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Preparation and characterisation of poly (lactide-co-glycolide) (PLGA) and PLGA/Bioglass® composite tubular foam scaffolds for tissue engineering applications

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Abstract

Polylactide-co-glycolide (PLGA) and PLGA/BioglassR foams of tubular shape have been prepared with a 1 wt.% 45S5 BioglassR content. Porous membranes with varying thickness and porosity were fabricated via a thermally induced phase separation process, from which tubes of controlled diameter and wall thickness in the range 1.5–3 mm were produced. Scanning electron microscopy (SEM) revealed that the structure of the tubular foams consisted of radially oriented and highly interconnected pores with two distinct pore sizes, i.e. macropores f 100-Am average diameter and interconnected micropores of 10-50-Am diameter. Foams with BioglassR inclusions showed similarly well-defined tubular and interconnected pore morphology. Cell culture studies using mouse fibroblasts (L929) were conducted to assess the biocompatibility of the scaffolds in vitro. L929 fibroblasts cultured in medium that was pre-conditioned by incubating with PLGA tubes containing BioglassR had a significant reduction in cell proliferation compared with fibroblasts grown in unconditioned medium ($p<0.0001$).
The PLGA and PLGA/Bioglass tubular foams developed here are candidate materials for soft-tissue engineering scaffolds, holding promise for the regeneration of tissues requiring a tubular shape scaffold, such as intestine, trachea and blood vessels.

Keywords*: PLGA; Bioactive glass; Composite; Tubular scaffold; Foam; Tissue engineering*

1. Introduction

Development of novel biomaterials and fabrication of scaffolds from these materials is an essential step towards being able to engineer tissues from different anatomical locations in the body [1]. Different therapeutic strategies can be adopted for tissue engineering of lost, injured or diseased tissue, which to a large extent depend on the tissue of interest. These include implantation of freshly isolated or cultured cells onto an implanted acellular scaffold, implantation of acellular scaffolds to support in situ tissue regeneration, or implantation of tissues grown on scaffolds in vitro.
Scaffolds must provide a three-dimensional structure to support tissue in-growth into the structure, resulting in neo-tissue with similar properties to the tissue under replacement [2].
Materials used to fabricate scaffolds for soft-tissue engineering are usually selected to provide transient structures, exhibiting adequate biological and mechanical properties. The use of biodegradable scaffolds negates the need for a permanent implant made of an engineered material remaining in the tissue. A great number of bioresorbable materials have been investigated as scaffolds for tissue engineering and tissue repair, including naturally occurring [2] and synthetic polymers [3,4], as well as porous bioactive ceramics and glasses [5] and polymer/ceramic composites [6,7]. Synthetic scaffolds are advantageous, as material composition, micro- and macro-structure can be precisely engineered and scaffold properties tailored for specific applications, thus enabling the attainment of optimal conditions for cell survival, proliferation, and subsequent tissue formation [3]. Synthetic bioresorbable polymers have been increasingly applied as tissue engineering scaffolds during the past 10 years, particularly polylactide (PLA), polyglycolide (PGA) and their copolymers [3,4,7].
For regeneration of soft and hard tissues containing a lumenal structure, i.e. blood vessels, peripheral nerves, long bones, urethers and intestine, the development of suitable scaffolds with a tubular conformation is the first requirement. Porosity of the tube walls is another key parameter to prevent invasion of scar tissue and to allow for cell infiltration as well as fluid and nutrient diffusion from the surrounding tissue to the lumen of the tube.

Different methods have been proposed to prepare biodegradable tubular scaffolds with porous walls including bonding of non-woven polymer meshes wrapped around a cylinder and spraying a polymer solution on them [8], dip-coating a mandrel with a suspension of porogen particles into a polymer solution followed by leaching off of the particulates [9-12], extrusion of polymer-salt composite followed by salt-leaching [13], and rolling foams pre-formed by salt-leaching of solvent-cast polymer/salt mixtures [14]. However, most of these methods rely up on the leaching of porogen particles to create porosity leading to pores with poor interconnectivity.
Bioglass® (grade 45S5) is a bioactive material which has been used in bulk form for more than 15 years in orthopaedic applications [15]. This material has been classified as being a ''class A bioactive material'', and it has been shown that it develops strong bond to both hard (bone) and soft tissues in vivo using different animal models [15,16]. There is increasing interest in assessing the application potential of 45S5 Bioglass® in tissue engineering of bone, lung and other tissues, both as filler or coating of polymers [6,17-19] and as porous structures [5,17].
This study describes a new processing method to fabricate tubular foam scaffolds from poly(D,L-lactide-co-glyco-lide) (PLGA) and PLGA/Bioglass®-filled composite foams. The main advantage of our process is that pores result from thermally induced solid-liquid phase separation (TIPS) of polymer solution followed by solvent sublimation leading to highly open-pore structures with a high degree of pore interconnection. Most interestingly, control of pore size and morphology can be achieved by adjusting the processing and formulation parameters for the TIPS process. The process can be conveniently modified for incorporation of filler particles, including hydroxyapatite [20] and Bioglass® [6,19] particles. Bioglass® particles should enhance the mechanical competence of the constructs, in particular stiffness and compression strength [19].
In the present study, the microstructural (porosity) characterisation of the tubular scaffolds and data on the compression strength of the scaffolds are presented. In addition, the biocompatibility of a PLGA/Bioglass® composite tubular scaffold (1 wt.% Bioglass®) to enable it to support soft-tissue infiltration, particularly that of fibroblasts, has been assessed. Specifically, cell culture studies using mouse fibroblasts (L929) were conducted. This investigation is a first step in order to assess the ability of the scaffolds to support in situ soft-tissue regeneration that might produce a cellularized tubular construct suitable for seeding with other cell types, such as surface epithelial cells. The effect of PLGA/Bioglass® degradation products on fibroblast cell proliferation was also assessed. In a previous study [21], similar tubular foam scaffolds (however without Bioglass® additions) have been evaluated by means of in vivo investigations using a rat model.

2. Materials and methods

2.1. Materials

PLGA membranes were fabricated from 75:25 poly(D,L-lactide-co-glycolide) (PLGA RG756, Boehringer-Ingelheim, Germany) following a thermally induced phase separation process which has been described in previous reports [4,19].
Basically, the polymer is dissolved in dimethylcarbonate at a given concentration and a certain volume of this polymer solution is cast onto a Petri dish (50 mm in diameter). The covered Petri dish is transferred into a 600-ml lyophilisation flask and frozen for 2 h into liquid nitrogen in order to induce a solid-liquid phase separation driven by nucleation and growth of the solvent crystals. The flask is then connected to vacuum (10^{-2} Torr) in order to remove the solvent by sublimation. The primary drying is carried out at – 10 °C for 48 h, followed by a secondary drying at 0 °C for subsequent 24 h. The material is finally dried at room temperature. Tubular foam constructs were fabricated by rolling the freeze-dried porous membranes into a tube. Slowly dissolving the edges in chloroform and pressing together joined the opposing edges. The polymer concentration (Cp) and casting volume (Vp) of polymer solutions were varied in order to optimise the tube external diameter and the nominal wall thickness. PLGA/Bioglass® composite tubular foams were prepared using the procedure described above by adding Bioglass® particles as filler during the foaming process. The Bioglass® powder used was grade 45S5 (US Biomaterials, FL, USA) with a mean particle size <5and composition (in weight percentage): 45% SiO_2, 24.5% Na_2O, 24.5% CaO and 6% P_2O_5 [15]. A given amount of Bioglass® powder was dispersed into the polymer solution and sonicated before freeze-drying to give a Bioglass® content of 1 wt.% in the PLGA matrix.
A total of five polymer foams were prepared in this study by varying three experimental parameters: (1) the concentration of the polymer solution, (2) the casting volume and (3) the addition of Bioglass® particles, as summarized in Table 1. The chosen concentration of Bioglass® particles added to the PLGA matrix was based on previous results which have shown that Bioglass® has a dose-dependant effect on cell viability and biocompatibility in soft-tissue engineering applications [22]. In fact the concentration of Bioglass® results as a compromise between the mechanical property effect, for which Bioglass® concentration should be as high as possible [19], and the bioactivity effect, which leads to an optimal concentration of Bioglass® additions, as

discussed elsewhere [22].

2.2. Microstructural characterisation

Freeze-dried foams were cross-sectioned to permit examination of the internal pore morphology by scanning electron microscopy (SEM). Both top and bottom surface were examined. Tubular foams were frozen in liquid nitrogen and fractured using a razor blade, thus avoiding compression damage. Samples were gold-coated and observed at an accelerating voltage of 15–25 kV.

2.3. Compression strength tests

The axial and diametrical compression strength values of selected PLGA/Bioglass® composite tubes were measured using an Instron 5565 machine, equipped with a 100N load cell. A strain rate of 1 mm/min was used. Axial compression tests were also conducted on cylindrical (bulk) foams for comparison. At least five samples were tested for each condition and the results were averaged. The cross-sectional area of the samples, needed to compute compression strength values from load data, was accurately measured by scanning the surface of the samples and by carrying out quantitative image analysis. The measured average cross-section area for the tubes and cylindrical foams was 17.00 ± 2.40 and 66.03 ± 4.19 mm^2, respectively.

2.4. In vitro experiments

Only tubes made from composition F5 (with 1 wt.% addition of Bioglass® particles to the PLGA matrix, see Table 1) were considered for this part of the study. Tubular scaffolds without Bioglass® additions have been studied in a previous work [21]. Fibroblasts (L929) derived from normal subcutaneous areolar and adipose tissue of a 100- day-old male C3H/An mouse [23,24] were obtained from European Collection of Cell Cultures (Wiltshire, UK) and maintained in Dulbecco's Minimum Essential Medium (DMEM; Sigma, UK) supplemented with 2 mM glutamine (Sigma), 1% penicillin and streptomycin (Life Technologies, UK) and 10% FBS (Life Technologies). Culture medium was changed every third day and cells were subcultured weekly after short treatment with 0.05% trypsin/0.02% EDTA in Hank's balanced salt solution and cultured in 5% CO_2 at 37 °C.

Pre-weighed PLGA/Bioglass® composite tubes were placed into sterile tubes containing cell culture medium (DMEM supplemented with 2 mM glutamine and 1% penicillin and streptomycin) at a ratio of 5% tube weight/volume of cell culture medium, as summarized in Table 2. The tubes were incubated at 37 °C for 24 h on a rocking platform.

Table 1
Experimental variables for preparation of PLGA and PLGA/Bioglass® foams by thermally induced phase separation process

Foam no.	Polymer concentration (Cp) (wt/v%)	Casting volume (Vc) (ml)	Addition of 45S5 Bioglass® particles
F1	5	6	no
F2	5	8	no
F3	15	6	no
F4	15	8	no
F5	5	6	yes (1 wt.%)

Table 2
Weight of PLGA/Bioglass® composite foams (F5) and culture medium volume used

	Tube 1	Tube 2	Tube 3
Weight	0.098 g	0.050 g	0.050 g
Volume of medium	2040	1960	1000

For cell proliferation studies, suspensions of L929 cells were seeded onto 96-well cell culture plates at 1×10^3 cells/ well. The plates were incubated for 24 h in 5% CO_2 at 37 °C. After 24 h, the medium was removed from each of the wells, replaced with conditioned medium+10% FBS, and incubated for a further 24 h. Changes in cell proliferation after 24 h were determined using a CytoTOX 96 cytotoxicty assay (Promega, UK), a non-radioactive colorimetric assay that quantitatively measures lactate dehydrogenase, a stable cytosolic enzyme present in the cytoplasm of intact cells, that is released upon cell lysis after adding 9% (v/v) Triton X-100 in water. The assay was performed to measure the absorbance (at 490 nm) of the total number of cells as previously

described [25]. Changes in the absorbance of the total number of cells at the end of the experimental period are directly related to changes in cell proliferation. For statistical analysis, each treatment condition was performed in replicates of six and results are shown as mean absor-bance values F standard deviation.

3. Results

3.1. Materials characterisation

A total of five polymer foams were prepared in this study by varying the concentration of the polymer solution, the casting volume and the addition of BioglassR particles, as shown in Table 1. The porosity of all foams, measured by mercury and helium pycnometry as reported elsewhere [26], was similar for all samples, in the range 93 ± 2% (corresponding to a pore volume off ~11.3 cm^3/g). The influence of the formulation and processing parameters on pore structure was examined by extensive scanning electron microscopy (SEM) of the resulting freeze-dried foams. Both top and bottom surfaces as well as cross-sections of polymer foams were analysed prior to processing them into tubes. As shown in Fig. 1, the samples exhibited a high porosity and possessed two distinct pore sizes, macropores ~100-Am average diameter and interconnected micropores of 10–50-Am diameter (Fig. 1a). This porous network is covered by a dense top surface (also refereed as ''skin surface'') (Fig. 1b). Varying the parameters for freeze-drying has an effect on the resulting pore structure of the foams. As shown in Fig. 1, increasing the polymer concentration from 5 to 15 wt.% resulted in a densification of the porous network with a slight decrease of the foam porosity (Fig. 1c), all the other conditions being the same. In case of foams prepared from high polymer concentration, i.e. 15 wt.%, the top surface of the foam was almost not porous (Fig. 1d). Fig. 1e and f showed the bottom surface of foams F2 and F4, respectively. In contrast to the top surface, these surfaces were almost as porous as the interior of the foams and the surface porosity decreased when the concentration was increased. In addition, foams produced from diluted solutions (i.e. 5 wt.%) were more flexible (less rigid) than ones produced from 15 wt.% solutions, which facilitates the processing of the foams into tubes. This different flexibility is due to the different concentration of polymer in solution used in each case; the more concentrated the polymer solution, the higher the density of the foam skeleton. Slight variations in porosity may also affect the flexibility, however, no quantitative data are available to correlate these two parameters. Generally, the tubes prepared from polymer foams F1– F4 (without Bioglass®) exhibit a regular interconnected tubular porous structure with a large pore size distribution (50-300m), as shown in Fig. 2. SEM micrographs showing radial sections of PLGA tubular structures for F1 foam (Fig. 2a), F2 foam (Fig. 2b), F3 foam (Fig. 2c) and F4 foam (Fig. 2d) document the interconnected pore structure achieved.
The foams thickness increased with the casting volume of polymer solution, resulting in tubes with a higher nominal wall thickness, i.e. 2–3 mm for foams F3 and F4 produced using a casting volume of 8 ml as compared to 1.5–2 mm for foams F1 and F2 produced using a casting volume of 6 ml. Cross-sections of tubular foams indicated that pores exhibit a radial direction with respect to the tube, as a result of pore alignment along the cooling direction during the freeze-drying of polymer foams, as shown in Fig.3.

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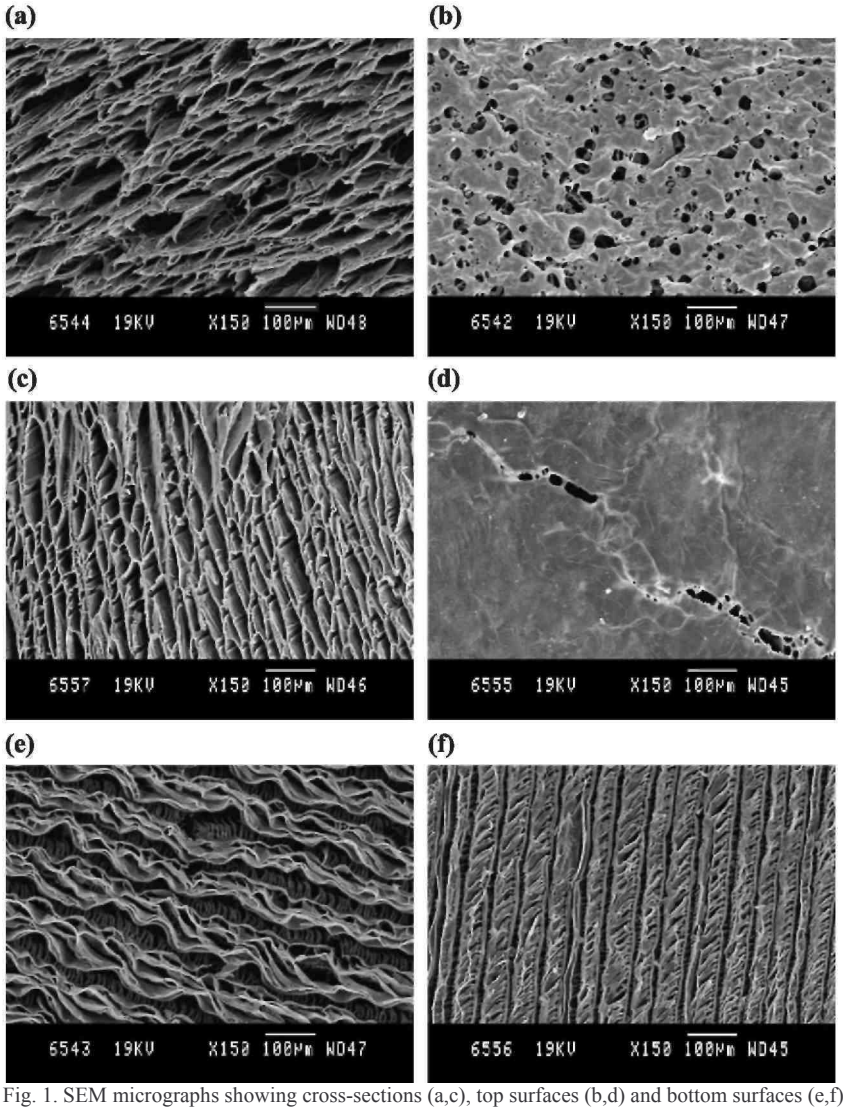

Fig. 1. SEM micrographs showing cross-sections (a,c), top surfaces (b,d) and bottom surfaces (e,f) of PLGA foams F2 (a,b,e) and F4 (c,d,f).

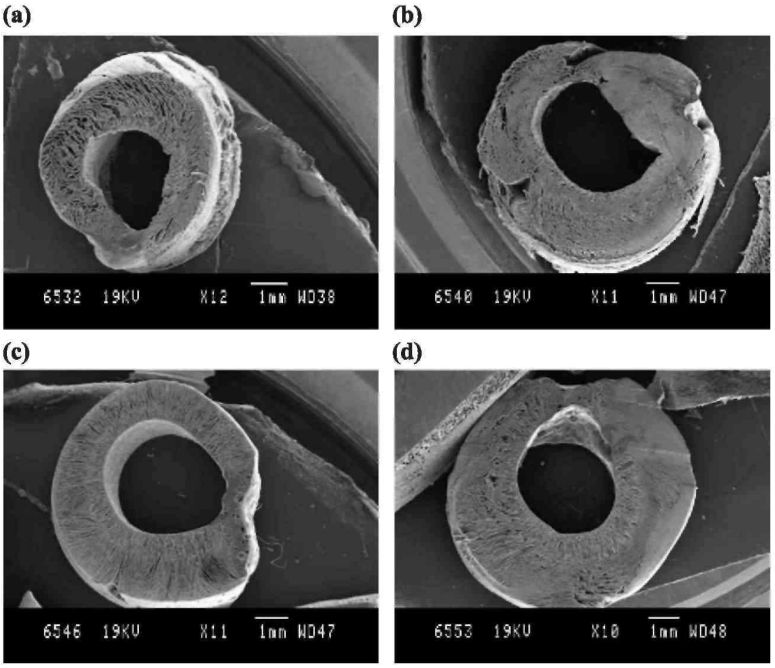

Fig. 2. SEM micrographs showing radial sections of PLGA tubular structures at low magnifications. Tubes have been prepared by rolling. (a) F1 foam, (b) F2 foam, (c) F3 foam, (d) F4 foam

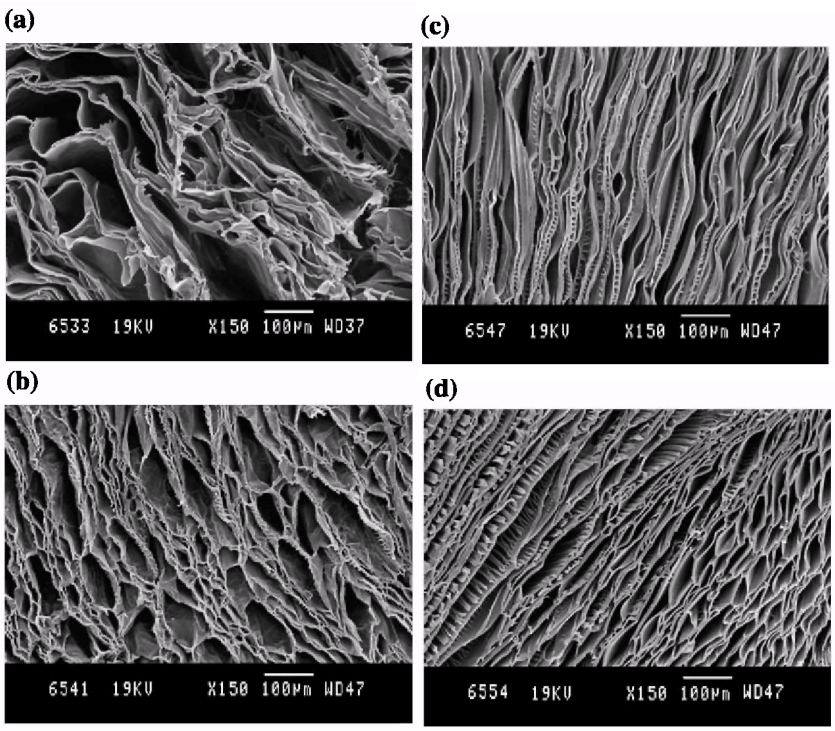

Fig. 3. SEM micrographs showing cross-sections of PLGA tubular structures at high magnifications. Tubes have been prepared by rolling. (a) F1 foam, (b) F2 foam, (c) F3 foam, (d) F4 foam.

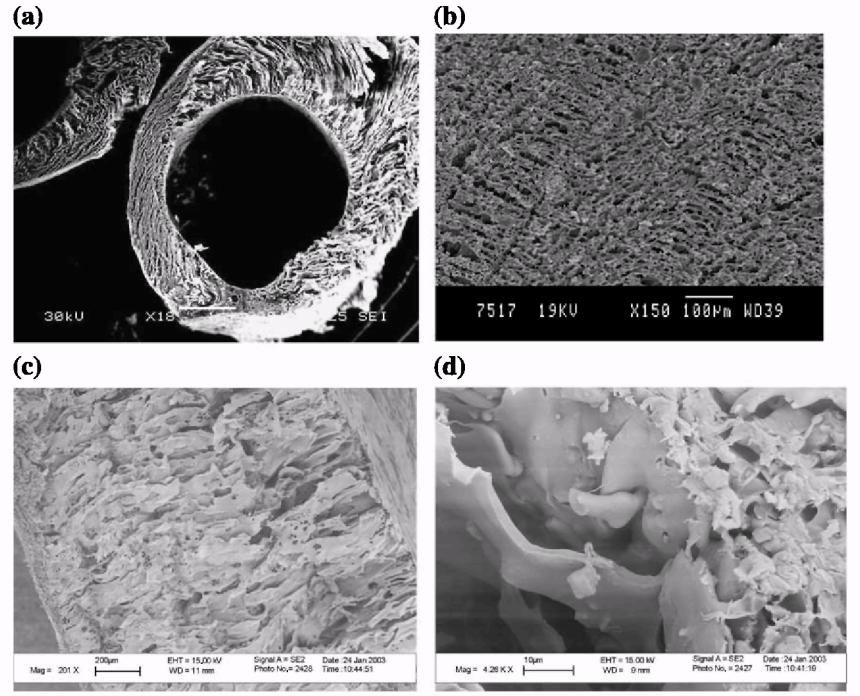

Fig. 4. SEM micrographs showing: (a) a radial section of PLGA/Bioglass® tubular foam (F5) at low magnification, (b) accumulation of Bioglass® particles at the surface of the composite foam, (c) an axial section of one edge of a cut PLGA/Bioglass® foam tube, and (d) Bioglass® particles on a fracture surface at high magnification.

High magnification SEM micrographs of cross-sections of PLGA tubes are shown for F1 foam (Fig. 3a), F2 foam (Fig. 3b), F3 foam (Fig. 3c) and F4 foam (Fig. 3d). For all tubes, the porosity is reduced around the joins of the edges. The composite foam structures with Bioglass® inclusions (F5) show similarly well-defined tubular and interconnected pore morphology (Fig. 4a). The accumulation of Bioglass® particles at the surface of the composite foams is illustrated in Fig. 4b, while Fig. 4c and d are high magnification SEM images showing the porosity structure of the cross-section of a tube and the presence of Bioglass® particles on a fracture surface,

respectively. The seam-like join has a dense surface and local reduced porosity, with concomitant reduction in tube thickness at this point, as evident in Fig. 4a. The presence of this dense area in the scaffold may have implications for the mechanical competence of the scaffold (see below) and it may also affect the scaffold biocompatibility. Deformation by rolling the flat membrane into a tube causes the structure to become locally rugose, resulting in reduced interconnectivity and porosity, which Fig. 4c demonstrates. Disparity in pore morphology and structure appears to result more from the rolling and joining process rather than from the effects of Bioglass® incorporation.

Table 3
Data for compression strength of tubular and bulk cylindrical PLGA/ Bioglass® foams (F5)

Scaffold	Axial compressive strength (10^2 MPa)	Diametrical compressive strength (10^2 MPa)
Tube	0.118 F0.031	0.052 F 0.008
Cylinder	0.412 F0.057–	-

The mechanical properties of the PLGA/Bioglass® tubes (composition F5, see Table 1) were assessed by compression strength tests. The results for both axial and diametrical compressive strength are shown in Table 3. For comparison, data for the compression strength of bulk cylindrical foams of the same composition are shown in Table 3, as discussed below.

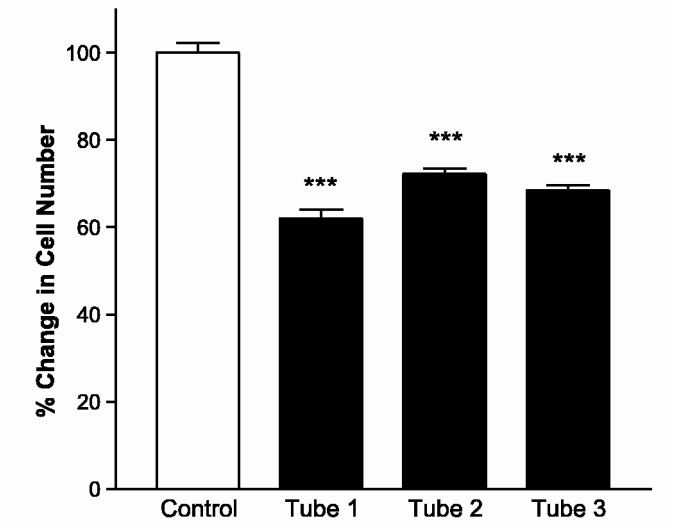

*Fig. 5. The relative change in cell number after incubation with conditioned medium (black bars) compared with cells grown in unconditioned medium (open bar). Each treatment condition was performed in replicates of six and results are shown as mean percentage values F standard deviation. Deviations from Gaussian distribution were checked for using the Kolmogorov–Smirnov test. Significant changes between cells grown in conditioned medium compared with unconditioned medium were tested for using the unpaired t-test. ***$p<0.0001$.*

3.2. In vitro experiments

Only tubes made from composition F5 (with 1 wt.% addition of Bioglass® particles to the PLGA matrix) were considered for this part of the study. L929 fibroblasts cultured in medium that was pre-conditioned by incubating with PLGA/Bioglass® tubes had a significant reduction in cell proliferation compared with control fibroblasts grown in unconditioned medium ($p< 0.0001$). The results of the in vitro experiments are summarized in Fig. 5. The mean value for percentage reduction in the number of cells compared with cells grown in unconditioned medium was 38.01 F4.995 for tube 1, 27.78 F 3.226 for tube 2 and 31.45 F2.820 for tube 3.

4. Discussion

The current study examines the feasibility of producing PLGA and PLGA/Bioglass® foams of tubular shape as scaffold for use in the engineering of lumenal tissues. The phase separation process, which has been developed in the previous studies for the fabrication of PDLLA/Bioglass® composite foams of cylindrical shape [6,18,19], was adapted to create tubular structures. This foaming process offers the possibility to control the macro- and the microstructure of the foams leading to possible control of the tubes geometry like diameter and nominal wall thickness as well as other characteristics such as the tube porosity and flexibility. The discrepancy between the

porosity of top and bottom surface of the foams is another advantage for providing tubes with semi-permeable wall structures. If the conduit needs to be seeded with cells for transplantation, then the ''skin surface'' of the polymer foams will provide tubes with a dense wall for maintaining cells in the lumen while fluids nutriments and oxygen could diffuse to the tube lumen through the outer porous layer. This may also prevent scar tissue from invading the tube lumen, which would be detrimental to tissue regeneration. In this study, the foams have been rolled such as the dense top surface constituted the inner wall of the tubes. By varying processing and formulation parameters for casting and freeze-drying, foam porosity and thickness can be modulated to control the tube geometry such as the external diameter and nominal wall thickness. We have previously shown that by casting a given volume of 5 wt/v% polymer solution followed by freeze-drying it, tubes of 3-mm diameter, 20-mm length and a nominal wall thickness of 1.5 mm were produced, that meet geometric requirements for intestinal regeneration scaffolds [21]. Foams made by the freeze-drying process are generally soft and flexible which makes them much more pliable than the one produced by particulate-leaching. Most interestingly, pores are radially oriented along the tube axis and their size distribution can be tailored by changing the polymer concentration.

Besides tube geometry and pore morphology, the mechanical stability of the tubular scaffold is an additional requirement for the success of tissue regeneration. In the particular case of tubular scaffolds, the foam requires a high degree of porosity to allow rapid tissue infiltration, while having enough matrix material to retain its structural integrity for a certain time during the degradation process. The foams used in the current study were fabricated as membranes prior to rolling into tubular constructs. The scaffolds exhibited attractive porous structure in terms of pore size and degree of pore interconnectivity and they were able to maintain their tubular structure, even after manual compression. However, a previous in vivo study using a rat model [21] has shown that 1 week after implantation, the neat PLGA tubes (no Bioglass® addition) had become compressed, reducing the volume of the lumen. After 4 weeks, the lumen had started to become filled with connective tissue deposits. The pore structure of the foams enabled infiltration of cells and blood vessels from surrounding tissue fairly rapidly, with complete cellularization occurring before the foams had completely degraded [21]. One aim of future use for these scaffolds is to seed the lumenal surface of the cellularized scaffold with other cell types, such as epithelial cells. This may be difficult if the lumen becomes compressed or is congested with connective tissue. We have previously shown that addition of 45S5 Bioglass® particles as a filler into PLGA foams can increase the compression modulus of the composite foams as compared to neat polymer foams (no Bioglass® addition): the compression strength of PLGA foams increased about three times with the addition of 10 wt.% Bioglass® particles [26].

The addition of rigid fillers to polymer matrices is common practice in composite technology to improve the stiffness, mechanical strength and structural integrity of components. In case of porous scaffold materials, however, the interrelationship between filler concentration and pore structure and their combined effect on mechanical properties is complicated, as discussed recently by Zhang et al. [27]. In particular, they investigated the effect of glass volume fraction and porosity content on elastic modulus and tensile strength of porous polysulfone/bioactive glass composites for materials with strong glass/polymer interfaces. In the present composites, strong interfacial bonding at the Bioglass®
/PLGA interface may be assumed based on previous results on compression strength of similar composites [26]. Applying the model developed by Zhang et al. [27], which is based on a previous equation by Ishai and Cohen [28], it is possible to estimate that the addition of 1 wt.% Bioglass® particles in our composites should lead to a 5% increase of elastic modulus of neat PLGA materials of equal porosity, assuming that all Bioglass® particles are incorporated in the polymer matrix and do not remain on pore surfaces. Moreover, the increase of composite stiffness should correlate with an increase of the breaking stress of the composites [27].

Thus, the possibility to improve the mechanical competence of tubular PLGA constructs by addition of rigid glass particles was one of the reasons why PLGA/Bioglass® composite foams were produced here, and their compression strength values were measured, as shown in Table 3. Although the present data do not allow to quantify the extent of improvement achieved by the very low concentration of Bioglass® particles added, the results of the compression stress tests show that PLGA/Bioglass® tubes of relatively thin wall thickness are able to sustain axial and diametrical compressive loads without collapsing. The authors have not been able to find relevant data in the open literature on similar tubular constructs, which could be used to correlate these values and more research efforts in this area are required. It is worthwhile noticing that the lower axial compressive strength exhibited by the tubular specimens in comparison to bulk cylindrical specimens (Table 3) is likely due to the difference in pore direction. The pores are orientated in the loading direction for the cylindrical specimens; conversely the principal pore orientation of the tubular foams is orthogonal to the applied load. Moreover, pore morphology and the overall pore structure are less homogeneous in the tubes than in bulk cylindrical foams, as rugosity is introduced in their manufacturing by rolling them into tubular shape. The seam produced in the process of forming the tubes, by joining the opposing edges using chloroform, is also likely to cause weaknesses and heterogeneity in the structure. Even if the measured compressive strength values of the tubular composites, both axial and diametrical values are lower that those measured on cylindrical foams, PLGA/Bioglass® tubular

composites produced in this study may improve the required in vivo structural integrity in comparison to unreinforced PLGA constructs. Thus, for the first time in this study, Bioglass® has been incorporated into a PLGA matrix to produce optimised foam composites of tubular shape.
In addition to improving mechanical properties, the incorporation of a bioactive particulate phase (45S5 Bioglass®) to the polymer matrix follows from the previous studies that have indicated the positive effect of Bioglass® to enhance the biocompatibility of artificial polymer scaffolds, as assessed by cell culturing methods for both hard and soft-tissue applications [22,29,30]. Thus, the incorporation of 45S5 Bioglass® into resorbable polymer porous matrices is seen as a convenient way towards tissue engineering scaffolds for both hard and soft-tissue engineering.
The cell culture research carried out in the present study was focused on assessing the biocompatibility of the tubular scaffold to enable it to support soft-tissue infiltration, particularly that of fibroblasts. Pre-conditioned medium has an inhibitory effect on fibroblast proliferation in vitro. The reasons for this are not known but may be due to changes in pH of the medium affecting cell differentiation. The current study focuses specifically on the development
of a PLGA/Bioglass® composite material for use as a tissue engineering scaffold, therefore, it is not certain whether the inhibitory effect is due to either one of the components or the composite itself. Future studies are planned to elucidate the basis of this effect, which will include quantification of pH changes in the conditioned medium using pure PLGA and PLGA/Bioglass® composite scaffolds. Previous research has shown that 45S5 Bioglass® increases the pH of the environment in in-vitro assessments, whereas PLGA (and polylactide, PLA) degradation reduces it [19,26]. However, the inhibitory effect on cell proliferation was not observed in vivo [21], where the environment has a better buffering capacity. Even if inhibition does occur in vivo, it is to be expected that this may, in fact, be of benefit because a reduction in fibroblast infiltration/proliferation in response to a bioactive material might prevent fibroblast overgrowth, enabling other cell types (e.g. endothelial cells) to migrate into the tissue engineering scaffold that might otherwise be overwhelmed by fibroblasts. It remains to be seen whether other cell types, such as epithelial and endothelial cells, have a similar reduction in cell proliferation in response to these scaffolds, the outcome of which could have profound implication on its use as a tissue engineering scaffold. The in vivo assessment of the PLGA/Bioglass® tubular foams is therefore the next characterisation step planned in this research effort.

5. Conclusions

The successful production of porous PLGA and PLGA/ 45S5 Bioglass® tubular foams by the thermally induced phase separation process has been shown. Both neat PLGA and PLGA/Bioglass® scaffolds exhibited high porosity of regular shape and dimensions. The material retained reasonable structural integrity, qualitatively assessed by manual compression and quantitatively by axial and diametrical compression strength tests. In vitro characterisation has been conducted with the aim to optimise tubular scaffolds to support lumenal intestinal epithelial cell growth, which complements previous in vivo studies. Pre-conditioned medium has an inhibitory effect on fibroblast proliferation in vitro. The reasons for this are not known but may be due to degradation products of the scaffolds affecting cell differentiation. It is likely that this effect will not occur in vivo due to the buffering capacity of tissue fluids, but even if it did still occur it is to be expected that this may be of benefit because a reduction in fibroblast infiltration/proliferation in response to a bioactive material might prevent fibroblast overgrowth, enabling other cell types (e.g. endothelial cells) to migrate into the scaffold.
Improvements in the production of tubular structure scaffolds by TIPS, for example, using double layered containers to avoid the rolling process used here, may be explored in the future. Further research from the materials science perspective will concentrate on measuring the time-dependant variation of mechanical properties upon in vitro degradation of the PLGA/BioglassR composites. Scaffolds developed following the procedure presented here should gain great interest for use in the engineering of tubular tissue constructs such as trachea, small intestine and oesophagus.

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