pores of scaffolds, which may be due to solid-liquid phase separation or heat transfer direction during freeze-drying.

The current investigation has demonstrated that HA nanoparticles could be incorporated successfully into composite scaffolds. The HA-incorporated composite scaffolds retained similar porous morphology as that of scaffolds made from PHBV/PLLA blends although a high HA loading (i.e. 20%) could result in scaffolds which were brittle in nature and possessed smaller pores. EDX analyses were conducted at different locations of composite scaffolds and the results confirmed the presence of HA nanoparticles inside pore walls (Figure 3).

In the current investigation, the polymer or HA/polymer emulsions were made in glass beakers, with the top of beakers being wrapped with an aluminium foil and the glass beakers being kept in a freezer at -35°C for freezing. Due to the temperature gradient (the emulsion temperature was around $45\text{--}50^{\circ}\text{C}$ whereas the freezer temperature was -35°C), the solvent solidification front proceeded mainly from the bottom to the top of the solution within a few hours. The scaffolds made therefore had channels parallel to the direction of solidification which may be due to the solid-liquid phase separation. The solid-liquid phase separation can be attributed to the crystallisation of the solvent. The crystallisation of the solvent took place when the temperature of polymer solution was lower than the freezing point (crystallisation temperature) of the solvent.

It is possible to accomplish phase separation during the freeze-drying cold stage by freezing the polymer solution below the solution's theta temperature and ultimately below the freezing point of the solvent [21]. A solution's theta temperature can be defined as the temperature at which the coiled polymer molecules expand to their full contour lengths and become rod shaped. It is also known as Flory temperature [22]. It was reported that forcing the phase separation by changes in the free energy of mixing is a more general approach which allows gels of polymers to be prepared. The "incompatibility" of

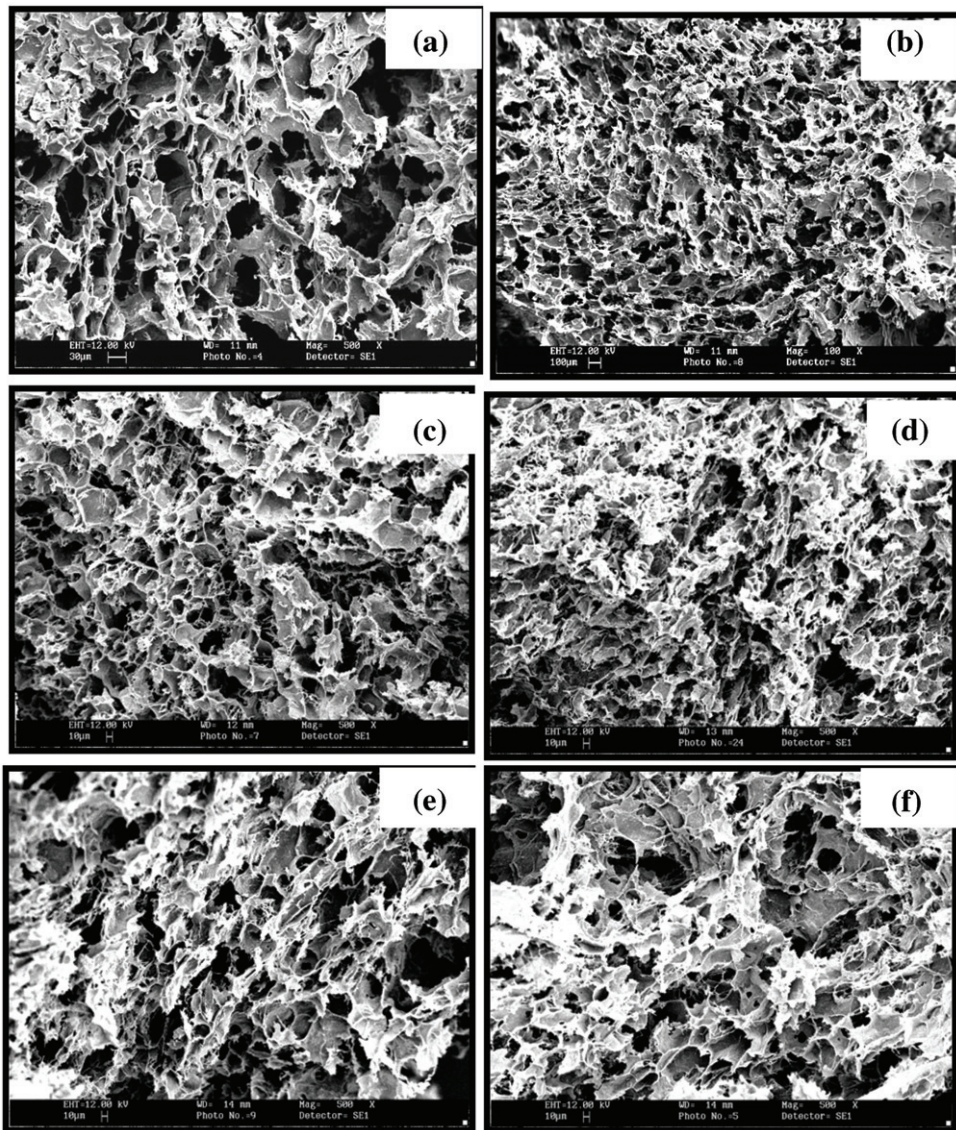


Figure 2. SEM micrographs of scaffolds: PHBV/PLLA scaffolds of (a) 75/25, (b) 50/50 blend ratios; PHBV/PLLA composite scaffolds (all with the 75/25 PHBV/PLLA matrix) of (c) 5% of HA, (d) 10% of HA, (e) 15% of HA, (f) 20% of HA.

solvent/polymer associated with mixing has a large entropic component and the removal of the solvent from these two-phase systems results in a foam structure [21]. It was also demonstrated that the quenching rate of the original homogeneous polymer solution had a profound effect on the scaffolds prepared by freeze-drying [23]. A faster quenching usually results in a decrease of the average pore size due to the rapidly frozen two-phase structure leaving small pores upon solvent sublimation. When the cooling is slow, phase

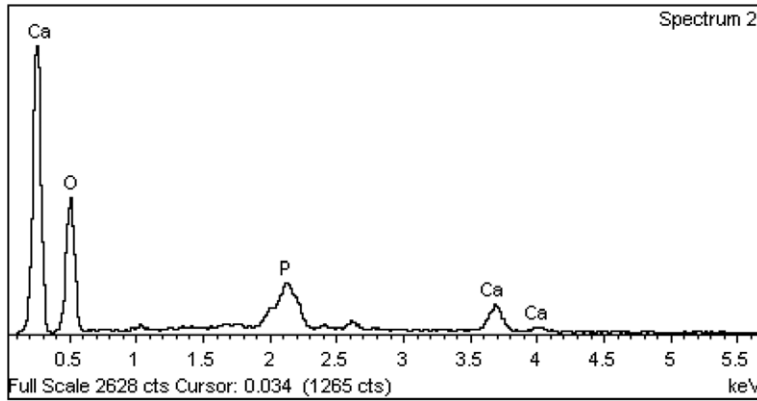


Figure 3. An EDX spectrum obtained from a composite scaffold.

coalescence occurs in order to decrease the interfacial energy, which ultimately results in larger pores. It should be borne in mind that the physical mechanism of convection is related to the heat conduction through the thin layer of fluid adjacent to the heat-transfer surface. Fourier's law is applicable in both conduction and convection. Besides, fluid mechanics must be brought into play in the convection problem in order to establish the temperature gradient [24]. It can also be demonstrated that the initial cooling rate is faster at the bottom and on the surface of the mould than the final cooling rate at the centre of the mould. The phase separation front proceeds more rapidly through the solution with the freeze front falling further and further behind as the thermal conductivity of the organic solution is greater than that of the frozen polymer/solvent mixture. The time lag allows different microstructures to be formed at the surface and the centre of a sample.

The characteristic internal ladder-like structure noted in the current investigation was also observed by other researchers [23,25]. It seems that the polymers were rejected from the solvent crystal front with the formation of radial sheets of an internal ladder-like structure. High-concentration emulsion and relatively fast freezing are known to be favorable for this particular inner morphology for scaffolds. Similar porous morphology was exhibited by semicrystalline PLLA scaffolds with a higher degree of organisation than the amorphous scaffolds. The temperature gradient along the solidification direction from the sample surface to the sample center may also lead to anisotropic pore structure. It was reported [25] that the direction of temperature gradient had a great influence on producing ladder-like structures. It was also reported that at high polymer concentrations, the architecture of oriented ladder-like (parallel microtubules with thin partitions) structures could be achieved if the solvent type and the temperature gradient were maintained the same. Directional freezing and subsequent freeze-drying can be used to align the structural elements both in the form of three-dimensional porous structures and two-dimensional oriented surface patterns [26].

3.3. Crystallinity

DSC curves were obtained for PHBV/PLLA scaffolds (Figure 4). With the melting enthalpies determined from DSC analyses, the degree of crystallinity, X_c , of scaffold

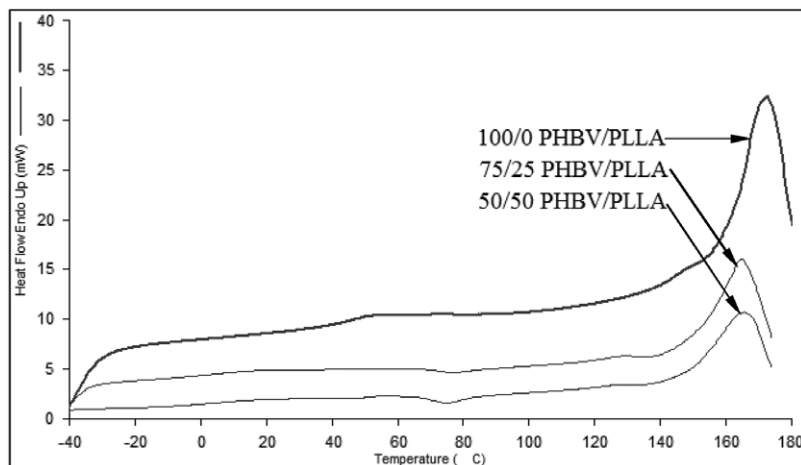


Figure 4. DSC curves of PHBV/PLLA scaffolds: (a) 100/0 PHBV/PLLA scaffold (upper curve), (b) 75/25 PHBV/PLLA scaffold (middle curve), (c) 50/50 PHBV/PLLA scaffold (lower curve).

samples was calculated using Iannace et al.'s method [19]. For pure polymer (PHBV or PLLA), its crystallinity can be calculated using the following equation:

$$X_C = \frac{\Delta H_m}{\Delta H_{m100\%}} \times 100\% \quad (1)$$

where ΔH_m and $\Delta H_{m100\%}$ are the melting enthalpy of the polymer scaffold and the theoretical enthalpy of melting of 100% crystalline polymer, respectively (For PHBV, $\Delta H_{m100\%} = 114 \text{ J/g}$; for PLLA, $\Delta H_{m100\%} = 93.7 \text{ J/g}$). According to Iannace et al. [19], the theoretical value of crystallinity of the polymer blend is the weighted sum of the crystallinity of the two pure polymers, therefore

$$X_C(\text{blend})_{\text{theoretical}} = W_{\text{PHBV}}X_C(\text{DSC})_{\text{PHBV}} + W_{\text{PLLA}}X_C(\text{DSC})_{\text{PLLA}} \quad (2)$$

where W_{PHBV} and W_{PLLA} are weight fractions of respective polymers in the polymer blend, and $X_C(\text{DSC})_{\text{PHBV}}$ and $X_C(\text{DSC})_{\text{PLLA}}$ are respective degrees of crystallinity of PHBV and PLLA which were calculated using DSC curves of 100/0 PHBV/PLLA and 0/100 PHBV/PLLA scaffold samples. In their study [19], Iannace et al. calculated the crystallinity of polymer blends using the aforementioned method and also determined the crystallinity of the polymer blends using the X-ray diffraction method. The values of crystallinity obtained from the two methods were in agreement [19]. Their DSC-based method for determining crystallinity of polymer blends is thus proven valid and was therefore used in the current investigation.

The degree of crystallinity of PHBV/PLLA scaffolds was found to decrease with the addition of PLLA polymers (Table 1). The decrease in crystallinity will lead to increased degradation rate.

PHB-based polymers are hydrophobic and their abiotic hydrolysis is relatively slow. It is known that the surface area and thickness of films have little effect on the biodegradation rate of PHB or PHBV. Several factors can affect the degradation

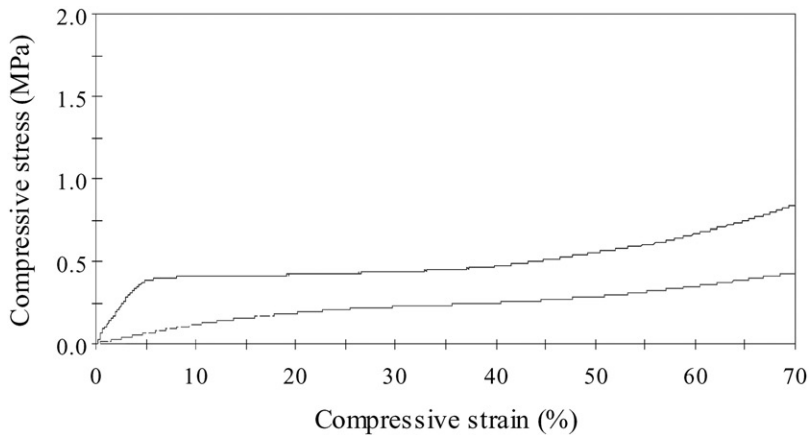


Figure 5. Compressive curves of a PHBV/PLLA scaffold (lower curve) and a PHBV/PLLA composite scaffold containing 15% of HA (upper curve).

rate of polymers. These factors include the configuration of the polymer, copolymer ratio, crystallinity, molecular weight, morphology, stresses, amount of residual monomer, porosity of the scaffold produced, the site of implantation, etc. The degree of crystallinity is the major factor which controls the hydrolysis rate [7] and hence the degradation rate. It was also reported that films of PHB/PLLA blends were more hydrophilic than PHB due to the higher hydrophilicity of PLLA compared to PHB [12], and hence a faster hydrolytic attack was expected for PHBV/PLLA blends. It has been shown that the rate of enzymatic degradation of blend films was higher than that of each single-component film. Therefore, the scaffolds produced in this investigation, which were made of PHBV/PLLA blends, are expected to have higher degradation rates than scaffolds based on PHB or PHBV only.

It was found in a previous study [27] that the addition of HA particles had led to lower degrees of crystallinity of the PHBV matrix in composite scaffolds. The same effect was observed for the PHBV/PLLA composite scaffolds containing HA nano-particles. As crystallinity of the matrix polymer is a major factor for controlling the degradation rate of polymeric scaffolds, from the results obtained in the current investigation, it can be expected that PHBV/PLLA composite scaffolds containing HA nano-particles will have higher degradation rates *in vitro* and *in vivo* than both PHBV scaffolds and scaffolds made of purely PHBV/PLLA blends. The next phase of the study is naturally the investigation into the long-term degradation behaviour and mechanism(s) of these scaffolds. It is envisaged that the degradation rate of PHBV/PLLA composite scaffolds containing osteoconductive calcium phosphate nanoparticles can be carefully controlled through varying the PHBV/PLLA blend ratio and the amount of the nanoparticles in the scaffolds.

3.4. Compressive mechanical properties

Typical compressive curves of PHBV/PLLA and HA/PHBV/PLLA scaffolds are shown in Figure 5. The scaffolds were made from 75/25 PHBV/PLLA polymer blend. Even though polymer blends of different blend ratios (PHBV/PLLA: 100/0, 75/25, 50/50, 25/75, 0/100)

were used for making scaffolds, the current research focused on using the 75/25 PHBV/PLLA polymer blend for scaffolds due to a few reasons: increasing the degradation rate of PHBV-based scaffolds by having a sufficient amount of PLLA in the blend, blending enough PLLA with PHBV while avoiding the use of larger amounts of PLLA which is currently much more expensive than PHBV, etc. Although various amounts of nano-sized HA (5, 10, 15 and 20% by weight) were incorporated into composite scaffolds, the current investigation concentrated on scaffolds containing no more than 15 wt.% of HA. It was found previously [16, 17] that after the incorporation of a large amount of HA (>15 wt.%), the scaffold morphology changed significantly. A large amount of HA in the polymer solution perturbed solvent crystallisation and was found to result in closed pores, decreased porosity and reduced pore size. Therefore, Figure 5 only displays the curve from an HA/PHBV/PLLA scaffold containing 15 wt.% of HA in comparison with a PHBV/PLLA scaffold. The PHBV/PLLA composite scaffolds containing HA nanoparticles exhibited a higher level of resistance to compressive load than PHBV/PLLA scaffolds. This clearly demonstrates the benefit of using HA nanoparticles to reinforce polymeric scaffolds.

As shown in Figure 5, under the compressive force, PHBV/PLLA and HA-containing PHBV/PLLA composite scaffolds underwent three stages of deformation, which are commonly observed for porous structures (the so-called “cellular structures” in solid mechanics) [28]. Under compression, the scaffolds exhibited linear elasticity at low stresses followed by a long plateau of cell wall collapse and then a regime of densification in which the stress rose steeply. The linear elasticity is controlled by cell wall bending, the plateau is associated with collapse of the cells (of the “cellular structure”) and when the cells have almost completely collapsed, opposing cell walls touch, with further strain compressing the solid itself, giving the final region of rapidly increasing stress. The reinforcing effect of HA nanoparticles and the compressive deformation behaviour of PHBV/PLLA and HA-containing PHBV/PLLA scaffolds were similarly observed in the HA/PHBV system [17].

4. Conclusions

Tissue engineering scaffolds based on PHBV/PLLA polymer blends were produced through an emulsion freezing/freeze-drying process. Due to the fabrication technique used, the scaffolds had distinctive porous structures. The crystallinity of the scaffold matrix was found to be decreased due to polymer blending and addition of HA nanoparticles, which is expected to be conducive in enhancing the degradation rate of the PHBV-based matrix. Composite scaffolds containing HA nanoparticles also possessed improved mechanical properties. It is expected that the composite scaffolds will provide a favourable environment for cell attachment and tissue formation in bone tissue engineering.

Acknowledgements

N. Sultana thanks the University of Hong Kong (HKU) for providing her with a postgraduate research studentship. This work was supported partly by an HKU research grant and partly by a CERG grant (HKU 7182/05E) from the Research Grants Council of Hong Kong. Assistance provided by technical staff in the Department of Mechanical Engineering, HKU, is acknowledged.

References

- [1] E. Bell, *Tissue engineering in perspective*, in *Principles of Tissue Engineering*, R.P. Lanza, R. Langer, and J. Vacanti, eds., Academic Press, San Diego, 2000, pp. xxxv–xli.
- [2] B.D. Ratner, A.S. Hoffman, F.J. Schoen, and J.E. Lemons (eds), *Biomaterials Science: An Introduction to Materials in Medicine*, 2nd ed., Academic Press, San Diego. 2004.
- [3] D.W. Hutmacher, *Scaffold design and fabrication technologies for engineering tissue-state of the art and future perspectives*, *J. Biomater. Sci. Polym.* 12 (2001), p. 107.
- [4] P.X. Ma, *Scaffolds for tissue fabrication*, *Mater. Today* 7(May Issue) (2004), p. 30.
- [5] S.J. Hollister, *Porous scaffold design for tissue engineering*, *Nat. Mater.* 4 (2005), p. 518.
- [6] A. Kumarasuriyar, R.A. Jackson, L. Grondahl, M. Trau, V. Nurcombe, and S.M. Cool, *Poly(β -hydroxybutyrate-co- β -hydroxyvalerate) supports in vitro osteogenesis*, *Tissue Eng.* 11 (2005), p. 1281.
- [7] W. Amass, A. Amass, and B. Tighe, *A review of biodegradable polymers: Uses, current developments in the synthesis and characterization of biodegradable polyesters, blends of biodegradable polymers and recent advances in biodegradation studies*, *Polymer international* 47 (1998), p. 89.
- [8] C. Bastioli (ed.), *Handbook of Biodegradable Polymers*, Rapra Technology, UK 2005.
- [9] I.B. Kim, M.C. Lee, I.S. Seo, and P.K. Shin, *The effects of properties on the biodegradation of biodegradable polymers*, *Polymer Korea* 19 (1995), p. 727.
- [10] R.P. Lanza, R. Langer, and J. Vacanti (eds.), *Principles of Tissue Engineering*, 2nd ed., Academic Press, San Diego, 2000.
- [11] E. Blumm and A.J. Owen, *Miscibility, crystallization and melting of poly (3-hydroxybutyrate)/poly (L-lactide) blends*, *Polym.* 36 (1995), p. 4077.
- [12] L. Zhang, C. Xiong, and X. Deng, *Miscibility, crystallization and morphology of poly (β -hydroxybutyrate)/poly (d, l-lactide) blends*, *Polym.* 37 (1995), p. 235.
- [13] B.M.P. Ferreira, C.A.C. Zavaglia, and E.A.R. Duek, *Films of poly (L-Lactic acid)/Poly (Hydroxybutyrate-co-valerate) blends: degradation*, *Mater. Res.* 4 (2001), p. 34.
- [14] M. Wang, *Bioactive materials and processing*, in *Biomater. Tissue Engin.*, D.-L. Shi (ed.), Springer, Berlin, 2004, pp. 1–82.
- [15] ———, *Developing bioactive composite materials for tissue replacement*, *Biomater.* 24 (2003), p. 2133.
- [16] N. Sultana and M. Wang, *Fabrication and characterization of polymer and composite scaffolds based on polyhydroxybutyrate and polyhydroxybutyrate-co-valerate*, *Key Engin. Mater.* 334–335 (2007), p. 1229.
- [17] ———, *Fabrication of HA/PHBV scaffolds through the emulsion freezing/freeze-drying process and characterization of the scaffolds*, *J. Mater. Sci.: Mater. Med.* 19 (2008), p. 2555.
- [18] W.Y. Zhou, M. Wang, W.L. Cheung, B.C. Gao, and D.M. Jia, *Synthesis of carbonated hydroxyapatite nanospheres through nanoemulsion*, *J. mater. Sci. mater. Med.* 19 (2008), p. 103.
- [19] S. Iannace, L. Ambrosio, S.J. Huang, and L. Nicolais, *Poly(3-hydroxybutyrate)-co-(3-hydroxyvalerate)/Poly-L-Lactide blends: thermal and mechanical properties*, *J. Appl. Polym. Sci.* 54 (1994), p. 1525.
- [20] K. Whang and K.E. Healy, *Processing of polymer scaffolds: freeze-drying*, in *Methods of Tissue Engineering*, A. Atala and R.P. Lanza, eds., Academic Press, San Diego, 2002, pp. 697–703.
- [21] L.L. Whinnery, W.R. Even, J.V. Beach, and D.A. Loy, *Engineering the macrostructure of thermally induced phase separated polysilane foams*, *J. Polym. Sci, Part A: Polym. Chem.* 34 (1996), p. 1623.
- [22] I. Teraoka, *Polymer Solutions an Introduction to Physical Properties*, John Wiley & Sons, New York, 2002.

- [23] Ch. Schugens, V. Maquet, C. Grandfils, R. Jerome, and Ph. Teyssie, *Biodegradable and macroporous polylactide implants for cell transplantation: 1. Preparation of macroporous polylactide supports by solid-liquid phase separation*, Polym. 37 (1996), p. 1027.
- [24] J.P. Holman, *Heat Transfer*, McGraw-Hill, New York, 1997.
- [25] P.X. Ma and R. Zhang, *Microtubular architecture of biodegradable polymer scaffolds*, J. Biomed. Mater. Res. 56 (2001), p. 469.
- [26] H. Zhang, I. Hussain, M. Brust, M.F. Butler, S.P. Rannard, and A.I. Cooper, *Aligned two-and three dimensional structures by directional freezing of polymers and nanoparticles*, Nature Mater. 4 (2005), p. 787.
- [27] N. Sultana and M. Wang, *Nano-hydroxyapatite/Polyhydroxybutyrate-co-valerate Composite Scaffolds for Bone Tissue Engineering. Society For Biomaterials 2007 Annual Meeting*, Chicago, IL, USA, 2007, p. 365.
- [28] L.J. Gibson and M.F. Ashby, *Cellular Solids: Structure and Properties*, Cambridge University Press, Cambridge, 1997.