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REES RAWLINGS FESTSCHRIFT

Simple methods to fabricate Bioglass®-derived glass–ceramic scaffolds exhibiting porosity gradient

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Abstract The present paper discusses different processing technologies for fabrication of novel 45S5 Bioglass®-derived glass–ceramic scaffolds with tailored porosity gradient for potential application in bone tissue engineering. Different types of scaffolds with continuous or stepwise gradient of porosity were produced by the foam replication technique, using preformed polyurethane (PU) foams as sacrificial templates. After preforming the PU foams in metallic moulds, they were dipped in a 45S5 Bioglass®-based slurry and subsequently heat treated in a chamber furnace up to 1100 °C. During heating, the organic phase is burned out and the glass sinters and partially crystallises. By using this new approach, Bioglass®-derived glass-ceramic scaffolds with different shapes and porosity profiles were designed. Scanning electron microscopy (SEM) showed that all samples have highly interconnected porous structure, with specific porosity gradients. By modifying the shape and dimensions of the metallic mould, bioactive glass–ceramic scaffolds with complex shapes and different degrees of porosity gradient could be obtained.

Introduction

New biomaterials that best mimic the complex structure of bone tissue are being developed to be used as scaffolds in tissue engineering [1–5]. The bone structure has an optimised morphology exhibiting a gradient of porosity, which increases along the transversal section from the surface (cortical bone with dense structure) towards the inner part (cancellous bone with highly porous structure). This complex microstructure optimises the material's response to external loading [6]. It is well known that abrupt transitions in material composition and properties result in a sharp local concentration of stresses, whether stresses are internally or externally applied. The stress concentrations are greatly reduced if the transition from one material to another is made gradually [7], this being one of the reasons behind the development of functionally graded materials (FGMs).

Tissue engineering scaffolds should have optimal pore structure to exhibit the required mechanical and biological behaviour [2–5]. For example, macropores can provide the space for tissue ingrowth and vascularisation of new formed tissue, whereas a denser structure should improve the mechanical stability of the scaffolds [6]. Moreover a gradient of porosity can facilitate the specific cell migration during the tissue regeneration process [8]. Scaffolds with graded microstructure are also required in osteochondral tissue engineering for treating articular cartilage defects [9]. Composite scaffolds containing two distinct regions: one for cartilage integration (a polymeric based material) and the other one for bone contact (bioactive glass or ceramic material) have been fabricated [9–11]. These bi-layered porous scaffolds, containing a cartilage-like layer and a bone-like layer mimic the characteristics of cartilage and underlying subchondral bone.

One of the materials widely used for bone replacement is 45S5 Bioglass®, which has also been employed in the bone tissue engineering field in the last 10 years [12]. This paper presents a new and simple approach for manufacturing functionally graded scaffolds for bone replacement, based on 45S5 Bioglass®. The method is based on the replication

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technique with preformed polyurethane foams. The technique for producing preformed polyurethane foams with graded porosity has been used in the past for fabricating ceramic-metal composites via colloidal infiltration [13, 14], while the foam replication technique has been recently developed for fabrication of highly porous, foam-like Bioglass® based scaffolds [15]. In this paper the two methods were combined for the first time for the manufacturing of highly porous Bioglass® scaffolds with tailored porosity gradient. Different scaffold morphologies with continuous or stepwise gradient of porosity were produced.

Experimental

Materials and methods

The 45S5 Bioglass®-derived glass-ceramic scaffolds with tailored porosity gradients were fabricated by the replication technique using pre-moulded polymeric sponges (polyurethane PU). Two different types of scaffolds were produced: one type with continuous porosity gradient (2D or 3D gradient of porosity) and the other one with stepwise gradient of porosity (double layer structure). The fabrication processes developed for these different types of scaffolds were similar. The only difference consisted in the preforming process of the PU foam. It was anticipated that by modifying the PU foam shape, the mould profile and the mode of preforming, PU foams with different types of gradients of porosity could be obtained.

After obtaining the preformed PU sponge, the fabrication process of the glass–ceramic scaffold follows the same steps as in the traditional replication method [15]. Briefly, after preforming, the PU foam samples were dipped in a 45S5 Bioglass®-based slurry followed by burning out the polymer and sintering the bioactive glass to obtain the graded Bioglass®-derived glass–ceramic scaffolds. During the heat treatment, the preformed PU foam burns out leaving behind a graded pore structure.

In this study, two types of fully reticulated polyurethane foams (Recticel, UK) were used: 60 and 45 ppi (pores per inch). SEM images of the internal structure of the foams before preforming are presented in Fig. 1. Both foams are characterised by a highly interconnected pore network. The 60 ppi foam has pore size in the range 200–600 μm. The 45 ppi foam has larger pore size in the range 350–800 μm.

The moulds were made of aluminium foil of 1-mm thickness. The 45S5 Bioglass® used was a powder of particle size less than 5 μm (NovaMin, Florida, USA). For the slurry preparation, 3 wt.% of poly(D,L-lactic acid) (PDLLA) (Purac biochem, Gorinchem, Holland) was dissolved in dimethylcarbonate (DMC) (Sigma Aldrich), under stirring. When PDLLA was completely dissolved, 40 wt.% 45S5 Bioglass® powder was added. The obtained suspension was stirred for 1 h, using a magnetic stirrer. Small samples of preformed PU foam were immersed in the slurry and manually rotated to ensure a homogeneous slurry infiltration. The foams were then extracted from the suspension and squeezed out to remove the excess of slurry. The obtained samples (green bodies) were then dried at room temperature and subsequently heat treated in a chamber furnace, up to a temperature of 1100 °C for 1 h. During heat treatment, the organic components (PDLLA, DMC and the PU foam) burn out, leaving a fragile, foam-like glass structure. During sintering, the Bioglass® densifies and partially crystallises, forming a glass-ceramic structure with enhanced mechanical properties in comparison with non-crystallised (amorphous) Bioglass® [15].

By using the described method, different types of glass–ceramic scaffolds with tailored gradient of porosity were fabricated. Their microstructure depends on the initial morphology of the preformed PU foam. In the following paragraphs, the fabrication process of preformed PU sponge with continuous (2D or 3D) and stepwise porosity gradients is described in detail.

Fabrication of trapezoidal shape PU preforms with 2D continuous porosity gradient

Rectangular pieces of aluminium foil were cut and then shaped, to obtain a trapezoidal form mould. The shape of the mould can be tailored to obtain different compression

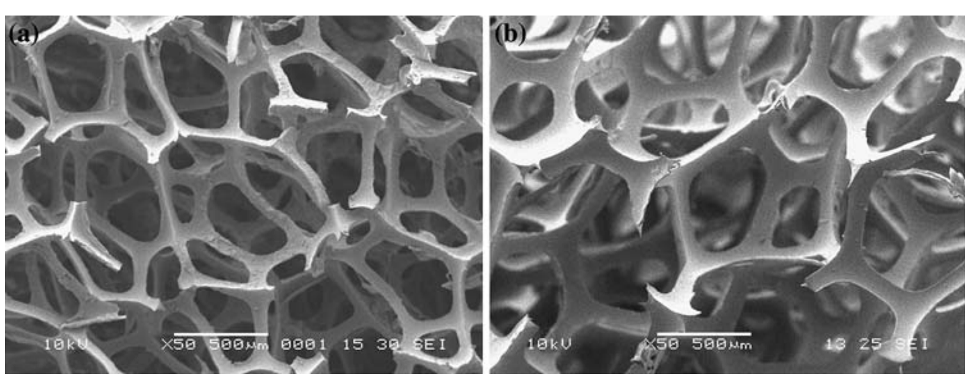

Fig. 1 SEM micrographs of (**a**) 60 ppi and (**b**) 45 ppi polyurethane foams, before performing

degrees of the PU sponge. Four types of moulds, for four different degrees of porosity gradients, were fabricated. The moulds were designed to obtain 0% compression at the top and 30, 50, 70 and 90% compression at the bottom of the mould, respectively. The preformed foams are named by the percentage of compression as: T2D-30, T2D-50, T2D-70 and T2D-90. Small pieces of 45 ppi PU foam were cut in rectangular shape ($20 \times 10 \times 10$ mm^3) and then compressed into the trapezoidal moulds at 200 °C for 30 min.

Fabrication of PU preforms with 3D continuous porosity gradient

PU foams with a 3D continuous gradient of porosity were obtained by compressing trapezoidal shape samples in a 3D mould with tetragonal prismatic shape. The mould was made by bending aluminium foils. Three types of moulds, for three degrees of porosity gradient, were fabricated. The moulds were defined by the length of the side of their square bases e.g.: 5, 7 and 10 mm, respectively. Small specimens of 45 ppi PU foam were cut in trapezoidal shape, similar to a truncated square pyramid. Samples with three different dimensions of the top face of the truncated pyramid were produced. These three dimensions correspond to the ones of the moulds: 5, 7 and 10 mm. The base length of all samples (of truncated square pyramid shape) was 20 mm and the height was 20 mm.

The PU foams were compressed inside the 3D mould so that 0% compression was obtained at the bottom of the mould. The degrees of compression at the top of the samples for both longitudinal and transversal directions were 75% (5 mm mould), 65% (7 mm mould) and 50% (10 mm mould), respectively. The PU foam samples are named by the degree of compression as: R3D-50, R3D-65 and R3D-75. The moulds containing the samples were maintained for 30 min inside the furnace at 200 °C.

Fabrication of PU preforms with stepwise gradient of porosity

For the preparation of scaffolds with stepwise gradient of porosity, the two different PU foams (45 and 60 ppi) were first assembled together in the aluminium mould and then heated in the furnace at 200 °C for 30 min. A double-layer sponge preform was thus obtained. The width of the mould (10 mm) was smaller than the sum of the widths of the two foam pieces (14 mm), to achieve a close bonding of the samples under compression. This sample was labelled R2D-DL.

The different types of fabricated samples are summarised in Table 1. The samples' names are defined by the shape of the samples after preforming (rectangular R or trapezoidal T), by the type of porosity gradient (2D or 3D) and by the degree of compression.

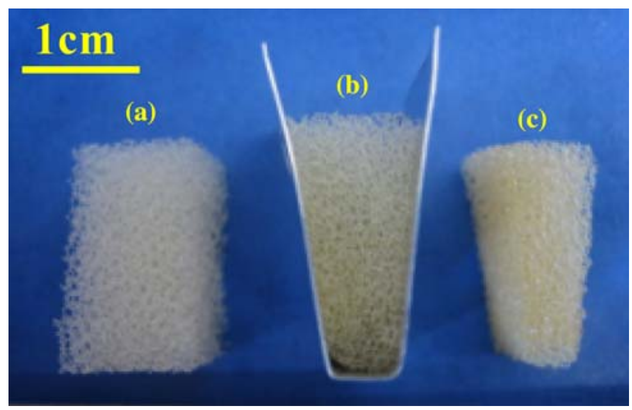

Fig. 2 Digital camera image of the T2D-50 trapezoidal shape foams with 2D continuous porosity gradient, during the different forming steps: (**a**) before preforming, (**b**) inside the mould, (**c**) final shape

Table 1 Summary of the different samples fabricated

Sample name	Sample shape after preforming[a]	Sample shape before preforming	Porosity gradient	Compression degree (%)
T2D-30	Trapezoidal (T)	Rectangular	Continuous 2D	30
T2D-50				50
T2D-70				70
T2D-90				90
R2D-50	Rectangular (R)	Trapezoidal	Continuous 2D	50
R2D-67				67
R2D-75				75
R3D-50	Rectangular (R)	Trapezoidal	Continuous 3D	50
R3D-65				65
R3D-75				75
R2D-DL	Rectangular (R)	Rectangular	Stepwise	30

[a] The shape of the samples after preforming is similar to the shape of the mould

Characterisation

The morphology of the internal structure of the PU foams before and after preforming and the microstructure of the Bioglass®-derived scaffolds after sintering were analysed by scanning electron microscopy (SEM) (JEOL JSM 5610LV). All samples were gold coated and observed at an accelerating voltage between 10 and 20 kV. A digital

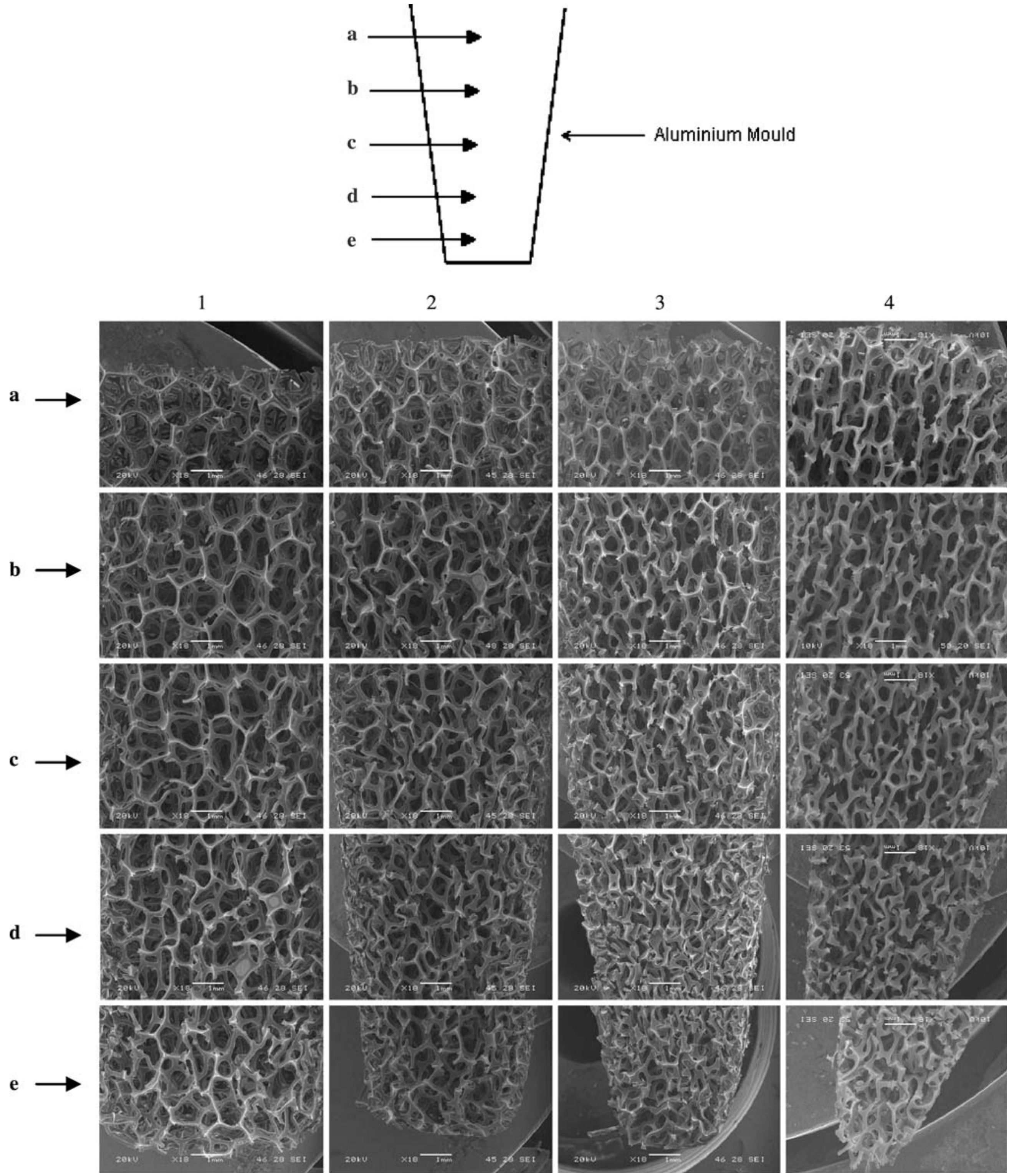

Fig. 3 SEM pictures of trapezoidal shape foams with 2D continuous porosity gradient before sintering at different degrees of compaction: (1) 30%, (2) 50%, (3) 70%, (4) 90%

camera was used for documenting the macroscopic structure of the samples.

Results

Scaffolds with 2D continuous porosity gradient

The different states of the T2D-50 foam before and after preforming are shown in Fig. 2. It can be noticed that the PU foams maintained the shape of the mould after preforming. Due to the specific shape of the mould, all samples were compressed at one side (bottom), but not at the other one (top). Therefore, a longitudinal gradient of porosity (increasing continuously from top to bottom) was created.

SEM images of the compressed foams before sintering are presented in Fig. 3. A continuous gradient of porosity is observed for each degree of compression. The highest degree of compression was obtained for sample T2D-90. It was observed that the pore size decreases gradually from top to the bottom of the foam, while the density has an inverse behaviour. The degree of compression was less visible for the sample T2D-30. All samples exhibited a position dependent porosity and the porosity gradient was shown to depend on the compression direction. On the non-compressed direction, there was no porosity gradient. Therefore, by using this technique, 2D porosity gradients in the compression direction were obtained.

During dipping of the preformed PU foam pieces in the Bioglass® slurry, the samples slightly swell due to the liquid infiltration. During the sintering process, considerable shrinkage (about 75%vol.) takes place [15], but scaffolds were shown to retain the continuous gradient of porosity and pore interconnectivity in the compression direction.

3D continuous porosity gradient scaffolds

A digital camera image of the R3D-50 foam during the different steps of preforming is shown in Fig. 4. SEM images of the three different compressed foams before sintering are illustrated in Fig. 5. A continuous gradient of porosity is clearly observed in all samples. As expected, the gradient of porosity increases with increasing degree of compression. The highest density was obtained for the R3D-75 sample, which appears almost compact at the bottom. By using a 3D mould, samples with continuous 3D gradient of porosity were obtained. Similar results in terms of the degree of compression in longitudinal and transversal directions are achieved.

After sintering, the Bioglass® scaffolds were seen to have the same morphology as the corresponding impregnated PU foams. Due to the high densification during the sintering process, shrinkage is quite significant, as mentioned above.

Typical SEM images of the sintered scaffolds are shown in Fig. 6. It was observed that a continuous gradient of porosity was achieved for all the samples and the highest densification was obtained for sample R3D-75. Some of the pores are seen to be closed due to the higher degree of compression. The most uniform sample was R3D-50, which presents a highly interconnected porous network.

Stepwise gradient of porosity scaffolds

A SEM image of the R2D-DL foam before sintering is presented in Fig. 7. Due to the slight compression exerted during preforming, a strong joining of the two PU foams is achieved. Figure 7 shows the interface between the two foams (45 and 60 ppi) after preforming, indicating that a stepwise gradient of porosity develops. The interface is seen to be quite homogeneous and a coherent interconnection of the two different foams has been accomplished.

It was also observed that the slurry dipping of the samples with Bioglass® powder did not distort the scaffold structure. SEM pictures of the R2D-DL scaffold after sintering are shown in Fig. 8A, indicating that no gaps appeared at the interface between the two foams after the sintering process and a continuous interface was obtained. A higher magnification SEM image of the interface between the two layers is shown in Fig. 8B, demonstrating the highly interconnected pore network.

Discussion

The present paper discusses a new technology for manufacturing highly porous scaffolds with graded porosity that mimic bone tissue. Spatially graded porosity is one

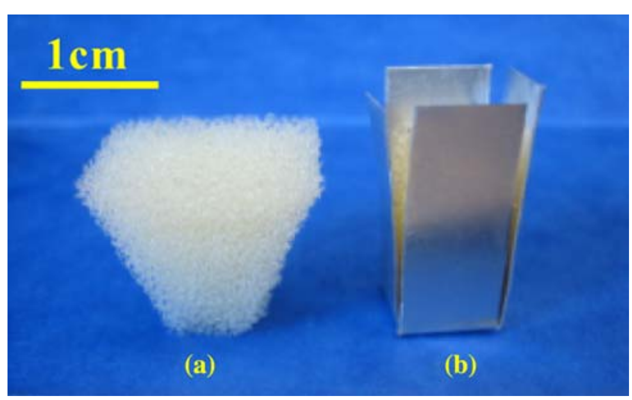

Fig. 4 Digital camera image of the R3D-50 foam with 3D continuous porosity gradient, during the different performing steps; (**a**) before preforming, (**b**) inside the mould

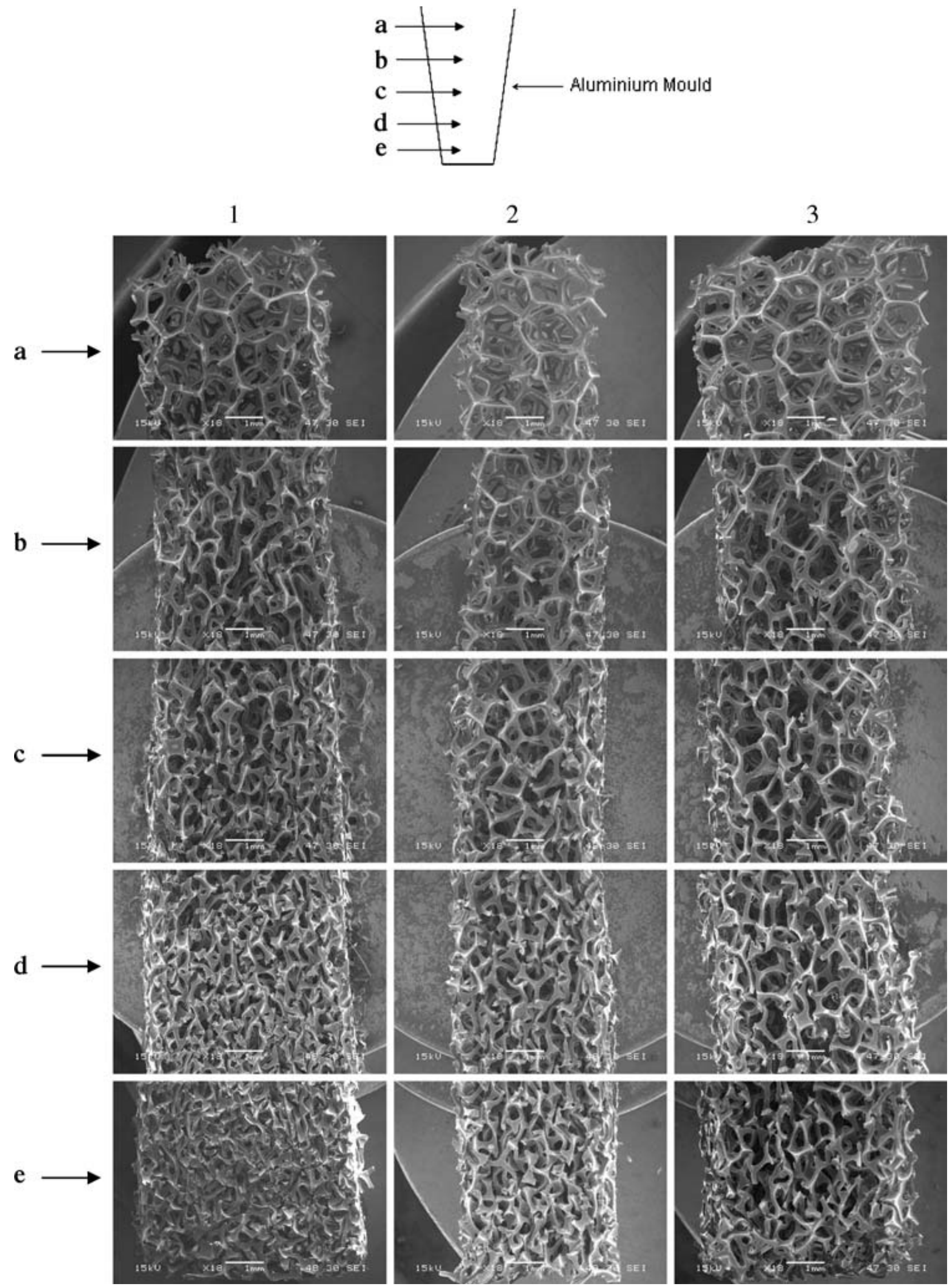

Fig. 5 SEM images of foams with 3D continuous gradient of porosity before sintering at different degrees of compaction: (1) 75%, (2) 65%, (3) 50%

characteristic feature of living tissue, and by developing bio-inspired materials that mimic the structure of natural tissues, the biological and mechanical properties can be optimised.

The present approach consists in adapting the technique used for preforming sacrificial polyurethane foams to produce highly porous scaffolds with tailored porosity gradient. The time and temperature of the heat treatment of the sacrificial polyurethane foams during the preforming process have been optimised. The optimal heat treatment was selected by maintaining the PU foams at 200 °C for 30 min. For lower temperatures, the foams were seen to loose their shape in contact with the Bioglass® slurry. For temperatures higher than ~250 °C, the internal structure of the foams was completely deformed and a higher degree of closed porosity was obtained, which is undesired for tissue engineering scaffolds. The sintering process was also optimised. For temperatures lower than 1000 °C, the strength of the resulted scaffolds was very low, in agreement with literature results [15]. For temperatures higher than 1100 °C, the degree of shrinkage was too high and the samples were completely distorted, with a negative effect

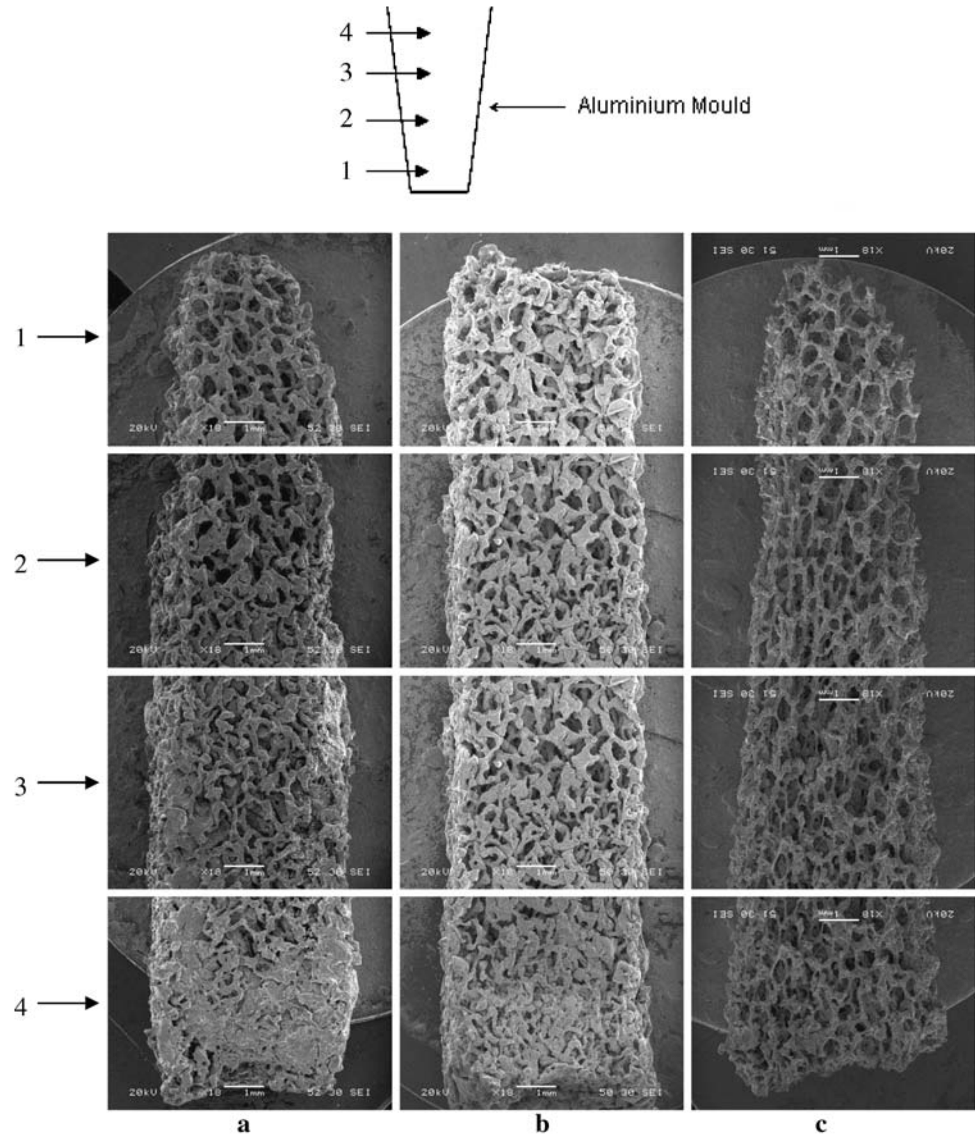

Fig. 6 SEM images of scaffolds with 3D continuous porosity gradient after sintering at different degrees of compaction: (a) 75%, (b) 65%, (c) 50%

on the interconnected pore network (i.e. higher degree of closed porosity). Therefore, a compromise between achieving optimal mechanical properties and highly interconnected porosity has been obtained. The chosen sintering temperature was 1100 °C, which was held for 1 h.

The infiltration technique of moulded PU foams with a Bioglass® slurry to produce graded porosity scaffolds is an attractive method with high versatility. In the present study several different shapes, more or less complex, were tested to obtain a pre-determined gradient of porosity. Polyurethane foams can be easily and accurately compression moulded to change the pore structure as desired. The gradient of porosity can be readily calculated by knowing the degree of compression of the foams and assuming isotropic compaction. For example, foam R3D-75 (75% compression) made from a 45 ppi sacrificial PU template will have on average pores of 560 μm in the uncompressed end and

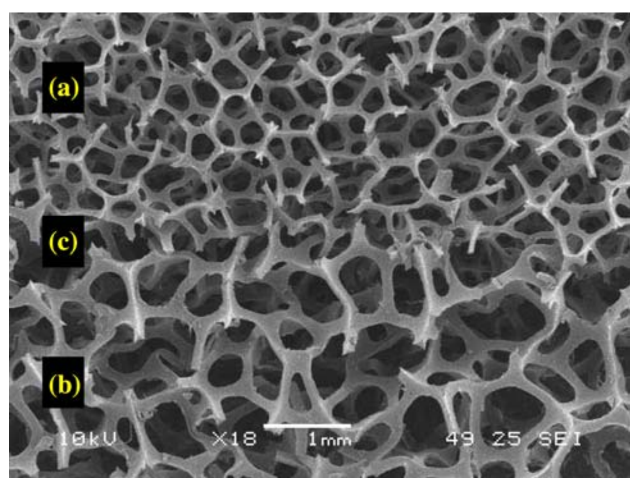

Fig. 7 SEM image of the R2D-DL preformed foam with stepwise gradient of porosity, before sintering: (a) 65 ppi foam, (b) 45 ppi foam and (c) interface

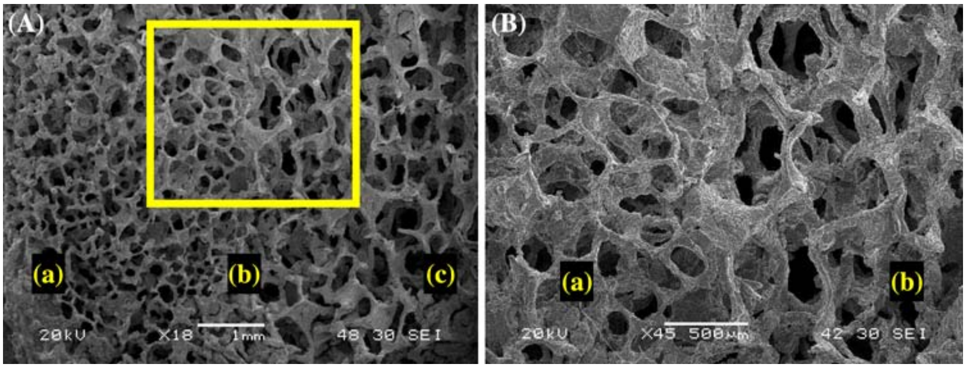

Fig. 8 (**A**) SEM image of the R2D-DL scaffold with stepwise gradient of porosity, after sintering: (a) 65 ppi foam, (b) 45 ppi foam and (c) interface; (**B**) inset of the marked area

pores of 140 μm at the compressed end. Thus the local pore size (*LP*) at a distance *x* from the large porosity side in a foam of length *l* could be calculated as:

$$\mathrm{LP} = 420(\mu\mathrm{m})/l(\mu\mathrm{m})x + 140(\mu\mathrm{m})$$

It is worthwhile noticing that the process developed offers also high flexibility in producing continuous or stepwise gradient of the pore structure. In addition, the technique is simple and easy to use for the fabrication of complex shape scaffolds. For example by combining both continuous and stepwise methods, different geometries can be designed. Moreover, by tailoring the shape and dimensions of both the PU foam and the mould, complex multilayer structures with porosity gradient can be produced. These multilayer structures are being considered for scaffolds intended for the engineering of the osteochondral area [9].

Conclusions

To meet both biological and mechanical requirements of bone and bone/cartilage tissue scaffolds, highly porous 45S5 Bioglass®-derived glass-ceramic foams with tailored gradient of porosity have been successfully produced. The innovative approach developed here consists in using preformed polyurethane foams with tailored gradient of porosity as sacrificial templates for the replication method. The preforming process was achieved by compressing the foams in aluminium moulds at low temperature (200 °C). By modifying the samples shape, the mould profile and the mode of preforming, PU foams with different porosity gradients, e.g. in 2D or 3D, can be obtained. Multilayer scaffolds with different pore structure in each layer are relevant for the engineering of the osteochondral area. Further investigations are required to assess the biological and mechanical behaviour of the scaffolds with graded pore structure for specific applications.

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