Melt-derived bioactive glass scaffolds produced by a gel-cast foaming technique

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Abstract

Porous melt-derived bioactive glass scaffolds with interconnected pore networks suitable for bone regeneration were produced without the glass crystallising. ICIE 16 (49.46% SiO₂, 36.27% CaO, 6.6% Na₂O, 1.07% P₂O₅ and 6.6% K₂O, in mol%) was used as it is a composition designed not to crystallise during sintering. Glass powder was made into porous scaffolds by using the gel-cast foaming technique. All variables in the process were investigated systematically to devise an optimal process. Interconnect size was quantified using mercury porosimetry and X-ray microtomography (μCT). The reagents, their relative quantities and thermal processing protocols were all critical to obtain a successful scaffold. Particularly important were particle size (a modal size of 8 μm was optimal); water and catalyst content; initiator vitality and content; as well as the thermal processing protocol. Once an optimal process was chosen, the scaffolds were tested in simulated body fluid (SBF) solution. Amorphous calcium phosphate formed in 8 h and crystallised hydroxycarbonate apatite (HCA) formed in 3 days. The compressive strength was measured to be approximately 2 MPa for a mean interconnect size of 140 μm between the pores with a mean diameter of 379 μm, which is thought to be suitable porous network for vascularised bone regeneration. This material has the potential to bond to bone more rapidly and stimulate more bone growth than current porous artificial bone grafts.

Keywords

bioactive glass; porous scaffolds; gel-casting; bone tissue regeneration; artificial bone graft

1. Introduction

There is a clinical demand for artificial bone graft materials (bioactive scaffolds) that can stimulate bone regeneration by acting as temporary templates for vascularised bone growth. Applications are spinal fusions, non-union fractures of bones and other less common defects such as the Hill-Sachs defect in the shoulder and defects resulting from tumor removal. The scaffold should have an interconnected pore structure, with interconnected pore sizes that are greater than 100 μm , be resorbable and possess the ability to stimulate bone growth (1).

There are many porous bioceramic artificial bone graft products commercially available, usually calcium phosphates. Bioactive glass particles have been found to be more bioactive than synthetic hydroxyapatite *in vivo* (2). Bioglass[®] 45S5 (46.1% SiO_2 , 24.4% Na_2O , 26.9% CaO , and 2.6% P_2O_5 , in mol%) was the first material found to bond to bone (3) and is commercially available in powder form (e.g. Novabone Products LLC, Alachua, FL, USA). Bioactive glasses have excellent osteogenic properties due to their dissolution products stimulating gene up-regulation in osteoblasts (4-5). Porous amorphous glass scaffolds have not been made from Bioglass[®] as it crystallizes to form a glass-ceramic when sintered (6), which greatly reduces its bioactivity. Glass-ceramic scaffolds based on 45S5 composition with an open pore structure have been produced by using sacrificial polymer foam templating, but compressive strength was less than 0.5 MPa, with ~85% porosity. An apatite layer was also not clearly formed until 4 weeks immersion in SBF (7). During scaffold production from glass particles, the particles must be sintered together and Bioglass[®] crystallises during this process because it has a very narrow sintering window, which is the temperature gap between its glass transition temperature (T_g) and its onset temperature for crystallisation ($T_{c\text{ onset}}$). In order to sinter a glass by viscous flow sintering, the temperature must be well above T_g but below the $T_{c\text{ onset}}$. Crystallisation reduces bioactivity of the Bioglass[®] and as bioactive

glasses exhibit strongly surface nucleated crystallisation, viscous flow sintering will be dramatically reduced if the glass crystallises. As a result of the poor processing characteristics of the 45S5 glass, alternative routes were pursued for making bioactive glass scaffolds such as the sol-gel foaming method (8-10). However sol-gel glass may degrade too rapidly for certain applications where the bone will take a long time to regenerate.

The first amorphous melt-derived bioactive glass scaffold produced with a pore structure suitable for bone ingrowth was developed by Fu *et al.* where the polymer foam replication technique was used with a glass composition 13-93 (54.6 mol% SiO₂, 22.1 mol% CaO, 6.0 mol% Na₂O, 7.7 mol% MgO, 7.9 mol% K₂O, 1.7 mol% P₂O₅). The composition expanded the sintering window compared to 45S5 glass and allowed the glass to be sintered without crystallisation. The scaffolds sintered well and established an interconnected pore network with pores in the range of 100-500 µm (~85% porosity) and achieved a compressive strength of 11 MPa, which is at the upper range of that of trabecular bone (2-12 MPa) (11). However the scaffolds only nucleated apatite in SBF after 7 days immersion, with the SBF being refreshed daily. This may be too low for a rapid bond to form *in vivo*. The foam replication process is also difficult to upscale for mass production, due to problems with removing excess glass slurry prior to sintering, in addition to the common problem of hollow centered struts that one would often find in this kind of scaffolds. The aim of this work was therefore to produce a porous scaffold with improved bioactivity using a processing method that can be up-scaled to the required ISO standards.

Gel-casting is a technique that has previously been used to produce dense or porous ceramic or metal structures. The process involves forming a gel by *in situ* polymerization of organic monomers (12-13), in doing so the gel binds the particles and is burnt out during sintering of

the particles. The process was adapted by Sepulveda *et al.* to produce porous hydroxyapatite (HA) foams by introducing a foaming step into the original process prior to gelation (14-17). The HA foams had superior interconnected pore networks to those produced by more conventional methods, such as sacrificial space holder technique, and improved mechanical properties over those produced by sacrificial polymer foam templating.

The aim of this work is to produce an amorphous melt-derived bioactive glass porous scaffold with a suitable pore network for bone in-growth. The glass composition was designed to allow sintering but also have a bioactivity similar to the 45S5 composition, by keeping the network connectivity (mean number of bridging oxygen bonds per silicon atom) as close to 2 as possible (18). Equation 1 was derived by Ray *et al.* (19) to calculate network connectivity of the glass, however a modified version that takes into account of phosphorus form Q^0 orthophosphate structure was used to calculate the network connectivity in this paper, an example of such calculation is displayed as Equation 2 in the case of Bioglass[®].

$$NC = 2 + \frac{\text{Total No. of bridging oxygens} - \text{No. of nonbridging oxygens}}{\text{Total No. of possible bridges}} \quad (1)$$

$$NC = 2 + \frac{2 \times \text{mol\% (SiO}_2\text{)} - 2 \times \text{mol\% (CaO + Na}_2\text{O)} + 2 \times \text{mol\% (P}_2\text{O}_5 \times 3\text{)}}{\text{mol\% SiO}_2} \quad (2)$$

The gel-cast foaming process was chosen because it has the potential to produce highly connected spherical pores and its ability of mass reproduction. The objectives were to adapt the gel cast foaming process for use with glasses by investigating the processing variables

and to produce a bioactive glass scaffold which has compressive strength similar to porous bone and an interconnected pore structure suitable for bone regeneration.

2. Materials and methods

2.1 Glass characterization

ICIE 16 (49.46% SiO₂, 36.27% CaO, 6.6% Na₂O, 1.07% P₂O₅ and 6.6% K₂O, in mol%) was the chosen glass composition (18). The relevant oxides were mixed together in their relative proportions and heated to 1420 °C, in a platinum crucible, and held for 1.5 h followed by quenching in water at room temperature. The coarse frit form of the glass was collected and dried overnight. The solid form of the glass was ground to a powder (Glen Creston Ltd. Gy-Ro Mill) and sieved at < 38 µm (Endecotts EFL 2000/1).

Differential Scanning Calorimetry (DSC) (Stanton Redcroft DSC 1500, Polymer Laboratories, Loughborough, UK) using a ramp rate of 10 °C /min to a final temperature of 950 °C, with matched platinum crucibles, was applied to study the differences in the glass transition temperatures and crystallisation behaviour of ICIE 16 and Bioglass[®] (46.1% SiO₂, 26.9% CaO, 24.4% Na₂O, 2.6% P₂O₅, in mol%). The modal particle sizes (D₅₀ value from laser scattering, CILAS 1064) of both glasses were 8 µm.

2.2 Verification of the gel-casting foaming process variables

Porous glass foams were produced by adapting the gel-cast foaming process, Figure 1 shows the steps in the process and Table 1 details the reagents and their role in the procedure, based on the work by Sepulveda *et al.* (14-17, 20).

Glass powder was mixed with deionised (18 Ω) water, the monomer (acrylamide) and the cross linker (N, N'-methylene bisacrylamide) to produce a slurry. The dispersant (Dispex) was added to help further dispersion of glass powder in the solution. The mixture was then vigorously agitated to produce a foam with the aid of the surfactant (Triton X100). Polymerization was activated by the addition of the initiator (ammonium persulfate solution, concentration 0.52 gml⁻¹) and the catalyst (tetramethylethylene diamine), which reacted with each other releasing a free radical particle. This free radical then reacted with both the acrylamide and the N, N'-methylene bisacrylamide, forming a polymer network. Viscosity increased over a period of 1 minute until gelation. Immediately (~2 or 3 seconds) prior to the gelation, the foam was cast into moulds. The pore structure was stabilized by the completion of gelation. It was then dried and sintered to burn out the organic matter, leaving the porous glass network.

Apart from the monomer (6 g), the crosslinker (3 g), the dispersant (2 drops) and the surfactant (0.1 ml), which were kept constant throughout, the influence of all other components on both the gelling time and the foam body was investigated systematically. Using a base protocol of 14 g glass, 20 ml water, 6 g monomer, 3 g crosslinker, 6 ml catalyst, 4 ml initiator, together with 2 drops of dispersant and 0.1 ml surfactant, the effect of varying water content (10 ml, 12 ml, 14 ml, 16ml, 18 ml, 20 ml, 22 ml, 26 ml, 28 ml and 30 ml) and catalyst content (3 ml, 4 ml, 5 ml and 6 ml) were investigated. Then the effect of particle size was observed using the base protocol with 3 ml catalyst. Two particle size ranges were collected after sieving the ground frit with a 38 μ m sieve: >38 μ m and <38 μ m. The glass powder loading in the slurry (10 g, 14 g and 30 g) in 20 ml water with 3 ml catalyst was then investigated. The effect of initiator (APS solution) freshness was also evaluated. As a result of this study, the base protocol was modified to: 20 g glass, 18 ml water, 6 g monomer, 3 g

crosslinker, 4 ml catalyst, 4 ml initiator, 2 drops of dispersant and 0.1 ml surfactant. The effect of initiator content (1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml and 7 ml) on gelling time and foam body volume was then verified.

2.3 Temperature Control

The effects of drying and sintering temperature were then assessed. The gelled foams were dried at different temperatures: 100 °C, 125 °C and 150 °C, followed by a two stage sintering programme where the temperature was ramped to 350 °C at 2 °C/min and held for 1 h; then ramped again to either 680 °C, 700 °C, 710 °C or 730 °C at 2 °C/min and held for 1 h; followed by furnace cooling to room temperature. X-ray diffraction spectrometry (Philips PW 1729 X-ray generator and PW1710 control system, operating at 40kV and 40mA) was performed on the samples (in powder form), and diffraction patterns were obtained in order to evaluate the sufficiency of sintering and the degree of crystallinity. The diffraction patterns were matched using the PANalytical X'Pert HighScore Plus (version 2.2b) software program.

2.4 Scaffold characterisation

The scaffolds were compared by imaging using scanning electron microscopy (SEM, JEOL JSM-5610) and X-ray microtomography (μ CT, Phoenix X-ray Systems and Services GmbH, operating at 100 kV and 100 μ A). A JEOL-6500F LEO FEG SEM was used to carry out energy-dispersive X-ray spectroscopy (EDX).

The compressive strength of the optimum scaffold was determined using a Zwick/Roell Z2.5 with a 2 kN load cell and a strain rate of 0.5 mm/min. Samples were cylinders, 6 mm in diameter 12 mm in height and the test was repeated 7 times.

The interconnect size was measured by mercury intrusion (Quantachrome Pore Master 33) and by applying a 4-step image analysis process to 3D μ CT images, which had to be modified from previous studies to account for the complexity of the pore network (21-23):

1. A $3 \times 3 \times 3$ median filter was applied to remove the noise and then the image was binarised into two phases: the struts and the background (void spaces which were identified as the pores). A $5 \times 5 \times 5$ morphological closing operator (dilation followed by erosion) was then applied to remove the fine intra-strut porosity, which otherwise reduces the watershed accuracy (step 3 below).
2. A distance map was then generated by successive dilation of the strut phase until all the pore voxels were filled. The distance function is then the number of steps need to reach that voxel in the pore, while the final voxels to be filled, or regional maxima in the distance in the 3D distance map, were tagged as the macropore centroids (21-22).
3. A 3D watershed algorithm (24) was then applied to the distance map and centroids. A watershed algorithm uses the rise and fall the distance map values to group voxels together where any water falling on those voxels would all run down to the same local minima. Here, the distance map is inverted and the lowest values at the struts are high point ridges where the water sheds into the centroid segmenting the space between struts into different macropores. Once the macropores are defined, the interconnects are easily indentified as groups of voxels touching two macropores.
4. After the pore space was segmented thoroughly, the sizes of each pore and interconnect could be represented by their equivalent diameters. The equivalent diameter of a pore was the diameter of a sphere that has the same volume to this pore. The equivalent

diameter of an interconnect was firstly quantified by using a principal component analysis (PCA) based method (23). This particular method found the best fit principal plane to each interconnect and projected all the voxels that belonged to that interconnect to the principal plane. The diameter of a circle with the same area of the projected voxels shade was the equivalent diameter of the interconnect.

The scaffolds were immersed in simulated body fluid (SBF) solution (25) (75 mg of glass per 50 ml of SBF) for up to 2 weeks in order to study dissolution rate of the glass and deposition rate of the hydroxycarbonate apatite (HCA) layer, which were investigated by applying inductively coupled plasma optical emission spectroscopy (ICP-OES, Thermo Scientific ICP 6000 series) and Fourier transform infrared (FTIR) spectroscopy respectively (Bruker Vector 22). Each data point was repeated at least 3 times in order to verify the error.

3. Results and discussion

Figure 2 shows the DSC traces of the 45S5 Bioglass[®] and ICIE 16. By modifying the composition of Bioglass[®] to produce ICIE 16, the T_g increased from just over 500 °C to 575 °C and the onset temperature for crystallization ($T_{c\ onset}$) increased from a little over 550 °C to over 725 °C whereas T_c increased from 600 °C to over 750 °C. The sintering window is defined as $T_{c\ onset} - T_g$. For ICIE 16 it was greater than 200°C compared to less than 100°C for Bioglass[®]. The DSC data indicates that sintering the ICIE 16 glass below 750°C should prevent crystallization.

To create an interconnected pore network suitable for bone regeneration, it is very important to maintain a certain volume of foam during agitation (e.g. 130 ml from an original suspension of 40 ml) until polymerization occurs (e.g. 90 seconds). This is because too high a

foam volume would result in very large pores and low mechanical strength whereas too little would give insufficient pore and interconnect size. Therefore to investigate the effect of process variables on the pore structure, the foam body volume was used. In pilot studies, a foam body volume of approximately 130 μm was found to produce modal interconnect diameters $> 100\mu\text{m}$ (10). The timing is critical because a rapid gelling time was not sufficient for the foam to develop but with too much time in agitation the foam collapsed and lost its stability. The glass composition and the particle size of the glass powder did not affect the gelling time and foam body volume. Water, catalyst and initiator content, on the other hand, played a significant role.

The amount of water in the system had a large impact on the gelling time and foam body. Figure 3a shows that as the water content increased from 10 to 30 ml, the average gelling time also increased from 50 to 114 seconds (127% increase), and the foam body increased from 50 to 192 ml (283% increase) respectively. The increase in gelling time was due to dilution of the reagents, but the increase in foam volume was due to the water increasing the efficiency of the surfactant.

The effect of catalyst concentration was different (Figure 3b). As catalyst content increased from 3 to 4 ml, there was a sharp decline in the gelling time from 126 to 96 s. The gelation time was approximately constant (83 s) as the catalyst content increased from 5 to 6 ml. The foam volume reduced gradually from 170 to 167 ml as the catalyst content increased from 3 to 4 ml and decreased sharply (from 158 to 110 ml) as the catalyst volume increased from 5 to 6 ml, even though the gelling time was approximately the same at these two data points. The ideal foam body was around 130 ml and an ideal gelling time for producing a stable

foam body was around 90 s, so 18-22 ml of water was suitable for the process. 18 ml of water and 4 ml of catalyst were chosen to be the standard quantities.

As the initiator content increased from 1 to 7 ml, the gelling time decreased (182 to 51 s) and the foam body volume increased from 130 to 170 ml (Figure 3c). The relationship was not linear over the range of concentrations used: as initiator content increased from 1 to 3 ml there was a sharp decline in gelling time from 182 to 84 s whilst the foam volume changed little, reducing from 130 to 124 ml. Then, as initiator content increased from 3 to 7 ml the gelation time decreased more slowly from 84 to 51 s and the relationship was approximately linear. The foam volume increased approximately linearly from 124 ml to 170 ml. Changing the initiator content changed the water content of the solution. The total change in water content through the range of initiator contents used was ± 3 ml. Figure 3a shows that ± 3 ml had little effect on foam volume or gelling time, therefore the differences observed in Figure 3c can be attributed to the initiator rather than to changes in water content.

In addition to the above components, the vitality of the initiator (APS) solution influenced the gelling time and the foam body volume of the suspension significantly. After mixing the APS powder with distilled water, it had to be left for at least 3 h before it could be used, otherwise the suspension would not foam at all, even though the gelling time was not affected. The solution became less reactive as time passed; therefore it was remade after 1 week. If the solution was left more than 1 week the gelling time was significantly prolonged.

The influence of the size of the glass particles played a critical role in sintering. Particle size measurements showed that for particles sieved to $<38\ \mu\text{m}$, their size distribution ranged from $1.31\ \mu\text{m}$ (D_{10} value) to $37.73\ \mu\text{m}$ (D_{90} value) with a mode (D_{50}) value of $8.65\ \mu\text{m}$. The >38

μm particles had a particle size range of 75 μm to 89.70 μm with a modal value of 84.15 μm . When >38 μm particles were used, sintering was not completed at 700 °C (Figure 4a). The foam struts were not fully dense and consisted of large particles embedded in a sea of smaller particles, which were loosely attached to one another, and the scaffold structure was fragile. The pore network collapsed during sintering. When <38 μm particles were used, the structure was intact and the scaffold was well sintered (Figure 4b). Particle packing and stacking during sintering was better for the <38 μm particles. The smaller particles have a greater driving force for sintering and must be used in order to allow efficient sintering at 700 °C. However, smaller particles have a larger surface area for crystal nucleation and are also likely to promote crystallization at lower temperatures (see later).

A side effect of using APS as the initiator was that crystallites were present in the glass after sintering (Figure 5a). These were not due to crystallization of the glass, but were identified by XRD (Figure 6) to be potassium sodium sulfate ($\text{K}_3\text{Na}(\text{SO}_4)_2$) (01-074-0398). Due to the glass being in contact with water during the process, sodium and potassium ions were dissolved from the glass in an ion exchange mechanism. The ions reacted with the sulfate, which was a by-product of the initiator (APS), causing nucleation of crystals on the surface of the glass particles. As the scaffold was sintered, the organic part was burnt out, leaving glass particles stacked in a porous glass structure, which then fused together as the temperature increased further. The crystals were therefore incorporated into the glass. Although the gelling time was short (90 s), the glass remained in contact with water during drying. Small amounts of $\text{K}_3\text{Na}(\text{SO}_4)_2$ may not affect biological properties (26-27) and materials such as calcium sulphates have been clinically used as synthetic bone graft material for over a century without failing (28), but methods to eliminate its formation were devised regardless.

Attempts were made to eliminate the $K_3Na(SO_4)_2$ formation by reducing the amount of water present and the amount of time the water is in contact with the glass, by increasing the drying temperature. Figure 5 shows SEM micrographs of the glass surface for scaffolds sintered at 700 °C but dried at 100 °C, 125 °C and 150 °C. The size of the crystals was significantly reduced from ~70 µm when dried at 100 °C (Figure 5a) to ~20 µm by drying at 150 °C (Figure 5c). However, drying at 150 °C caused the glass to crystallize, which could be seen in Figure 6c and therefore the optimum drying temperature was considered to be 125 °C.

Figure 6 shows XRD traces for the glass scaffolds for each sintering temperature following drying at 100 °C (Figure 6a), 125 °C (Figure 6b) and 150 °C (Figure 6c). Drying at 125 °C (Figure 6b) suppressed the $K_3Na(SO_4)_2$ formation to a minimum without the glass crystallizing too much. As the sintering temperature increased above 700 °C, scaffolds dried at all temperatures began to crystallise, the crystal phases were a mixture of potassium sodium sulfate ($K_3Na(SO_4)_2$) and sodium calcium silicate ($Na_2CaSi_3O_8$) (01-077-2189). This means that the gel-casting process combined with the slower heating rate had reduced the $T_{c\text{ onset}}$ of the glass to a lower temperature (<700 °C) compared to the $T_{c\text{ onset}}$ that was derived from the DSC trace in Figure 2 (725 °C).

Comparing Figure 6a and Figure 6b, there was less crystallisation of the glass in the scaffolds dried at 100 °C (Figure 6a), The degree of crystallinity increased as the drying temperature increased to 125 °C (Figure 6b), which means the crystallisation temperature of the glass was reduced and drying at higher temperature promoted the onset of glass crystallisation.

The higher drying temperatures may have also lowered the onset crystallization temperature of the glass by increasing the ion diffusion rate and therefore a low sintering temperature

would minimise crystallisation of the glass and growth of the $\text{K}_3\text{Na}(\text{SO}_4)_2$ crystals, but sintering was not efficient at temperatures below 680 °C and therefore the desired sintering temperature was between 680 °C and 700 °C.

Another option was to reduce the amount of initiator used in the process. Figure 7 shows that as the initiator volume decreased from 4 to 1 ml, the crystallization of the $\text{K}_3\text{Na}(\text{SO}_4)_2$ were suppressed as there was less sulfate available. No crystallites were observed on scaffolds made with 1 ml APS (Figure 7a). However 1 ml of the initiator could not efficiently gel the system, therefore 2 ml was the optimum amount of initiator to be applied in the gel-casting process.

In summary, small particle size promoted sintering at lower temperature but also promoted glass crystallization. As initiator content increased, gelling time decreased and pore size increased. However, more importantly, too much initiator (ammonium persulfate) resulted in the formation of potassium sodium sulfate crystals in the scaffold, due to the sulfate left from the initiator reacting with glass dissolution products in the aqueous slurry. The drying temperature was increased to reduce the amount of water and time that the glass was exposed to water; however this stimulated crystallization of the glass when it was raised too much. An optimal drying temperature was therefore chosen and the amount of initiator was therefore reduced to a minimum. Sintering temperature was also critical, as temperatures above 700 °C triggered crystallization and temperatures below 680 °C did not allow complete sintering of the particles.

An optimal protocol for the gel-cast foaming of ICIE 16 was therefore devised. The size of the glass powder should be around 10 μm , and the standard formula of the process was: 20 g

glass powder, 18 ml water, 6 g methacrylamide (monomer), 3 g N,N'-methylene bisacrylamide (cross-linker), 2 ml ammonium persulfate solution (initiator), 0.1 ml Triton X (surfactant), 4 ml tetramethylethylenediamine (TEMED) (catalyst). The foams were dried at 125 °C for 10 h, and then ramped at 2 °C/min to 350 °C, held for 1 h and was ramped up again at 2 °C/min to 700 °C, held for 1 h before furnace cooling.

The optimised scaffolds were characterized to determine whether the interconnect size was sufficient for blood vessel penetration and tissue ingrowth, i.e. greater than 100 µm. Figure 8a shows the data obtained by mercury intrusion porosimetry, from which the modal sizes of the interconnect were determined to be 128 µm (80% porosity). Figure 8b is a 3D µCT image of the structure of a representative optimized scaffold and is the visual evidence of the high interconnectivity and porosity of the scaffold. Three such scaffolds were scanned and the interconnect size distributions from µCT image analysis are shown in Figure 8c. ICIE16 1 is the sample imaged in Figure 8b. The mean modal interconnect size of the three samples was 140 µm. The image analysis of µCT images also allowed quantification of pore size (Figure 8d), which had a mean modal pore size of 379 µm.

Nine preliminary compression tests were carried out to investigate the compressive strength of the scaffolds and the results were summarized in Table 2. The individual values are shown in Table 2 since there was a small variation in porosity between samples. For a scaffold with 79% porosity, modal pore size of ~345 µm and modal interconnect size of 144 µm, its compressive strength was 1.9 MPa. Overall the compressive strength increased as the porosity decreased.

The scaffold with a bulk density of 0.52 gcm^{-3} and a porosity of 81% was immersed in SBF for the purpose of deposition and dissolution rate studies. The deposition of HCA was inspected by FTIR and the results were summarized in Figure 9a. It is clear that the deposition of calcium phosphate occurred after 8 h of immersion in SBF, as P-O bend bands were seen for the first time, which is likely to be amorphous calcium phosphate. Amorphous calcium phosphate deposition has previously been found to occur on bioactive glasses, prior to HCA formation, using synchrotron X-ray diffraction studies (29-30). The twin P-O bending bands at 576 and 611 cm^{-1} indicate the calcium phosphate crystallized into HCA after 3 days immersion. The fact that the P-O bending peaks corresponded to HCA was confirmed by XRD (Figure 9b). Apatite formation in SBF was therefore at a similar rate to sol-gel foam scaffolds that had a similar compressive strength (31) and more rapid than the 13-93 scaffolds. The reason of the more rapid HCA layer formation on the ICIE16 scaffolds is that the network connectivity of ICIE16 is 2.13, which is very close to that of Bioglass[®] (2.11); whereas the network connectivity of 13-93 is a lot higher, at 2.59, as the result of the higher silica content of 13-93. A higher network connectivity means that the glass is less susceptible to ion exchange and dissolution, which are the first stages of the HCA layer formation mechanism, therefore apatite nucleation is slower on 13-93.

Figure 10 shows the dissolution (ICP) data where Si, Ca, S, K and Na were all released slowly into the SBF. The phosphorus was taken up by the sample from the solution and none was left after 1 week of immersion, indicating P deposition on the glass. After 1 day in SBF, there was an increase of phosphorus in the solution but a decrease in all other elements except calcium. It is therefore possible that some amorphous calcium phosphate deposited on the glass and then redissolved before reprecipitating on the glass again. This has been observed in sol-gel derived bioactive glasses using synchrotron XRD (32-33). Approximately 35 ppm

of sulfur was released into the solution after 2 weeks, which was from the potassium sodium sulfate crystallites. Sulfur is non-toxic in its element form and is a vital component of all living cells, at a quantity this small it is unlikely to provoke any response from the body.

Conclusion

Bioactive glass foam scaffolds were produced of the ICIE 16 composition (49.46% SiO₂, 36.27% CaO, 6.6% Na₂O, 1.07% P₂O₅ and 6.6% K₂O, in mol%) by adapting the gel-casting foaming process. Open porous structures were achieved with interconnects exceeding 100 µm in diameter while maintaining the amorphous structure of the glass. The optimum scaffold was produced by applying the standard protocol that consists of 20 g glass powder, 18 ml water, 6 g monomer (acrylamide), 3 g crosslinker (N, N'-methylene bisacrylamide), 4 ml catalyst (TEMED), 2 ml initiator (APS Solution), 2 drops of dispersant and 0.1 ml surfactant (Triton X). All the parameters listed above influenced the gelling time and the porosity. The size of the glass powder is critical for sintering efficiency. Particles with a particle size range of 1 - 10 µm sintered well, while larger particles did not. The initiator concentration, the water concentration and the drying and sintering temperatures affected the amount of potassium sodium sulfate formation that occurred during processing. A decrease in any of these parameters resulted in less crystallization. 2 ml of initiator solution and 18 ml water, together with a drying temperature between 125 °C and 150 °C and a sintering temperature between 680 °C and 700 °C produced the optimum scaffold. Optimized scaffolds had modal interconnect sizes of 141 µm and modal pore size was 379 µm. The scaffolds also had a compressive strength at the lower end of that of trabecular bone (1.9 MPa). Calcium phosphate deposited on the scaffolds after 8 h of immersion in SBF and crystallized into HCA after 3 days immersion.

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Disclosures

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Figure Captions

Figure 1. Flow chart of the gel-cast foaming process.

Figure 2. DSC traces of 45S5 Bioglass[®] and ICIE16 (particles <38µm) with a heating rate of 10°C.

Figure 3. Gelling time and foam body volume of the gel cast foam as a function a) water content, b) catalyst content and c) initiator content.

Figure 4. SEM images of glass scaffolds produced with particles with size distributions of a) $>38\ \mu\text{m}$ and b) $<38\ \mu\text{m}$ particles. Both were dried at $100\ ^\circ\text{C}$ and sintered at $700\ ^\circ\text{C}$.

Figure 5. SEM images of scaffolds dried at a) $100\ ^\circ\text{C}$, b) $125\ ^\circ\text{C}$ and c) $150\ ^\circ\text{C}$, which were then sintered to $350\ ^\circ\text{C}$ followed by $700\ ^\circ\text{C}$.

Figure 6. XRD traces of scaffolds dried at a) 100 °C, b) 125 °C and c) 150 °C, sintered at different temperatures. Peaks marked with * represent the crystallization of potassium sodium sulfate, and the peaks with × mark the crystallization of the glass.

Figure 7. SEM images of bioactive glass foam scaffolds gel cast with initiator volumes of a) 1ml, b) 2ml, c) 3ml, and d) 4ml.

Figure 8. Pore network quantification: a) mercury porosimetry and b) a X-ray microtomography (μCT) image of a gel cast foam scaffold, c) interconnect diameter distribution from image analysis of μCT images, d) pore size distributions from image analysis of μCT images.

Figure 9. a) FTIR traces of the scaffolds immersed in SBF up to 2 weeks, and b) XRD traces of the ICIE16 glass powder ($<38 \mu\text{m}$) before processing and after immersion of the scaffold in SBF for 2 weeks.

Figure 10. Concentrations of the different ions released from the scaffolds after immersion in SBF for up to 2 weeks (ICP data)