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The double porogen approach as a new technique for the fabrication of interconnected poly(L-lactic acid) and starch based biodegradable scaffolds

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Abstract One of the most widely used fabrication methods of three dimensional porous scaffolds involves compression moulding of a polymer salt mixture, followed by salt leaching. However, the scaffolds prepared by this technique have typically limited interconnectivity. In this study, besides salt particles, an additional polymeric porogen, poly(ethylene oxide), PEO, was added to poly(L-lactic acid), PLLA, to enhance the interconnectivity of the scaffolds. Compression moulded specimens were quenched and put into water, where PEO crystallized and phase separated. Following the leaching of PEO fraction, the permeability and interconnectivity among the macropores formed by salt leaching could be observed. The porosities obtained in the prepared scaffolds were between 76 to 86%. Moreover, the highest porosity of 86% was obtained with minimum fraction of total porogen. The water absorption of the porous scaffolds prepared with PEO could vary between 280 to 450% while water uptake of pure PLLA scaffolds was about 93%. The increase of interconnectivity induced by compounding PLLA with PEO could also be obtained in porous PLLA/starch blends and PLLA/hydroxyapatite composites demonstrating the versatility and wide applicability of this preparation protocol. The simplicity of this organic solvent free preparation procedure of three-dimensional porous scaffolds with high interconnectivity and high surface area to volume ratio holds a promise for several tissue engineering applications.

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1 Introduction

Tissue engineering is an emerging field attempting to repair or replace the function of defective or damaged tissues or organs. The essential steps to engineer tissues are the development of adequate scaffolds for different anatomical locations in the body. Generally, the materials of scaffolds are either of natural origin or synthetic biodegradable polymers [1–5]. Synthetic polymers have design flexibilities in terms of materials composition, processability, control over micro and macro structures, and mechanical properties [3]. These properties can be tuned for specific applications. Moreover, natural based polymers are also used in biomedical applications for their excellent biocompatibility and biodegradability [5–7].

Poly(α-hydroxy acids) including poly(lactic acid), poly(glycolic acid) and their co-polymers are the widely-accepted polymers for most of the tissue engineering applications [1, 3, 8]. These thermoplastic polyesters have reasonable biocompatibility, biodegradability, mechanical strength and easy processability. Blends of PLLA and starch (SPLA) have also been proposed in our group to be used in the biomedical field, and were found from cell adhesion and proliferation tests, to interact positively with cells [9]. Poly(ethylene oxide) (PEO) is a thermoplastic and water soluble polymer. Moreover, PEO is biocompatible and currently being used in biomedical applications [10]. PEO has been used in this study as the second porogen to obtain interconnected matrix of PLLA.

Bioactive ceramics such as hydroxyapatite (HA) spontaneously forms bonelike apatite in living body and bonds to the host tissue [11]. However, the high elastic moduli and low fracture toughness constitute the drawbacks in the use of such materials as implants [12]. The blends of the biodegradable polymers with bioactive ceramics would allow processing the composites by conventional processing technique, improve the fracture toughness as well as introduce bioactivity in the polymer-ceramics composite scaffolds.

Several techniques have been used to develop porous scaffolds of different shape, size and 3D geometry. Interconnectivity of the pores or pore walls is one of the important requirements for supply of nutrients for cell growth and carrying away the metabolic wastes [13]. An interconnected network of pores could be prepared by freeze drying or thermally induced phase separation techniques as the solution is a continuous phase. The limitations of this process arise from poor solubility of polymers in most of the solvents and the potential toxicity from the residual organic solvents [14]. The macro pore sizes obtained by this process lie between 80 to 100 μm. The interconnected pore sizes could be increased by emulsification of polymer solvent solution [15]. The solvent casting/particulate leaching process involves mixing of particulates into polymer solution, casting the suspension to produce a membrane, and the porous scaffolds are obtained by removing salt and solvent [8].

The fabrication of porous scaffolds from the polymer melt is the most convenient method, as it allows the fast production of scaffolds of different shape and sizes in an efficient way. The most straightforward way is to melt a polymer/salt mixture, cool it and subsequently remove the salt particles producing the porous scaffolds. The desired porosity and pore sizes could be independently controlled by salt fraction and sizes of salt particles respectively. This technique produces scaffolds with trapped salt particles at the interior of polymer matrix and poor interconnectivity is often obtained [16].

In this paper, we describe an original technique for the preparation of porous three-dimensional PLLA based scaffolds using double porogens and compression moulding. The aim of this study was to develop a way to obtain an interconnected porous PLLA based scaffolds by simultaneous use of two porogens: (1) a particulate NaCl to create the macro-porosity; and (2) a polymeric porogen, PEO that will create a unique surface topography and permeability as well as the interconnectivity within the pore walls. Moreover, it must be noted that the polymeric porogen, PEO is miscible with PLLA. The proof of concept was extended to the enhancement of interconnectivity to porous scaffolds of PLLA/starch blends (SPLA) and PLLA/HA composites.

2 Materials and methods

2.1 Materials

A commercial grade PLLA of high stereoregularity from Cargill-Dow was used in this study. The specific optical rotation, $[\alpha]_D^{25}$ of this PLLA was 154.7 as measured in chloroform at a concentration of 1 g.dl^{-1} and 25°C (AA-1000 Polarimeter). PLLA was estimated to have L-lactide content of 99.6% by assuming $[\alpha]_D^{25}$ of poly(L-lactic acid) and poly(D-lactic acid) to be -156 and 156 respectively [17]. The PLLA had $M_n = 69{,}000$ and polydispersity of 1.73 as determined by gel permeation chromatography (Shimadzu LC,10A, Japan) in chloroform with the standard of polystyrene.

The PEO used in this study was Polyox WSR N-10, $M_n = 100{,}000$ from Dow Chemical company, USA. A 30/70 (wt%) blend of starch and PLLA (SPLA70) was used in this study. The SPLA70 was obtained by melt blending of 70 wt% PLLA and 30 wt% of corn starch. The PLA of SPLA70 had an L-lactide content of 94%. The hydroxyapatite (HA) used in this study was supplied by Plasma Biotal Ltd, U.K. This sintered HA had an average particle size of 10.1 μm.

2.2 Preparation of the scaffolds

2.2.1 Powder preparation and compounding

All individual polymers were pre-dried at 50°C for 4 h in a vacuum oven. PLLA and SPLA70 were cryogenically milled in an ultra-centrifugal mill (Retsch, ZM-100) with liquid nitrogen at 14000 rpm. NaCl particles of 250 to 500 μm particle sizes were hand-sieved with the stacked stainless steel sieves (ISO3310, Endecotts Ltd., England).

2.2.2 Compression moulding

The compression-moulded discs of 9 mm thickness and 80 mm diameter were prepared on a steel mould using a Moore hydraulic press (UK), with 50 ton capacity. The milled polymers were dried at 50°C for 4 h in a vacuum oven prior to compression moulding. PLLA and SPLA70 powders were individually mixed with PEO; then the mixture was blended with the sieved NaCl particles. The polymer/salt mixtures were put in between teflon release papers in the mould. The mould was placed in between the hot plates of the hydraulic press at top and bottom platen temperature of 180°C. To remove the trapped air bubbles, the pressure was slowly raised to 10 tons and released, and this process was repeated for five times. The polymer/salt mixture was then allowed to melt for 7 min at constant load of 151 kPa, the weight of top half of the mould. The mould was immediately quenched from 180°C to around 0°C using ice water. The initial compositions of the mixtures

Table 1 Volume (%) composition of the mixtures used to prepare the studied scaffolds

Scaffolds	PLLA	PEO	NaCl	PLLA: PEO
PLLA	43	0	57	100:0
PLLA-PEO	38	5	57	88:12
PLLA-PEO	33	10	57	77:23
PLLA-PEO	30	14	56	68:32
PLLA-PEO	26	18	56	59:41
SPLA70	43*	0	57	100:0
SPLA70-PEO	30*	14	56	68:32

*SPLA70 was used in place of PLLA.

that were used to prepare the studied scaffolds are shown in Table 1.

We have maintained a fixed and reasonably lower salt fractions to obtain an essentially closed porous matrix. The hypothesis was to check whether the relatively thicker pore walls and confined macropores could be interconnected with the addition of PEO, trying to keep the same initial (essentially) closed pore morphology created by the leaching of the salt particles.

2.2.3 Porogen leaching

The moulded discs were immediately sliced to 4.5 × 4.5 × 9 mm^3 and put into water. The leaching was performed in beakers filled with de-ionized water at 37°C. The water was replaced in every 4 h. The presence of NaCl content was checked by silver nitrate solution. The leaching protocol was continued till 72 h.

2.3 Bioactivity test

The apatite-forming ability of porous PLLA / hydroxyapatite composites was assessed by *in vitro* tests in a simulated body fluid, SBF [18]. Prior to incubating the porous scaffolds to SBF, a series of depressurization and repressurization cycles were performed in solution to remove the trapped air and to make wet the internal surface of the pores. Three scaffolds were immersed in each polyethylene test tubes containing 50 ml SBF at 37°C for 0, 1, 3, 7, 14 and 30 days. After the predetermined time periods, the samples were taken out of the test tubes, rinsed in deionised water (pH = 5.35) to remove the inorganic ions and dried at 30°C under vacuum.

2.4 Characterisation of the scaffolds

2.4.1 Water uptake and porosity

The density and porosity of the porous scaffolds were determined by measuring the dimensions and mass of the scaffolds. The density (ρ) of the scaffolds was calculated as follows $\rho = m / V$, where m is the mass and V is the volume of the porous scaffolds.

The porosity of the porous scaffolds was calculated as

$$Porosity(\%) = 100(1 - \rho/\rho_c), \quad (1)$$

where ρ is the density of the porous scaffold and ρ_c is the density of compact amorphous PLLA assumed to be 1.248 g/cm^3 [19].

The water absorption of the porous scaffolds was calculated using the following equation:

$$Water\ uptake(\%) = 100 \times (m_{wet} - m)/m, \quad (2)$$

where m_{wet} and m are the weights of wet and dried scaffolds, respectively.

2.4.2 Morphological characterisation

Porous morphologies and apatite formation of the scaffolds were examined using scanning electron microscopy (SEM) (Leica Cambridge S-360, UK) at 15 kV. The specimens were coated with gold using a sputter coater (JFC 1100, Jeol).

3 Results and discussion

Most of the polymer blends are immiscible because of unfavourable interactions and small entropy enhancement upon blending. A polymer pair tends to be miscible if the difference of solubility parameters is less than 0.5 $(cal.cm^{-3})^{1/2}$. The solubility parameter is defined as $(CED)^{1/2}$, where CED is the cohesive energy density. The solubility parameters of PLLA and PEO are 10.1 and 9.9 ± 1 $(cal.cm^{-3})^{1/2}$ respectively [20, 21]. The close solubility parameters provide an indication for thermodynamic miscibility of PLLA/PEO blend.

The glass transition temperature (T_g) of pure PLLA and pure PEO were 61°C and –54°C respectively. The melting points of pure PLLA and PEO were 162°C and 58°C respectively. The melting point depression of PEO and PLLA in blends compared with pure polymers supports the conclusion that the blends were miscible in melt [19, 22]. The miscibility was further supported by the detection of a single glass transition temperature in between the T_g 's of the pure polymers [22].

A number of PLLA/PEO/NaCl compact discs were compression moulded with PEO content up to 41 vol% and fixed fractions of NaCl particulates, as shown in Table 1. The melt miscibility was kept by quenching the melt from 180°C to 0°C, as the T_g of the blend was above 0°C [22]. After keeping 4 days at room temperature, the compact specimens were sliced and put into water at 37°C. The salt particles dissolve first as the solubility of NaCl in water is high. If only PLLA is

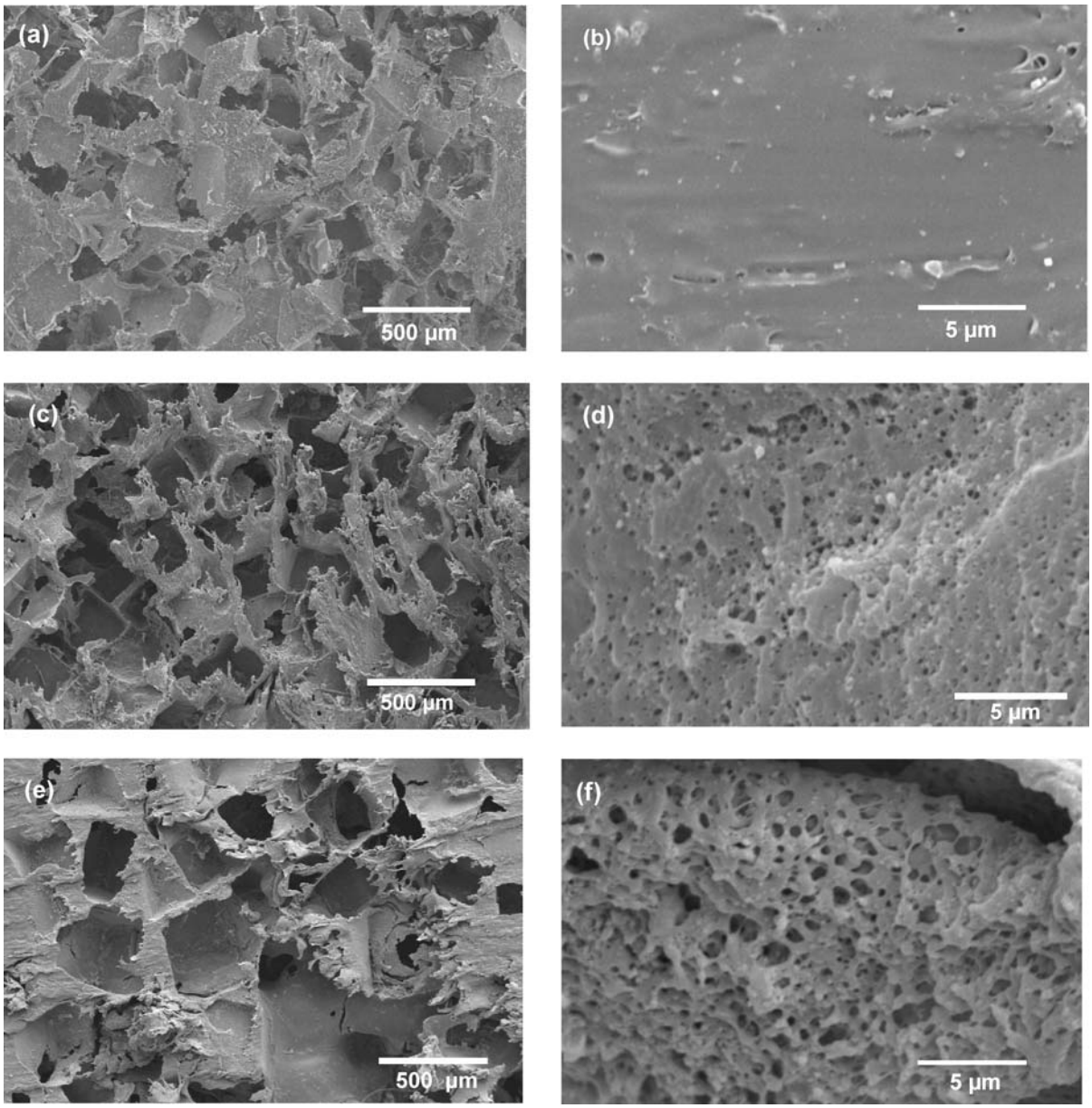

Fig. 1 SEM micrographs of PLLA scaffolds prepared from quenching of PLLA/PEO/NaCl composite melt, kept at room temperature for 4 days followed by leaching of porogens. NaCl particle size = 250–500 μm: (a, b) PLLA: PEO (100:0), (c, d) PLLA: PEO (88:12), (e, f) PLLA: PEO (68:32). Two magnifications are shown for each formulation. The low magnification reveals the interconnectivity of the pores and the higher magnifications shows the texture in the pore walls

mixed with NaCl particles, then a typical macroporous scaffold with low interconnectivity appears—see Figs.1(a) and (b). If a blend of PLLA/PEO is used with NaCl particle, then the walls separating the pores could be permeable with the dissolution of PEO, enhancing the interconnectivity.

Figure 1 shows the porous PLLA scaffolds obtained on delayed incubation of compact PLLA/PEO/NaCl followed by aqueous dissolution of porogens. The shapes of macropores are almost similar to that of pre-seeded salt particles and interconnected pores are scarcely visible. However, the macropore sizes appeared to be increased with increasing PEO fractions (Figs. 1(c) and (e)). Moreover, the micropores originated from dissolution of PEO are visible at higher magnification (Figs. 1(d) and (f)). The shapes of the micropores were similar to droplets and sizes of $\sim$1 μm, the sizes of the micropores were increased with increasing PEO fraction. At room temperature, the PEO may get phase separated with time from initially homogeneous PLLA/PEO with the crystallization of PEO [17, 22]. Upon immersion of this compact PLLA/PEO/NaCl into water, the NaCl and the phase separated PEO were leached out and the micropores were created.

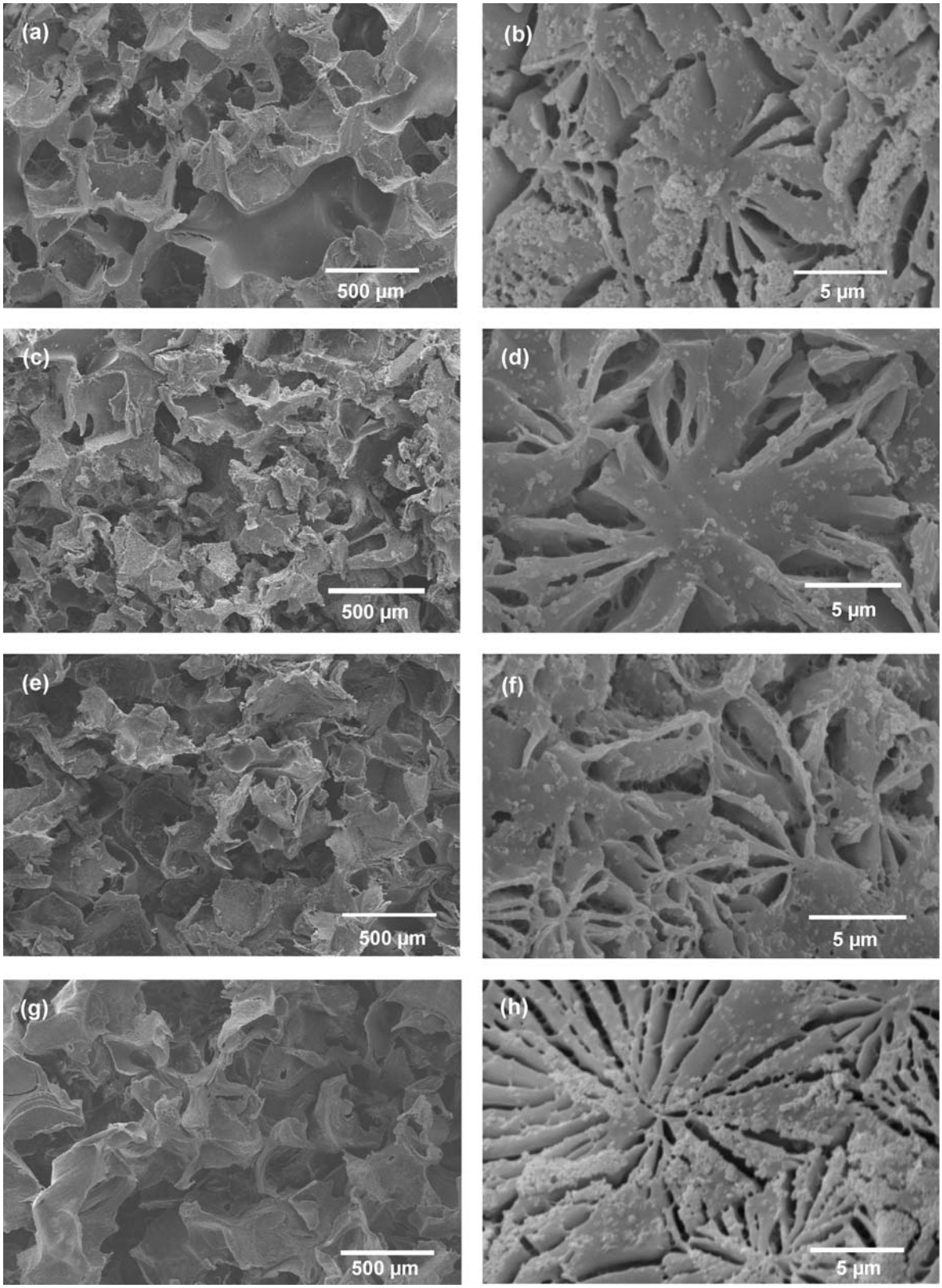

Fig. 2 SEM micrographs of PLLA scaffolds prepared from quenching of PLLA/PEO/NaCl composite melt followed by immediate leaching of porogens. NaCl particle sizes = 250–500 μm: (a, b) PLLA: PEO (88:12); (c, d) PLLA: PEO (77:23); (e, f) PLLA: PEO (68:32); (g, h) PLLA: PEO (59:41). Two magnifications are shown for each formulation. The low magnification reveals the interconnectivity of the pores and the higher magnifications shows the texture in the pore walls

The data in Fig. 1 suggest that with the delayed incubation, the PLLA and PEO phase separates prior to incubation to water. It is thus expected that different porous microstructures could be obtained if the compact and quenched PLLA/PEO/NaCl specimens were immediately put into water at 37°C (see below).

Figure 2 shows the porous PLLA scaffolds obtained on immediate incubation of compact PLLA/PEO/NaCl followed by aqueous dissolution of porogens. PLLA is a hydrophobic polymer, whereas PEO is a hydrophilic polymer. Although, PLLA is hydrophobic, the hydrophilicity of homogeneous PLLA/PEO blend increases with increasing PEO content. When the blend is put in water, the homogeneous PLLA/PEO blend will absorb water and the absorbed water plasticizes the blend and reduces the T_g further. The system can swell until PEO crystallizes and phase separates. The phase separated PEO absorbs water and progressively leaches out. During crystallization, some of the PLLA rich phase may be entrapped within the PEO spherulitic structure. Therefore, upon PEO leaching, the remaining PLLA rich phase will reveal the spherulitic morphology developed during PEO crystallization. The SEM micrographs at higher magnification (Figs. 2(b), (d), (f) and (h)) clearly showed the spherulitic mark created by PEO crystallization followed by leaching. We can conclude that the proposed method can create a microstructure in the pore walls that cannot be obtained using conventional compression moulding and salt leaching process. It could be worth to mention that this kind of pattern could only be prepared by manipulating the miscible phase. The substrate topography created by this technique could play an important role in regulating cell behaviour [23]. Generally, the micro and sub-micro pores are created on substrates by photolithographic techniques [24]. In our study, the permeable pores created by dissolution of PEO may be useful for enhancing cell-substrate interaction.

For sufficiently high PEO contents, a phase separation between PEO -rich phase and homogeneous PLLA/PEO phase will occur. Such PEO—rich phase will be dissolved in water creating pores within the structure that will add enhancement of interconnectivity of the scaffolds as shown Figs. 2(c), (e) and (g). The lower magnification SEM micrographs in Fig. 2 show an increase in pore interconnectivity with increasing fraction of PEO. Therefore, with higher amount of PEO fraction, it is possible to obtain the permeable pores from miscible PLLA/PEO phase, comparatively bigger interconnected pores from immiscible PEO fraction besides the macropores created from dissolution of salt fraction. The miscibility of PEO in PLLA depends on molecular weight of PEO, the stereoregularity of PLLA, and the processing history of the blend [17, 22, 25]. Lower molecular weight PEO is more soluble in PLA whereas PEO is less soluble in higher stereoregular PLA. Under the present experimental set up, the miscibility of PEO in PLLA was in between 12 and 23 vol% of PEO. However, this could be checked using complementary techniques.

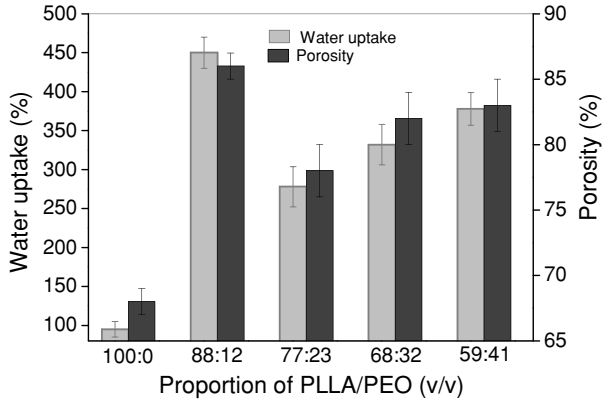

Fig. 3 Water uptake capability and porosity of the PLLA scaffolds obtained from PLLA/PEO/NaCl composite melt followed by leaching of porogens

Figure 3 shows the water uptake capability and porosity of the macroporous PLLA scaffolds prepared by the double porogen approach. PLLA is a hydrophobic polymer and uptakes little water as shown in Fig. 3. Through the introduction of permeable pores by dissolution of PEO from miscible PLLA/PEO blend and the dissolution of PEO rich phase, the overall water absorption (%) of PLLA matrices was increased by 200 to 350%—see Fig. 3. This increase of water uptake could be due to the increase of surface area to volume ratio of scaffolds as the sizes the micropores are of few microns. Moreover, these results indicate that the micro pores created by this technique are accessible to water.

The porosity exactly followed the same trend of water absorption. The overall increase of scaffolds sizes of all the PEO extracted scaffolds indicates that the PLLA/PEO blend swelled before the blends phase separated and the obtained porosities (76 to 86%) were higher than the fractions of preseeded porogen fractions. However, the obtained porosities could be further augmented with the addition of higher fraction of salt.

An unexpected result can be seen in Fig. 2 where the PLLA/PEO 88:12 formulation exhibits higher values of both water uptake and porosity with respect to those with higher PEO content. A possible explanation will be given, related to the different swelling capabilities of the different formulations. For the PLLA/PEO 88:12 formulation, the system was found to swell in a much higher extent than for the pure PLLA scaffold. The hydrophilicity of homogeneous PLLA/PEO blend increases with increasing PEO content. However, the diffusion coefficient of water in the homogenous blend may decrease with increasing PEO content owing to a decrease in fractional free volume [26]. Therefore, the permeability of water in a homogeneous blend of a hydrophobic polymer like PLLA and a hydrophilic polymer like

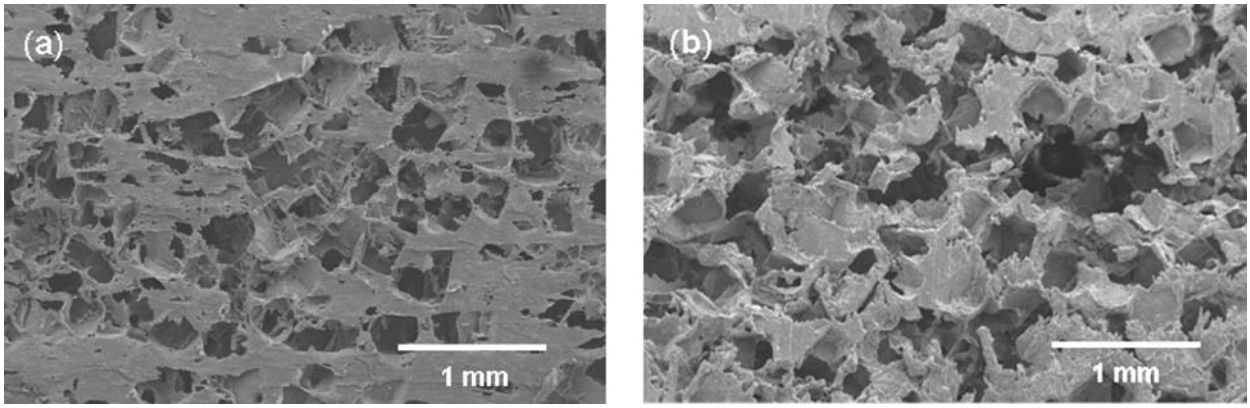

Fig. 4 SEM micrographs of SPLA70 scaffolds prepared from SPLA70/PEO/NaCl composite melt followed by leaching of porogens. NaCl particle size = 250–500 μm: (a) SPLA70/PEO (100:0), (b) SPLA70: PEO (68:32)

PEO would be a balance between hydrophilicity and rate of diffusion. With 88: 12 PLLA: PEO, the permeability could be optimum, where the homogeneous blend absorbs more water. As the T_g of water is -138°C, this absorbed water plasticizes the blend and the blend T_g lowered further. The rate of diffusion may increase due to higher segmental mobility of the plasticized blend. Moreover, the plasticized blend could swell for extended period before phase separation as the difference between the T_g of PLLA and T_c of PEO was increased. We observed that the 88:12 PLLA: PEO formulation took longer time to reach the equilibrium water uptake than all other combinations.

For the formulations with higher PEO content, the water uptake and porosity of the phase-separated blends are quite different from the homogeneous blend. Two possible explanations could interpret the equilibrium water uptake of the scaffolds originated from the phase separated blends. The phase separated PEO fraction is more hydrophilic, uptakes water fast and forms a cluster (hydrated PEO or a viscous PEO solution) around the homogenous blend. The clustering may reduce the rate of diffusion of water to the homogeneous blend [26]. Moreover, the rate of diffusion of water in saturated blend is lower than the unsaturated blends. These two diffusion rates control the water uptake behaviour of phase separated blends. The compositions with 68:32 and 59:41 PLLA/PEO had higher volume fraction of phase separated PEO. The leaching of phase separated PEO increases the pore volume fraction and thereby the water absorption of the scaffolds were increased.

In the process of immediate incubation to water after quenching, the scaffolds gained a set of important attributes i.e. textured spherulitic surface, bigger interconnected pores, higher surface area to volume ratio, excess porosity obtained by swelling and the extent of interconnectivity. The spherical micropores observed on scaffolds with delayed incubation (Fig. 1) appeared more like the ones originated from immiscible polymer blend. During the reviewing process of this work, it has been reported that interconnected porous scaffolds could also be obtained from immiscible polymer blends and salt particle; with precise control of shear rates, viscosity and volume fractions of constituent polymer, quenching followed by selective leaching of porogens [27]. The porous scaffolds originated from immiscible polymer blends closely resembled with the scaffolds originated on delayed incubation.

Figure 4 shows the porous structures of the studied PLLA/starch blend, SPLA70, formed after aqueous dissolution of SPLA70/NaCl (43:57) and SPLA70: PEO: NaCl (30:14:56) compact discs. As expected, the porous SPLA70 matrix created by dissolution of salt reveals only the macroporous structure as shown in Fig. 4(a). Moreover, the interconnectivity was found to be limited. The matrices created with the inclusion of PEO and subsequent dissolution showed noticeable increase in interconnectivity in the porous network (Fig. 4(b)). The porous SPLA70 scaffolds created by double porogen swelled in water by around 20 vol% compared with SPLA70 scaffolds without PEO. Therefore, it can be concluded that an interconnected porous network could be obtained by double porogen approach to the analyzed PLLA/starch containing blends.

In order to assess the role of interconnected micropores on bioactive materials like HA-based composites, two sets of porous scaffolds were created from aqueous dissolution of PLLA/HA/NaCl and PLLA/HA/PEO/NaCl compact specimens. Figure 5 shows the *in vitro* bioactivity of the porous scaffolds after two weeks of incubation in SBF. The scaffolds with higher interconnectivity induced by PEO dissolution often showed more zones of improved bioactivity with cauliflower like apatite growth (Fig. 5(b)) as compared with the scaffolds prepared without PEO (Fig. 5(a)). The higher bioactivity could be assigned to the more hydrophilic character of the matrix created by inclusion of PEO that will allow for better transport and absorption of ions from the solution [28]. The increase in surface area induced by leaching of

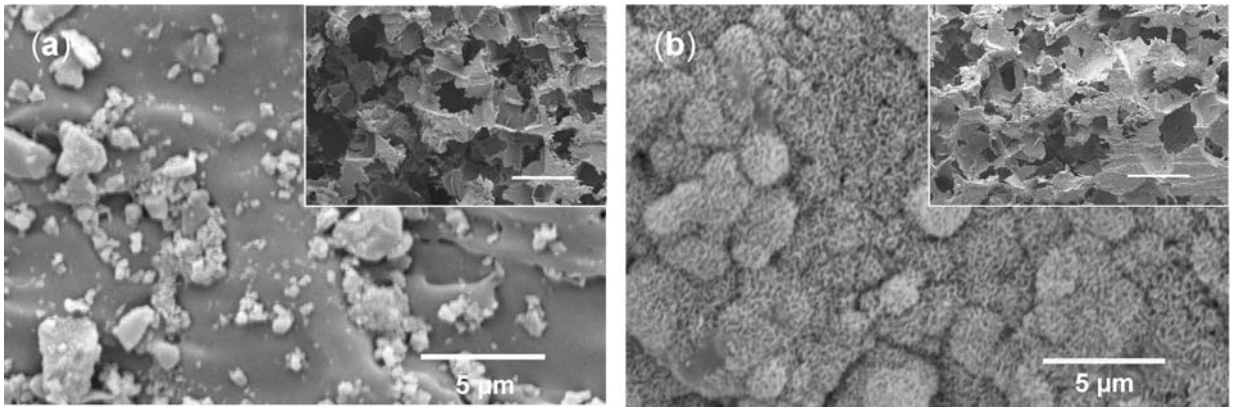

Fig. 5 SEM micrographs of the porous PLLA/HA composite scaffolds after two weeks incubation in SBF: (a) PLLA: HA (86:14); (b) PLLA: HA: PEO (72:12:16). The composite scaffolds were prepared from PLLA/HA/PEO/NaCl composite melt followed by leaching of porogens. The insets are on lower magnification with scale bar = 500 μm

PEO could increase the available nucleation points for the development of the apatite like layer. This result indicates that bioactive materials like HA could also be incorporated in the matrix prepared by double porogen approach and could improve the bioactivity with respect to scaffolds processed by conventional compression moulding and salt leaching.

4 Conclusions

A new technique for the preparation of PLLA-based three-dimensional porous polymeric scaffolds was developed that involved simultaneous melting of two miscible polymers in which the salt particles were dispersed. This technique allowed the fabrication of porous scaffolds where: (i) the global pore sizes and void fraction could be controlled by the size and the fraction of salt; (ii) a spherulitic—like microporous pattern could be produced in the pore walls upon leaching of the PEO fraction crystallized from PLLA/PEO phase that could improve cell attachment and proliferation and (iii) for higher PEO fraction, interconnected pores could be formed in the pore walls by leaching of immiscible PEO fraction.

The porous scaffolds have homogeneous pore structure and the obtained porosities were between 76 to 86% with constant salt fractions of around 57%. Moreover, the highest porosity i.e. ∼86% was obtained with the addition of only 5 vol% of polymeric porogen.

The 350% increase in water uptake indicates that micropores are accessible to water and increases the overall hydrophilicity of the porous scaffolds.

This processing technique of preparing 3D porous scaffolds by compression moulding, quenching and leaching of porogens is a simple and convenient process without any complicated process controls. Scaffolds with higher thicknesses could be prepared by this technique.

It was also found that the interconnectivity of porous scaffolds prepared from this SPLA blend with NaCl could also be increased by compounding with PEO.

Bioactive fillers like hydroxyapatite could be incorporated in the scaffolds prepared by this method. The interconnected composite scaffolds prepared with the double porogen approach exhibited enhanced bioactivity when compared with the composite scaffolds prepared using the conventional compression moulding and salt leaching process.

This simple and organic solvent free processing technique offers the versatility of preparing PLLA-based three-dimensional porous scaffolds with adequate morphology for different tissue engineering applications.

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References

1. L. E. FREED, D. A. GRANDE, Z. LINGBIN, J. EMMANUAL, J. C. MARQUIS and R. LANGER, *J. Biomed. Mater. Res.* **28** (1994).
2. W. L. MURPHY, D. H. KOHN and D. J. MOONEY, *J. Biomed. Mater. Res.* **50** (2000) 50.
3. J. C. MIDDLETON and A. J. TIPTON, *Biomaterials* **21** (2000) 2335.
4. J. M. CHUPA, A. M. FOSTER, S. R. SUMNER, S. V. MADIHALLY and H. W. T. MATTHEW, *Biomaterials*. **21** (2000) 2315.
5. R. L. REIS and J. SAN ROMAN, in "Biodegradable Systems in Tissue Engineering and Regenerative Medicine" (CRC Press, 2005).
6. A. P. MARQUES, R. L. REIS and J. A. HUNT, *Biomaterials* **23** (2002) 1471.

7. M. E. GOMES, R. L. REIS, A. M. CUNHA, C. A. BLITTERSWIJK and J. D. DE BRUIJN, *Biomaterials* **22** (2001) 1911.
8. A. G. MIKOS, A. J. THORSEN, L. A. CZERWONKA, Y. BAO, R. LANGER, D. N. WINSLOW and J. P. VACANTI, *Polymer*. **35** (1994) 1068.
9. A. P. MARQUES, H. R. CRUZ, O. P. COUTINHO and R. L. REIS, *J. Mater. Sci.- Mater. Med.* **16** (2005) 833.
10. J. LI, X. NI and K. W. LEONG, *J. Biomed. Mater. Res. Part A* **65** (2003) 196.
11. J. D. DE BRUIJN, C. A. VAN BLITTERSWIJK and J. E. DAVIES, *J. Biomed. Mater. Res.* **29** (1995) 89.
12. J. A. JUHASZ, S. M. BEST, R. BROOKS, M. KAWASHITA, N. MIYATA, T. KOKUBO, T. NAKAMURA and W. BONFIELD, *Biomaterials* **25** (2004) 949.
13. W. L. MURPHY, M. C. PETERS, D. H. KOHN and D. J. MOONEY, *Biomaterials* **21** (2000) 2521.
14. S. FORMAN, J. KAS, F. FINI, M. STEINBERG and T. RUML, *J. Biochem. Mol. Toxicol.* **13** (1999) 11.
15. K. WHANG, C. H. THOMAS, K. E. HEALY and G. NUBER, *Polymer*. **36** (1995) 837.
16. Q. HOU, D. W. GRIJPMA and J. FEIJEN, *Biomaterials* **24** (2003) 1937.
17. Y. HU, Y. S. HU, V. TOPOLKARAEV, A. HILTNER and E. BAER, *Polymer*. **44** (2003) 5711.
18. T. KOKUBO, H. KUSHITANI, S. SAKKA, T. KITSUGI and T. YAMAMURO, *J. Biomed. Mater. Res.* **24** (1990) 721.
19. D. SHIN, K. SHIN, K. A. AAMER, G. N. TEW, T. P. RUSSELL, J. H. LEE and J. Y. JHO, *Macromolecules* **38** (2005) 104.
20. E. MEAURIO, E. ZUZA and J. R. SARASUA, *Macromolecules* **38** (2005) 1207.
21. J. BRANDRUP and E. H. IMMERGUT, in "Polymer Handbook." (Wiley, NewYork, 1989) p. 555.
22. A. J. NIJENHUIS, E. COLSTEE, D. W. GRIJPMA and A. J. PENNINGS, *Polymer* **37** (1996) 5849.
23. R. G. FLEMMING, C. J. MURPHY, G. A. ABRAMS, S. L. GOODMAN and P. F. NEALEY, *Biomaterials* **20** (1999) 573.
24. J. B. RECKNOR, D. S. SAKAGUCHI and S. K. MALLAPRAGADA, *Biomaterials* **27** (2006) 4098.
25. H. TSUJI, R. SMITH, W. BONFIELD and Y. IKADA, *J. Appl. Polym. Sci.* **75** (2000) 629.
26. K. A. SCHULT and D. R. PAUL, *J. Polym. Sci, Part B: Polym. Phys.* **35** (1997) 655.
27. J. REIGNIER and M. A. HUNEAULT, *Polymer,* **47** (2006) 4703.
28. P. LI, D. BAKKER and C. A. VAN BLITTERSWIJK, *J. Biomed. Mater. Res.* **34** (1997) 79.