

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
15 February 2007 (15.02.2007)

PCT

(10) International Publication Number
WO 2007/017756 A2

- (51) International Patent Classification: **Not classified**
- (21) International Application Number:
PCT/IB2006/002438
- (22) International Filing Date: 7 August 2006 (07.08.2006)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
0516157.5 5 August 2005 (05.08.2005) GB
- (71) Applicant (for all designated States except US): **IMPERIAL COLLEGE INNOVATIONS LIMITED** [GB/GB]; 47 Prince's Gate, Exhibition Road, London SW7 2QA (GB).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **BOCCACCINI, Aldo, Roberto** [IT/GB]; Department of Materials, Imperial College of London, South Kensington, London SW7 2AZ (GB). **CHEN, Qizhi** [CN/GB]; Department of Materials, Imperial College of London, South Kensington, London SW7 2AZ (GB). **REZWAN, Kurosch** [CH/DE]; Universität Bremen, Am Biologischen Garten 2, IW 3, Room 2180, 28359 Bremen (DE).
- (74) Agent: **CLYDE-WATSON, Zoe**; D Young & Co, 120 Holborn, London EC1N 2DY (GB).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**
— without international search report and to be republished upon receipt of that report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: PROCESS

(57) **Abstract:** The present invention provides a method of preparing a bioactive glass scaffold, said method comprising the steps of: (i) preparing a slurry comprising bioactive glass powder and a binder; (ii) contacting a polymer template with said slurry so as to form a coated polymer template; (iii) allowing the coated polymer template obtained in step (ii) to dry at ambient temperature; (iv) forming a bioactive glass scaffold by heat treating the coated polymer template obtained in step (iii) to remove the polymer template; and (v) sintering the bioactive glass scaffold obtained in step (iv).



WO 2007/017756 A2

PROCESS

The present invention relates to the field of bioactive and biodegradable glass. In particular, the invention relates to a bioactive and biodegradable glass which is useful in the fabrication of scaffolds for applications in tissue engineering. The invention also relates to a process for the preparation of bioactive and biodegradable glass scaffolds.

BACKGROUND TO THE INVENTION

Tissue engineering seeks to promote the regeneration ability of the host tissue through a designed biodegradable scaffold. Ideally, the scaffold should be 3-dimensionally porous to enable cell penetration, tissue ingrowth, rapid vascular invasion, and nutrient delivery (R. Langer, J.P. Vacanti, Science, 1993, 260, 920; J.S. Temenoff, L. Lu, A.G. Mikos, in Bone Engineering, J.E. Davies, Eds., EM Squared, Toronto, 2000, chapter 42; L.L. Hench, J.M. Polak, Science, 2002, 295, 1014).

The specific criteria for ideal scaffolds used in bone regeneration are as follows (S.P. Bruder, A.I. Caplan, in Principles of Tissue Engineering, R.P. Lanza, R. Langer, J. Vacanti, Eds., Academic Press, California, 2nd Edition, 2000, pages 683-696; Jones JR, Boccaccini AR. Cellular ceramics in biomedical applications: tissue engineering. In: Cellular Ceramics: Structure, Manufacturing, Processing and Applications. Scheffler M, Colombo P (Eds.). 2005 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, pages 550-573):

- (i) Ability to deliver/support cells: the material should not only be biocompatible, but should also foster cell attachment, differentiation, and proliferation;
- (ii) Osteoconductivity and bioactivity: the material should encourage osteoconduction with host bone. Osteoconductivity not only eliminates the formation of encapsulating tissue but also brings about a strong bond between the scaffold and host bone;

- (iii) Controllable biodegradability: the composition of the material, combined with the porous structure of the scaffold, should enable biodegradation *in vivo* at rates appropriate to tissue regeneration;
- (iv) Porous structure with a porosity ~90 % and pore diameters >400-500 μm : the scaffold should have an interconnected pore structure with diameters >400-500 μm for cell penetration, tissue ingrowth, new vascularization, and nutrient delivery;
- (v) Proper mechanical properties: the mechanical strength of the scaffold, which is determined by both the properties of the biomaterial and the porous structure, should be sufficient to provide mechanical stability to constructs in load bearing sites prior to synthesis of new extracellular matrix by cells.
- (vi) Irregular shape fabrication ability: the material and scaffold production methods should possess desired fabrication features, e.g., it should be feasible to produce irregular shapes of scaffolds that match bone defects of individual patients; and
- (vii) Potential for commercialisation: the synthesis of the material and fabrication of the scaffold should be cost-effective and suitable for commercialisation.

Bioactive glasses constitute one of the major material systems used in bone engineering. The glasses satisfy the first three criteria mentioned above i.e. the ability to deliver cells (A.M. Gatti, G. Valdre, O.H. Andersson, *Biomaterials*, 1994, 15, 208), excellent osteoconductivity and bioactivity (Wilson, G.H. Pigot, F.J. Schoen, L.L. Hench. *J. Biomed. Mater. Res.* 1981, 15, 805), and tailorable biodegradability (L.L. Hench, J. Wilson, *Science*, 1984, 226, 630). These advantages make bioactive glasses promising materials for scaffold design and fabrication (T. Livingston, P. Ducheyne, J. Garino, *J. Biomed. Mater. Res.*, 2002, 62, 1; H. Yuan, J.D. de Bruijn, X. Zhang, C.A. van Blitterswijk, K. de Groot, *J. Biomed. Mater. Res. Appl. Biomater.*, 2001, 58, 270).

The most popular bioactive glass composition (45S5 Bioglass[®]) has a composition containing 45% SiO₂, 24.5% Na₂O, 24.4% CaO and 6% P₂O₅ by weight percent. 45S5 Bioglass[®] has been approved by the US Food and the Drug Administration and

has been applied clinically as a filling material for bone repair and dental implants for the last 20 years (L.L. Hench, J. Wilson, Science, 1984, 226, 630).

To date, however, highly porous bioactive glass-based scaffolds (foams) have always suffered from a lack of mechanical strength, i.e. a failure to meet criterion
5 (v). This major hurdle has arisen due to the following apparently irreconcilable issues of this glass.

In order to improve the mechanical properties of the scaffold, extensive densification is required to strengthen the struts of the foam, which would otherwise be made of loosely bonded particles and thus be too fragile to handle. This densification can be
10 achieved by sintering the foam at high temperature. However, before significant densification takes place, full crystallisation of the glass will occur (D.C. Clupper, L.L. Hench, J. Non-Crys. Solid., 2003, 318, 43) and reports have shown that crystallisation may turn a bioactive glass (such as 45S5 Bioglass®) into an inert material (P. Li, F. Zhang, T. Kokubo. J. Mater. Sci. Med., 1992, 3, 452).

15 According to these three factors, to obtain a 45S5 Bioglass®-based scaffold having satisfactory mechanical properties, one must sinter it at a high temperature to ensure that extensive densification of struts occurs; but the bioactivity of the 45S5 Bioglass®-based foam is expected to be severely retarded or even suppressed altogether by crystallisation occurring before densification. Consequently, to
20 maintain the bioactivity of 45S5 Bioglass®, one should sinter the foam at a relatively low temperature at which crystallisation does not take place or does not occur to a great extent. However, this will be at the expense of mechanical strength as sufficient densification by sintering will not take place under these conditions, ultimately resulting in a very fragile scaffold made of loosely packed 45S5 Bioglass®
25 particles.

To date, this problem has impeded the development of an “ideal” bioactive glass scaffold meeting all of the desired criteria (i) to (vii) set forth above.

The present invention seeks to address this problem and provide a porous bioactive and biodegradable glass scaffold (and process for the production thereof) which

exhibits the desired degree of mechanical strength. In particular, bioactive glass scaffolds prepared by the process of the invention exhibit improved properties which have implications in the field of tissue engineering.

Aspects of the invention are set forth in the accompanying claims and description.

5 **STATEMENT OF THE INVENTION**

A first aspect of the invention relates to a method of preparing a bioactive glass scaffold, said method comprising the steps of:

- (i) preparing a slurry comprising bioactive glass powder and a binder;
- (ii) contacting a polymer template with said slurry so as to form a coated
10 polymer template;
- (iii) allowing the coated polymer template obtained in step (ii) to dry at ambient temperature;
- (iv) forming a bioactive glass scaffold by heat treating the coated polymer template obtained in step (iii) to remove the polymer template; and
- 15 (v) sintering the bioactive glass scaffold obtained in step (iv).

Advantageously, the bioactive glass scaffolds prepared in accordance with the claimed method exhibit a relatively high mechanical strength, whilst at the same time retaining the other desirable properties of a bioactive glass scaffold as outlined above, for example, in terms of pore size, bioactivity, biodegradation rate etc.

- 20 A second aspect of the invention relates to a bioactive glass scaffold obtainable by the above method.

A third aspect of the invention relates to a medical article comprising a bioactive glass scaffold as defined above.

- 25 A fourth aspect of the invention relates to the use of a bioactive glass scaffold as defined above in tissue engineering.

A fifth aspect of the invention relates to method of preparing a bioactive glass scaffold, said method comprising the steps of:

- (i) preparing a slurry comprising bioactive glass powder and a binder;
- (ii) contacting a polymer template with said slurry so as to form a coated polymer template;
- (iii) allowing the coated polymer template obtained in step (ii) to dry at ambient temperature; and
- (iv) forming a bioactive glass scaffold by heat treating the coated polymer template obtained in step (iii) to remove the polymer template.

10

A sixth aspect of the invention relates to method of preparing a bioactive and biodegradable glass scaffold comprising $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$, said method comprising the steps of:

- (i) preparing a slurry comprising bioactive glass powder and a binder, wherein the bioactive glass powder contains 45 % SiO_2 , 24.5 % Na_2O , 24.4 % CaO and 6 % P_2O_5 by weight;
- (ii) contacting a polymer template with said slurry so as to form a coated polymer template;
- (iii) allowing the coated polymer template obtained in step (ii) to dry at ambient temperature; and
- (iv) forming a bioactive glass scaffold by heat treating the coated polymer template obtained in step (iii) to remove the polymer template; and
- (v) sintering the bioactive glass scaffold obtained in step (iv).

15

20

25 The present invention is further described with reference to the detailed description below, and the following figures wherein:

Figure 1 shows the spongy-bone like structures of 45S5 Bioglass[®]-based foams sintered at (a) 950°C for 2 hours and (b) 1000°C for 1 hour. The inset in (b) shows the cross-section of a hollow strut.

Figure 2 shows the microstructure of struts in the 45S5 Bioglass[®]-based foams that were sintered at (a) 900°C for 5h; (b) 950°C for 2h; and (c) 1000°C for 1h. The inset in (c) shows the fine crystals of Na₂Ca₂Si₃O₉ phase.

Figure 3 shows the X-ray diffraction spectra of 45S5 Bioglass[®] powder and 45S5 Bioglass[®] powder sintered at 900°C for 5h and at 1000°C for 1h. All spectra were obtained using 0.1g powder. The major peaks of the phase Na₂Ca₂Si₃O₉ are marked by (▽).

Figure 4 shows the experimental and theoretical compressive strengths and, the three-point bending strength of scaffolds of the present invention sintered at 1000°C for 1h.

Figure 5 shows the X-ray diffraction spectra of 45S5 Bioglass[®]-based foams sintered at 1000°C for 1h, and immersed in simulated body fluid for 1, 2, and 4 weeks. All spectra were obtained using 0.1g powder. The major peaks of Na₂Ca₂Si₃O₉ and hydroxyapatite are marked by (▽) and (•), respectively.

Figure 6 shows the (a) cauliflower-shaped hydroxyl-apatite aggregation and (b) fine hydroxyapatite crystal needles (in the circled area) on the strut surface of 45S5 Bioglass[®]-based foams sintered at 1000°C for 1h and immersed in simulated body fluid for 2 and 4 weeks, respectively.

Figure 7 shows a flowchart of the polymer-sponge method for innovative production of Bioglass[®]-based foams.

Figure 8 shows the heat treatment program designed for burn-out of polyurethane templates and sintering of 45S5 Bioglass[®] green bodies.

Figure 9 shows (a) Porous β-tricalcium phosphate by solid free-form technique; (b) porous hydroxyapatite by dry powder method (powders and porogen additives are directly packed by pressing into moulds and thus form green bodies); (c) Bioactive glasses by sol-gel technique; (d) alumina by phase separation/freeze-drying method;

(e) porous hydroxyapatite by polymer-sponge method similar to the one of the present invention; and (f) spongy bone.

Figure 10 shows a schematic diagram of the electrophoretic deposition (EPD) cell used for obtaining a carbon nanotube (CNT) coating on Bioglass[®] scaffolds.

- 5 Figure 11 shows SEM micrographs showing the interconnected porous structure of Bioglass[®] scaffolds prepared using polyurethane foams with (a) 45 ppi and (b) 60 ppi.

Figure 12 shows SEM micrographs of a Bioglass[®] scaffold coated with CNT by EPD at 55V for 4 min: (a-b) low and (c-d) high magnification images showing partial
10 covering of the pore surfaces by CNT.

Figure 13 shows SEM micrographs of a Bioglass[®] scaffold coated with CNTs by EPD at 15V for 20 min: (a-b) low and (c-d) high magnification images, showing complete coating of the struts by CNT.

- Figure 14 shows an aluminium mould which can be used in the preparation of 45S5
15 Bioglass[®]-derived glass-ceramic scaffolds with gradient porosity

Figure 15 shows the realisation of a polyurethane preform with gradient porosity (a) a piece of polyurethane sponge after cutting; (b) compressing the polyurethane sponge in the aluminium mould; and (c) polyurethane sponge after heat treatment at 200°C/30min.

- 20 Figure 16 shows overlapped SEM images of a polyurethane preform with gradient porosity.

Figure 17 shows a 45S5 Bioglass[®]-derived glass-ceramic scaffold with a gradient of porosity after sintering at 1100°C/3h.

- Figure 18 shows overlapped SEM images of a sintered Bioglass[®]-derived glass-
25 ceramic scaffold with gradient porosity.

Figure 19 shows the silanization of the Bioglass® surface by the sol-gel precursor APTS.

Figure 20 shows the surface composition measured by x-ray photoemission spectroscopy for sintered Bioglass®-derived glass-ceramic pellets with and without surface functionalisation with APTS (a) before and (b) after 3 days in simulated body fluid.

Figure 21 shows cell proliferation data up to 6 days (relative growth vs control, Bioglass® sintered and sintered + functionalised).

Figure 22 shows scanning electron micrographs of cultured MG 63 osteoblast-like cells on sintered 45S5 Bioglass®-derived glass-ceramic pellets.

(a)-(b) Cultured for 1 day, showing that cell dividing was the main activity of cells; meanwhile, attachment was also undergoing.

(c)-(d) Cultured for 6 days, showing initial stage of attachment and well-attached cells.

Figure 23 shows scanning electron micrographs of cultured MG 63 osteoblast-like cells on sintered and surface-functionalised Bioglass®-derived glass-ceramic pellets.

(a)-(b) Cultured for 1 day; (c)-(d) cultured for 6 days.

DETAILED DESCRIPTION

A first aspect of the invention relates to a method of preparing a bioactive glass scaffold as set forth above. The process is based on a technique known as “replication” in which the bioactive glass scaffold is prepared using a sacrificial polymer template, usually in the form of a foam, which is subsequently removed to leave a porous bioactive glass scaffold.

Porous polymer and ceramic materials and their composites can be fabricated by a variety of processes (M.S. Widmer, A.G. Mikos in *Frontier in Tissue Engineering*, C.W. Patrick Jr, A.G. Mikos, L.V. McIntire, Eds., Elsevier Science, New York,

1998, pages 107-120; A. Atala, R.P. Lanza (editor), *Methods of Tissue Engineering*, Academic Press, California, 2002; D.W. Hutmacher, *Biomaterials*, 2000, 21, 2529; V. Maquet, A.R. Boccaccini, L. Pravata, I. Notingher, R. Jerome, *Biomaterials*, 2004, 25, 4185). Examples of these processes are outlined in Table 5. Among them,
5 the replication technique produces porous ceramic structures that are most similar to those of spongy bone (S.C. Cowin (editor), *Bone Mechanics*, CTC Press, Boca Raton, 1989, pages 1-4 and 10-1 - 10-23). The replication technique has been used for example to produce hydroxyapatite foams (Callcut S, Knowles JC. Correlation between structure and compressive strength in a reticulate glass-reinforced
10 hydroxyapatite foam. *J Mater Sci Mater Med*, 2002, 13, 485-489; Kim HW, Knowles JC, Kim HE. Hydroxyapatite porous scaffold engineered with biological polymer hybrid coating for antibiotic vancomycin release. *J Mater Sci Mater Med*, 2005, 16, 189-195). Other silicate based foams have been fabricated by sol-gel based methods (Jones JR, Hench LL. Factors affecting the structure and properties of
15 bioactive foam scaffolds for tissue engineering. *J Biomed Mater Res B: Apply Biomater*, 2004, 68B, 36-44). Finally, hydroxyapatite and calcium phosphate based porous scaffolds have been also produced by gel-casting techniques (Sepulveda P, Binner JGP, Rogero SO, Higa OZ, Bressiani JC. Production of porous hydroxyapatite by the gel-casting of foams and cytotoxic evaluation. *J Biomed Mater*
20 *Res*, 2000, 50, 27-34; Sepullveda P, Bressiani AH, Bressiani JC, Meseguer L, Konig Jr B. *In vivo* evaluation of hydroxyapatite foams. *J Biomed Mater Res*, 2002, 62, 587-59; Ramay HRR, Zhang M. Preparation of porous hydroxyapatite scaffolds by combination of the gel-casting and polymer sponge methods. *Biomaterials*, 2003, 24, 3293-3302; Ramay HRR, Zhang M, Biphasic calcium phosphate nanocomposite
25 scaffolds for load bearing bone tissue engineering. *Biomaterials*, 2004, 25, 5171-5180).

The replication method involves preparation of green bodies of ceramic (or glass) foams by coating a polymer (e.g. polyurethane) foam with a ceramic (or glass) slurry. The polymer, having the desired pore structure, serves as a sacrificial
30 template for the ceramic coating. The polymer template is immersed in the slurry, which subsequently infiltrates the structure and ceramic (glass) particles adhere to

the surfaces of the polymer. Excess slurry is squeezed out leaving a more or less homogeneous coating on the foam struts. After drying, the polymer is slowly burned out in order to minimise damage to the ceramic (glass) coating. Once the polymer has been removed, the ceramic (or glass) network is sintered to a desired density.

- 5 The process replicates the macrostructure of the starting sacrificial polymer foam, and results in a rather distinctive microstructure within the struts.

The porosity and pore size distribution of foams produced by the replication technique can easily achieve a porosity: ~90% and pore size greater than 400-500 μm (criterion (iv) mentioned above). This method also allows the fabrication of
10 irregular shapes (criterion (vi)), as a polymer template, from which the ceramic (or glass) scaffold copies the shape, can be produced in an irregular or complex shape that matches the defect in the bone of an individual patient. Finally, the replication technique does not involve the use of any toxic chemicals and is suitable for commercialisation in view of its simplicity and low cost (criterion (vii)).

15

Importantly though, the replication technique has to date never been considered in the context of producing scaffolds from bioactive glass.

The replication technique has a number of advantages compared to other prior art
20 processes currently used for the fabrication of porous bioceramic scaffolds.

Firstly, the porous structure produced by the replication technique is the most similar to spongy bone: i.e. highly porous and exhibiting open and interconnected pores (Figure 9), compared with the structures obtained by the other techniques.

Secondly, the technique is simple, and thus more suitable for commercialisation,
25 compared with SFF, sol-gel, and gel-casting techniques.

Thirdly, the technique does not involve any toxic chemicals, compared with sol-gel and gel-casting techniques.

Finally, the replication technique can produce a scaffold in an irregular or complex shape, as required for the tissue engineering application, compared with standard dry powder processing.

5 In one preferred embodiment of the invention, the polymer template is a foam.

Preferably, the polymer template is a polyurethane foam.

10 Preferably, the polyurethane foam is a fully opened foam having at least 60 pores per inch.

In one preferred embodiment, step (ii) comprises immersing the polymer template in the slurry. Preferably, the template is immersed for at least 5 minutes, more preferably at least 10 minutes, even more preferably at least 15 minutes.

15

Preferably, excess slurry is removed from the polymer template by squeezing the polymer template. Preferably, the coated template is rubbed manually until the coating appearance is homogenous by visual inspection. The coated template is then allowed to dry, preferably at ambient temperature, preferably for at least 12 hours.

20

In one preferred embodiment, step (iii) comprises turning the coated polymer template during the initial drying phase to allow homogenous drying.

25 Preferably, the coated polymer template is turned about once a minute for about 15 minutes during the initial drying phase.

In one particularly preferred embodiment, the bioactive glass contains 45 % SiO₂, 24.5 % Na₂O, 24.4 % CaO and 6 % P₂O₅ by weight. This bioactive glass is known as 45S5 Bioglass® (L.L. Hench, J. Wilson, Science, 1984, 226, 630).

30

Preferably, the bioactive glass powder has an average particle size of $\leq 10 \mu\text{m}$, more preferably from about 5 to about $10 \mu\text{m}$. In another preferred embodiment, the bioactive glass powder has a mean particle size of $< 5 \mu\text{m}$.

- 5 In one preferred embodiment, the slurry of step (i) comprises a mixture of bioactive glass powder and binder in a ratio of from about 20:80 to 50:50 w/w.

In one highly preferred embodiment, the ratio of bioactive glass powder to binder is about 40: 60 w/w.

10

In one preferred embodiment, the binder is an aqueous solution of polyvinyl alcohol.

Preferably, the concentration of polyvinyl alcohol is from about 0.005 mol/L to about 0.1 mol/L, more preferably from about 0.008 to about 0.04 mol/L.

15

In one particularly preferred embodiment, the concentration of polyvinyl alcohol is about 0.01 mol/L.

- 20 In one especially preferred embodiment, the slurry consists essentially of about 40 % of the bioactive glass 45S5 Bioglass[®] and about 60 % of an aqueous solution of polyvinyl alcohol.

In another preferred embodiment, the binder comprises PDLLA in DMC solution.

- 25 As used herein, "PDLLA" refers to poly-DL-lactide.

As used herein, "DMC" refers to dimethyl carbonate.

- 30 The slurry recipe using DMC solution/PDLLA produces a more homogeneous and thicker coating on a green body than the slurry recipe using PVA.

Preferably, the ratio of PDLLA:DMC solution is from about 0.5 g/60ml to about 2g/60ml, more preferably, from about 0.8 g/60ml to about 1.8 g/60ml.

5 In one particularly preferred embodiment, the ratio of PDLLA:DMC solution is from about 1.5 g/60 ml to about 1.8 g/60ml.

In one particularly preferred embodiment, the ratio of PDLLA:DMC solution is about 1.5 g/60 ml.

10 In another particularly preferred embodiment, the ratio of PDLLA:DMC solution is about 1.8 g/60 ml.

In one especially preferred embodiment, the slurry consists essentially of about 40 % of the bioactive glass 45S5 Bioglass® and about 60 % of PDLLA in DMC solution.

15 In one preferred embodiment, step (iv) comprises burning out the polymer template. Thus, in one preferred embodiment, step (iv) comprises heating the coated polymer template to a temperature sufficient to vaporise the polymer template.

20 In one especially preferred embodiment, step (iv) comprises heating the coated polymer template to a temperature of at least 200°C, more preferably, to at least 350°C.

25 In an even more preferred embodiment, step (iv) comprises heating the coated polymer template to at temperature of about 400°C at a rate of from about 1 to about 5°C/minute, more preferably, at a rate of about 2°C/minute.

Preferably, the coated polymer template is maintained at a temperature of 400°C for at least 30 minutes, preferably about an hour.

In another preferred embodiment, step (iv) comprises heating the coated polymer template to a temperature of about 550°C at a rate of from about 1 to about 5°C/minute, more preferably, at a rate of about 2°C/minute.

- 5 Preferably, the coated polymer template is maintained at a temperature of 550°C for at least 30 minutes, preferably about an hour.

Step (v) of the process involves sintering the bioactive glass scaffold that remains after the polymer template has been burned off. Without wishing to be bound by
10 theory, it is believed that for 45S5 Bioglass[®], sintering leads to densification of the struts of the foam-like scaffolds and the formation of fine crystals of Na₂Ca₂Si₃O₉, which confer the scaffolds the highest possible compressive and flexural strength. Importantly, the mechanically stiff phase Na₂Ca₂Si₃O₉ can transform into a
15 biodegradable amorphous calcium phosphate after immersion of the scaffold in simulated body fluid (SBF) for up to 4 weeks, thereby allowing the kinetics of this transformation and thus the degradation of the scaffold to be controlled.

In one preferred embodiment, step (v) comprises sintering the bioactive glass scaffold at a temperature of from about 900°C to about 1200°C.

20

More preferably, step (v) comprises sintering the bioactive glass scaffold at a temperature of from about 950°C to about 1100°C.

Even more preferably, step (v) comprises sintering the bioactive glass scaffold at a
25 temperature of about 1100°C.

In one especially preferred embodiment, step (v) comprises sintering the bioactive glass scaffold at a temperature of from about 900°C to about 1100°C for a period of up to about 5 hours.

30

In a further preferred embodiment, step (v) comprises sintering the bioactive glass scaffold at a temperature of from about 900°C to about 1100°C for a period of about 3 hours.

- 5 In one highly preferred embodiment, step (v) comprises sintering the bioactive glass scaffold at a temperature of about 1000°C for about 3 hours.

In another preferred embodiment, step (v) comprises sintering the bioactive glass scaffold at a temperature of from about 950°C to about 1000°C.

10

Even more preferably, step (v) comprises sintering the bioactive glass scaffold at a temperature of about 1000°C.

- 15 In one especially preferred embodiment, step (v) comprises sintering the bioactive glass scaffold at a temperature of from about 900°C to about 1000°C for a period of up to about 5 hours.

- 20 In a further preferred embodiment, step (v) comprises sintering the bioactive glass scaffold at a temperature of from about 900°C to about 1000°C for a period of about 1 hour.

In one highly preferred embodiment, step (v) comprises sintering the bioactive glass scaffold at a temperature of about 1000°C for about 1 hour.

- 25 Preferably, after sintering, the bioactive glass scaffold is cooled at a rate of about 1 to about 5°C/minute, more preferably, about 5°C/minute.

- 30 In one highly preferred embodiment, the method involves applying the heat treatment program set out in Figure 8, i.e. heating the coated polymer template obtained in step (iii) up to about 400°C at a rate of about 2°C/minute; maintaining the temperature at about 400°C for about 1 hour; heating up to a temperature of about 900 to about 1000°C at a rate of about 2°C/minute; maintaining the temperature at

about 900 to about 1000°C for up to 5 hours; cooling the temperature at a rate of about 5°C/minute until room temperature is reached.

Another aspect of the invention relates to a method of preparing a bioactive glass scaffold, said method comprising the steps of:

- (i) preparing a slurry comprising bioactive glass powder and a binder, wherein the bioactive glass powder contains 45 % SiO₂, 24.5 % Na₂O, 24.4 % CaO and 6 % P₂O₅ by weight;
- (ii) contacting a polymer template with said slurry so as to form a coated polymer template;
- (iii) allowing the coated polymer template obtained in step (ii) to dry at ambient temperature;
- (iv) forming a bioactive glass scaffold by heat treating the coated polymer template obtained in step (iii) to remove the polymer template; and
- (v) sintering the bioactive glass scaffold obtained in step (iv) at a temperature of from about 900°C to about 1000°C.

The present invention not only represents the first successful development of the replication technique to produce Bioglass[®]-based foam scaffolds, but more significantly provides a tailored sintering schedule which challenges the conventional wisdom in this field, namely, that to maintain bioactivity, bioactive glasses should be heat treated at such temperature that they remain in amorphous state (P. Li, F. Zhang, T. Kokubo. J. Mater. Sci. Med., 1992, 3, 452; L.L. Hench, J. Wilson, An Introduction to Bioceramics, Word Scientific, London, 2nd Edition, 1999).

The present invention is based on the idea that different crystalline phases formed in sintered 45S5 Bioglass[®] material have different bioactivities and biodegradabilities; some of them might be completely biologically inert, others might not. Moreover the bioreaction kinetics of a highly porous network is remarkably different from that of a dense product of the same material due to a high surface area. Hence, the invention provides a new sintering protocol leading to mechanically competent foams through

extensive densification of the struts, while inducing the formation of bioactive and biodegradable crystalline phases.

Advantageously, all criteria for an ideal tissue engineering scaffold, including that related to mechanical competence, can be satisfied by 45S5 Bioglass[®] foams fabricated by the replication method in combination with the prescribed sintering conditions.

In a preferred embodiment, the sintering conditions were optimised as follows: temperature range: 900-1000°C, sintering holding time: 0-5h; with heating and cooling rates being 2°C/min and 5°C/min, respectively. The selected sintering temperatures are much higher than those usually applied for Bioglass[®] heat treatment (<800°C) (T. Livingston, P. Ducheyne, J. Garino, J. Biomed. Mater. Res., 2002, 62, 1; L.L. Hench, J. Wilson, An Introduction to Bioceramics, Word Scientific, London, 2nd Edition, 1999).

All 45S5 Bioglass[®]-based scaffolds sintered under different conditions were highly porous resembling spongy bone (Figure 1). The inset in Figure 1b shows the hollow nature of the foam struts. The porosities of all synthesised foams were in the range 89-93%. The pore sizes were in the range 510-720µm in foams sintered at 1000°C for 1h. Scanning electron microscopy (SEM) observations showed that extensive densification did not occur at 900°C after 5h sintering (Figure 2a), but it occurred largely when the foams were heated up to 950°C and above (Figure 2b). A significant reduction in porosity was observed after sintering at 1000°C for 1h (Figure 2c). This indicates the very narrow temperature window for densification of this particular glass. Fine crystals of ~0.5µm in diameter were detected by SEM observation in foams sintered at 950-1000°C (inset of Figure 2c). The crystallisation of the foams was analysed using X-ray diffraction (XRD) (Figure 3). It was found that crystallisation had already occurred extensively in samples sintered at 900°C for 5h, though bonding of partially sintered particles was not obvious at this sintering condition (Figure 2a). This observation confirmed the finding of Clupper and Hench (D.C. Clupper, L.L. Hench, J. Non-Crys. Solid., 2003, 318, 43) that extensive

crystallisation usually occurs prior to significant viscous flow sintering in 45S5 Bioglass® and in related bioactive glasses. The crystalline phase in the present foams was identified to be Na₂Ca₂Si₃O₉, as the angle and intensity of all peaks in Figure 3 match well with the standard PDF #22.1455.

Thus, 1000°C/1h represents an optimum sintering condition, which leads to significant densification of the struts while the high macroporosity of the foams as well as their macroscopic shape are retained. The low microporosity and fine crystal particles in the foam struts (Figure 2c) are expected to lead to improved mechanical properties. Hence compressive strength and three-point bending strength tests were carried out on foams sintered under these conditions. In Figure 4, the raw data of compressive or bending strength are plotted against the foam porosities. It can be seen that the compressive and bending strength values are in the ranges 0.3-0.4MPa and 0.4-0.5MPa, respectively, when porosity is ~91%. The bending strength is higher than the compressive strength, the ratio being ~1.4. This is in agreement with early studies on ceramic materials (R.W. Davidge, Mechanical Behaviour of Ceramics, Cambridge Univ. Press, Cambridge, 1979, page 139).

The theoretical compressive strength (σ_{theo}) can be expressed as a function of the relative density ($\rho_{\text{foam}}/\rho_{\text{solid}}$) of a cellular structure and the size of the central hollow struts by equation (1) (L.J. Gibson, M.F. Ashby, Cellular Solids: Structure and Properties, Pergamon, Oxford, 2nd Edition, 1999, pages 118, 211 and 442):

$$\frac{\sigma_{\text{theo}}}{\sigma_{\text{fs}}} = 0.2 \left(\frac{\rho_{\text{foam}}}{\rho_{\text{solid}}} \right)^{\frac{3}{2}} \frac{1 + \left(\frac{t_v}{t} \right)^2}{\sqrt{1 - \left(\frac{t_v}{t} \right)^2}}, \quad (1)$$

where t_v/t is the ratio of the void and strut sizes on a cross-section of a strut (see inset of Figure 1b) and σ_{fs} is the modulus of rupture of the strut.

Statistical calculations show that the modulus of rupture is typically 1.1 times larger than the tensile strength in ceramics (L.J. Gibson, M.F. Ashby, Cellular Solids:

Structure and Properties, Pergamon, Oxford, 2nd Edition, 1999, pages 118, 211 and 442).

In the present application, the tensile strength (42MPa) of annealed 45S5 Bioglass[®] (L.L. Hench, J. Wilson, An Introduction to Bioceramics, Word Scientific, London, 2nd Edition, 1999) was used as an approximation for the strength of the partially crystallised material. The ratio t_v/t was estimated to be ~0.5 according to microstructural observations (e.g. Figure 1b). Equation (1) has been derived assuming that the ceramic phase in the tubular strut walls is fully dense. Using the above equation, the theoretical compressive strength of the Bioglass[®]-based foams was calculated and found to be in good agreement with experimental values, a narrow gap being in the scattering range of the experimental data, as shown in Figure 4.

The bioactive glass scaffolds of the invention are advantageous for the following reasons:

- a) the theoretical strength (i.e. maximum achievable strength) has been reached by virtue of nearly full densification of foam struts at 1000°C for 1h;
- b) the tensile strength of sintered 45S5 Bioglass[®] (42MPa) of the present application is very close to that of hydroxyapatite (HA) (40MPa) (L.L. Hench, J. Wilson, An Introduction to Bioceramics, Word Scientific, London, 2nd Edition, 1999), hence theoretically, the mechanical strength of a 45S5 Bioglass[®]-based scaffold should be as high as, if not higher than, that of a HA scaffold with a similar pore structure; and
- c) for the same relative density, the foam becomes stronger with increasing t_v/t ratio (L.J. Gibson, M.F. Ashby, Cellular Solids: Structure and Properties, Pergamon, Oxford, 2nd Edition, 1999, pages 118, 211 and 442).

To date, there has been no report on the mechanical properties of highly porous bioactive glass scaffolds made from melt-derived powder. Comparing with HA foams of similar porosities, the foams produced by the invention are the strongest:

e.g. compressive strengths of 0.175 and 0.21MPa were reported for HA foams with ~85% porosity (H.W. Kim, J.C. Knowles, H.E. Kim, *J Mater. Sci. Mater. Med.*, 2005, 16, 189; S. Callcut, J.C. Knowles, *J. Mater. Sci. Mater. Med.*, 2002, 13, 485).

In fact the compressive strength values of the foams of the present invention are in the range of values measured for spongy bone, which is 0.2-4MPa when porosity is ~90% (L.J. Gibson, M.F. Ashby, *Cellular Solids: Structure and Properties*, Pergamon, Oxford, 2nd Edition, 1999, pages 118, 211 and 442). The foams (1cm³ in volume) could keep integrity when dropped from one metre high onto a hard marble floor. This indicates that a strength in the range 0.3-0.4MPa is sufficient for the foams to be handled, for example, when manipulating during SBF tests and cutting samples for mechanical tests.

Assessment of bioactivity by detecting the formation of HA phase on the surface of foam struts in simulated body fluid (SBF) was carried out on samples sintered at 1000°C for 0.5h and 1h. Figure 5 shows the XRD spectra of 45S5 Bioglass[®]-based foams (sintered at 1000°C for 1hr) after immersion in SBF for 1, 2 and 4 weeks. Figure 6a is a SEM micrograph showing the formation of HA-like phase on the foam strut surface. In addition to the growing peaks related to the HA-like phase detected in the XRD spectra of soaked samples, a significant phenomenon documented in Figure 5 should be noted: it is observed that the crystallinity of the sintered 45S5 Bioglass[®]-based foams decreases with increasing immersion time in SBF. Eventually the sharp diffraction peaks of the Na₂Ca₂Si₃O₉ phase disappear from the XRD spectrum after soaking in SBF for 4 weeks, leaving a typical broad halo (produced by an amorphous phase) overlapped by the sharp diffraction peaks of the HA phase. This indicates that (at least under the detection limits of XRD) the sintered 45S5 Bioglass[®]-based material was mainly composed of an amorphous phase and crystalline apatite after soaking in SBF for 4 weeks. SEM examination (Figure 6b) confirmed that the fine Na₂Ca₂Si₃O₉ crystals have disappeared from the strut microstructure after immersion in SBF for 4 weeks. Instead typical fine crystalline needles of HA were observed embedded in an amorphous matrix. Similar XRD spectra and SEM morphologies were also obtained for foams sintered at

1000°C for 0.5h, except that the kinetics of apatite formation and of transformation of $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$ to an amorphous phase were about one week faster in foams sintered at 1000°C for 0.5h than in those sintered at 1000°C for 1h.

The mechanisms behind the transformation of $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$ to an amorphous phase
5 might be based in the well-known bone-bonding mechanisms of bioactive glasses, which were originally proposed by Hench (L.L. Hench, J. Wilson, Science, 1984, 226, 630). In the sequence of interfacial reactions on the surface of Bioglass® in contact with body fluids, the bioactive glass first dissolves to form a silica-gel layer; then an amorphous calcium phosphate is formed from the hydrated silica-gel; and
10 finally apatite crystallites nucleate and grow from the amorphous calcium phosphate. The general idea of the reaction sequence should be applicable to $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$ crystallites as well, although these dissolve at a slower rate than the glass phase. Hence, it is very likely that the amorphous phase detected by XRD after immersion in SBF for 4 weeks (Figure 5) is a calcium phosphate, according to Hench's theory
15 (L.L. Hench, J. Wilson, Science, 1984, 226, 630; L.L. Hench, J. Wilson, An Introduction to Bioceramics, Word Scientific, London, 2nd Edition, 1999). Although the kinetics of the transformation has yet to be fully understood, it is believed that the high surface area (including the hollow centre of the struts) in the porous network, are of importance in maintaining bioactivity and biodegradability of the
20 sintered 45S5 Bioglass®-based foams. The energy provided by the high surface area should ensure that the transformation of $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$ to the amorphous calcium phosphate phase occurs at a reasonably fast rate at the body temperature. This presumption is supported by the fact that the bioactive reactions only occur at the surface of a bulk solid glass. It is based on this fact that bioactivity is defined as
25 being the interfacial ability to bond to bone (L.L. Hench, J. Wilson, Science, 1984, 226, 630).

The significance of the transformation of the $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$ crystalline phase to the amorphous calcium phosphate phase in a body solution environment is that it could realise the dream of achieving an ideal scaffold for bone engineering. Bone
30 engineering demands a scaffold that can provide a temporary mechanical support

and that degrades later at a rate matching the regeneration rate of new bone. Unfortunately this is not the manner conventional bioceramics behave in biological conditions. Crystalline HA, for instance, can provide strong support as a scaffold material, but degrades very slowly in contact with body fluids (indeed it is
5 fundamentally wrong to expect crystalline HA to degrade quickly in the body, considering that nano-sized HA is a stable component of natural bone). Amorphous HA, as well as related amorphous calcium phosphates, degrades fast; but these phases are too fragile to be used for production of highly porous scaffolds.

In contrast, in the present Bioglass[®]-based scaffolds, the nearly full densification of
10 struts and the fine crystals of $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$ confer the scaffold a suitable mechanical performance before the crystalline phase transforms to the amorphous calcium phosphate phase at later stages of contact with simulated body fluid. The transformation of $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$ to the amorphous calcium phosphate phase in SBF ensures the bioactivity and the degradability of the scaffold. More importantly, the
15 kinetics of the transformation and degradation can be controlled by factors such as initial crystallinity, microporosity in struts, and crystal size of $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$, all of which can be tailored by the sintering conditions. Accordingly, the goal of an ideal scaffold that provides proper mechanical support temporarily while maintaining bioactivity, and that can biodegrade later at a rate tailored to match the rate of bone
20 regeneration at an anatomical site, is achievable with 45S5 Bioglass[®]-based foams.

Another aspect of the invention relates to a bioactive glass scaffold obtainable by the method set forth above.

As mentioned above, the present scaffolds exhibit a number of advantages over
25 existing ceramic foams. Compared with foams produced by other methods, the porous structure of the present scaffolds is most similar to that of spongy bone. Moreover, the porosity of the present 45S5 Bioglass[®]-based foams is much higher than that of previously reported 45S5 Bioglass[®]-based foams fabricated using dry powder process; the latter exhibit a porosity of only 21-40%, which is much lower
30 than the required porosity of an ideal scaffold.

Finally, the foams of the present invention are advantageous when compared with other foams produced by the replication technique. For example, hydroxyapatite (HA) and other inert bioceramic foams, such as ZrO_2 , Al_2O_3 , and TiO_2 , have been fabricated by the same technique used by this invention. HA degrades very slowly, whereas ZrO_2 , Al_2O_3 , and TiO_2 are all bioinert, non-degradable ceramics. In contrast, the present 45S5 Bioglass[®]-based foams exhibit tailorable biodegradation kinetics and are the strongest scaffolds among those produced by the replication method.

10 Bioactive Glass Scaffolds having Porosity Gradients

The present invention further provides a method of preparing bioactive glass scaffolds with tailored porosity gradients using the above replication technique. Glass-ceramic scaffolds with tailored porosity gradients can be produced by using a pre-moulded polymeric sponge. The process for the preparation of these scaffolds involves pre-treating the polymer template in a metallic mould at temperatures of 150-200°C in order to obtain a gradient sponge preform which is then used as the polymer template in the methods of the invention as described above.

Preferably, the polymer template is a polyurethane foam. More preferably, the polyurethane foam is a fully opened foam having at least 45 pores per inch.

As used herein, the term "porosity gradient" means that the porosity is non-uniform, i.e. the bioactive glass scaffold has a higher porosity in one area than in another.

25 In an especially preferred embodiment, the porosity gradient is continuous.

Preferably, the polymer template is pre-treated by:

- (a') compressing the polymer template into a mould; and
- (b') heating the compressed polymer template in the mould.

30

This pre-treatment leads to a gradient sponge preform which is then used as the polymer template in the above-described methods.

In one preferred embodiment, the mould is a metallic mould.

In one particularly preferred embodiment, the mould comprises aluminium, preferably aluminium foil.

5

In one preferred embodiment, step (b') comprises heating the compressed polymer template in the mould at a temperature of at least 150°C.

10

Even more preferably, step (b') comprises heating the compressed polymer template in the mould at a temperature of about 150°C to about 200°C, more preferably at about 200°C.

15

In one especially preferred embodiment, step (b') comprises heating the compressed polymer template in the mould at a temperature of about 150°C to about 200°C for a period of up to about 60 minutes, more preferably for about 45 minutes and even more preferably for about 30 minutes.

20

In one highly preferred embodiment, step (b') comprises heating the compressed polymer template in the mould at a temperature of about 200°C for a period of about 30 minutes.

Carbon Nanotube Coating for Bioactive Glass Scaffolds

In one preferred embodiment of the invention, the process further comprises the step of depositing carbon nanotubes onto the bioactive glass scaffold obtained in step (v).

25

Thus, optionally, carbon nanotubes can be deposited onto the above-mentioned bioactive glass scaffolds. The scaffold's pore structure remains invariant after the CNT coating, as assessed by SEM. Advantageously, the incorporation of CNTs induces a nanostructured internal surface of the pores which is believed to be beneficial for osteoblast cell attachment and proliferation.

- Carbon nanotubes (CNTs) have been a subject of extensive research since the paper published in 1991 by Iijima [Letters to Nature; 354, 56, 1991]. Due to their impressive structural, electrical and mechanical properties as well as their small size and mass, carbon nanotubes have become one of the most promising materials for future developments and have opened a new era in materials science and nanotechnology (M. S. Dresselhaus and H. Dai, April 2004 issue: Advances in Carbon Nanotubes. MRS Bulletin, P. J. F. Harris, "Carbon nanotube composites", International Materials Reviews, 49 (2004) [1], 31-43).
- 10 In recent years, considerable efforts have been devoted to apply CNTs in the biological and medical fields (Wang W, Omori M, Watari F, Yokoyama A, Novel bulk carbon nanotube materials for implant by spark plasma sintering, Dental Mater. Journal 24 (4) (2005): 478-486; Zhang DH, Kandadai MA, Cech J, Roth S, Curran SA, Poly(L-lactide) (PLLA)/multiwalled carbon nanotube (MWCNT) composite: 15 Characterization and biocompatibility evaluation, J. Phys Chem. B 110 (26) (2006): 12910-12915; .Sinha N, Yeow JTW, Carbon nanotubes for biomedical applications, IEEE Trans. Nanobioscience 4 (2) (2005) 180-195). Previous reports have shown that the electrical conductance of carbon nanotubes is highly sensitive to the environment and depends on the electrostatic charge variation and surface absorption of different 20 molecules (J.Kong, N. R. Franklin, C. W. Zhou, M. G. Chapline, S. Peng, K. J. Cho and H. Dai, "Nanotube molecular wires as chemical sensors", Science, 287 (2000) 622-625). Moreover, the results of related research have suggested the possibility of fabricating of CNT-based miniature sensors for detecting biological molecules in fluids (Y. Lin, S. Taylor, H. Li, K. A. Shiral Fernando, L. Qu, W. Wang, L. Gu, B. 25 Zhou and Y. P. Sun, "Advances toward bioapplications of carbon nanotubes", Journal of Materials Chemistry, 14, (2004) 527-541).

Since CNTs have the ability to be shaped into 3D architectures, they are ideal for cell seeding and *in vitro* cell modelling, and thus for the fabrication of novel 30 nanostructured tissue engineering scaffolds and other biomedical applications. Several studies have been carried out on the interactions between CNT and a variety of cells including osteoblasts, focusing on the biocompatibility of CNT (L. P.

- Zanella, B. Zhao, H. Hu and R. C. Haddon, "Bone cell proliferation on carbon nanotubes", *Nano Letters*, 6 [3] (2006), 562-567, M. A. Correa-Duarte, N. Wagner, J. Rojas-Chapana, C. Morszeck, M. Thie and M. Giersig, "Fabrication and biocompatibility of carbon nanotube-based 3D networks as scaffolds for cell seeding and growth", *Nano letters*, 4 [11], (2004) 2233-22236). These studies suggest the possibility of using CNTs as an alternative material for the treatment of bone pathologies in combination with bone cells, with the potential for enhanced osteoblast proliferation and bone formation. Moreover, recent studies have shown that CNTs and carbon nanofibres are highly biocompatible and provide efficient substrates to stimulate robust tissue formation (T. J. Webster, M. C. Waid, J. L. McKenzie, R. Price, J. Ejiofor, "Nano-biotechnology: carbon nanofibres as improved neural and orthopaedic implants", *Nanotechnology*, 15, (2004) 48; L. P. Zanella, B. Zhao, H. Hu and R. C. Haddon, "Bone cell proliferation on carbon nanotubes", *Nano Letters*, 6 [3] (2006), 562-567)
- Electrophoretic deposition (EPD) is a materials processing technique based on the movement of charged particles in liquid suspensions and their deposition on a substrate acting as electrode in the EPD cell (P. Sarkar and P. S. Nicholson, "Electrophoretic deposition (EPD): mechanics, kinetics and application to ceramics", *J. Am. Ceram. Soc.*, 79, (1996) 1987-2002). EPD is a method of low cost, short formation time, few substrate shape restrictions and simple experimental equipment useful to produce coatings and films of homogeneous microstructure and controlled thickness on different substrates (A. R. Boccaccini and I. Zhitomirsky, *Application of electrophoretic and electrolytic deposition techniques in ceramics processing. Current Opinion in Solid State & Materials Science*, 6(3), (2002) 251-260). This processing technique is being increasingly considered for the production of nanostructured coatings and layers on a variety of substrates for numerous applications, including wear and oxidation resistance, bioactive coatings for biomedical implants and devices as well as functional coatings for photocatalytic, electronic, magnetic and related applications (A. R. Boccaccini, et al., *The electrophoretic deposition of inorganic nanoscaled materials. A Review*, *J. Ceram. Soc. Japan* 114 (2006) 1-14).

In this context, electrophoretic deposition (EPD) has been shown to be a very convenient method to manipulate CNTs and to produce CNTs assemblies and layers on planar substrates (B. J. C. Thomas, M. S. P. Shaffer, S. Freeman, M. Koopman, K. K. Chawla and A. R. Bocaccini, "Electrophoretic deposition of carbon nanotubes on metallic surfaces", *Key Engineering Materials*, 314, (2006) 141-146; C. Du, D. Heldbrandt and N. Pan, "Preparation and preliminary property study of carbon nanotubes films by electrophoretic deposition", *Materials Letters*, 57, (2002) 434-438). For example, Du *et al.* (C. Du, D. Heldbrandt and N. Pan, "Preparation and preliminary property study of carbon nanotubes films by electrophoretic deposition", *Materials Letters*, 57, (2002) 434-438) deposited CNT on metallic substrates by EPD using ethanol/acetone mixed suspensions. Further studies were carried out by Thomas *et al.* (B.J.C. Thomas, A. R. Bocaccini, "Multi-walled carbon nanotube coatings using electrophoretic deposition (EPD)", *Journal of the American Ceramic Society*, 88 [4], (2005) 980-982) where homogeneous deposition of CNT assemblies using aqueous suspensions was accomplished on stainless steel substrates. However, no previous research has been conducted focusing on production of CNTs coatings on porous substrates such as scaffolds for tissue engineering applications.

The current studies explore the possibility of using EPD to produce uniform deposits of CNTs on porous Bioglass[®]-based scaffolds intended for bone tissue engineering. The incorporation of CNTs into the scaffolds has a number of purposes, such as to encourage cell adhesion and proliferation, to work as a crack inhibiting mechanism on the scaffold surfaces and to confer biosensing (electrical conduction) properties while maintaining the bioactivity and interconnected porous network of the scaffold.

In one preferred embodiment of the invention, the CNTs are deposited by electrophoretic deposition.

In another preferred embodiment, the CNTs are deposited by electrophoretic deposition using an aqueous suspension comprising CNTs.

Preferably, the ratio of CNTs : water is about 0.1 mg/ml to about 10 mg/ml, more preferably about 0.5 to about 6 mg/ml, more preferably still, about 0.6 mg/ml to about 5.1 mg/ml and even more preferably about 5.1 mg/ml.

- 5 In one particularly preferred embodiment, the aqueous suspension further comprises a surfactant.

As used herein, the term “surfactant” includes any surface-active agent, i.e. any material that lowers the surface tension.

10

In one especially preferred embodiment, the surfactant is an anionic surfactant.

In a highly preferred embodiment, the surfactant is polyethylene glycol *tert*-octylphenyl ether (Triton X-100).

15

The high dispersion efficiency of Triton X-100 is due to its chemical structure, where the aromatic group is responsible for the strong hydrophobic interaction between Triton X-100 and the CNTs. Without wishing to be bound by theory, it is believed that the π -like stacking of the benzene rings of the surfactant onto the surface of graphite increases the binding and surface coating of surfactant molecules onto graphite significantly (M. F. Islam, E. Rojas, D. M. Bergey, A. T. Johnson and A. G. Yodh, “High weight fraction surfactant solubilization of single-wall carbon nanotubes in water”, Nano Letters, 3 [2], (2003) 269-276).

20

- 25 In another particularly preferred embodiment, the aqueous suspension further comprises a charger.

As used herein, the term “charger” includes a material which enhances particle charging in the solution.

30

In an especially preferred embodiment, the charger is iodine. Without wishing to be bound by theory, it is believed that iodine works as a charger due to its ability to form complexes with water.

- 5 In another particularly preferred embodiment, the aqueous suspension is sonicated before electrophoretic deposition to help the particles overcome the attractive van der Waals forces.

10 Preferably, the aqueous suspension is sonicated for about 1 to about 10 hours, more preferably, for about 2 to about 8 hours and even more preferably, for up to about 4 to about 5 hours.

15 In yet another particularly preferred embodiment, the aqueous suspension is centrifuged before electrophoretic deposition in order to remove the large CNT agglomerations and avoid their deposition during electrophoretic deposition.

20 Preferably, the aqueous suspension is centrifuged for up to about 60 minutes, more preferably, for up to about 30 minutes and even more preferably for up to about 15 minutes.

Preferably, the aqueous suspension is centrifuged at up to about 5000 rpm, more preferably, at up to about 4000 rpm and even more preferably, at up to 3000 rpm.

25 In another preferred embodiment, electrophoretic deposition is carried out at an applied electric field in the range of about 1 V/cm to about 100 V/cm, more preferably, in the range of about 5 V/cm to about 70 V/cm and even more preferably, in the range of about 10V/cm to about 55V/cm.

30 In a particularly preferred embodiment, the applied electric field is about 15V/cm.

In yet another preferred embodiment, the deposition time is in the range of about 1 minute to about 60 minutes, more preferably, in the range of about 2 minutes to

about 30 minutes and even more preferably, in the range of about 4 minutes to about 20 minutes.

In a particularly preferred embodiment, the deposition time is about 20 minutes.

5

In another preferred embodiment, the aqueous suspension comprises carbon nanotubes : water in a ratio of about 5.1 mg/ml, polyethylene glycol *tert*-octylphenyl ether (Triton X-100) and iodine, the applied electric field is about 15V/cm and the deposition time is about 20 minutes.

10

Two series of EPD experiments were carried out. One set of experiments involved constant deposition time and different applied voltages in the range 10-55V. In the second experiment series, the deposition time was varied in the range 4-20 minutes, while the applied voltage was kept constant at values between 10 and 55V.

15

Experiments were carried out with dilute or concentrated suspensions, as shown in Table 6. Typical examples are shown below to document the effect of the different variables investigated and to determine the optimal conditions.

20

Figure 12(a-d) are SEM images of a bioactive glass scaffold coated with CNTs by EPD using a voltage of 55 V and deposition time of 4 minutes, using the diluted suspension (Table 6). It can be seen from the SEM images that the scaffold is partially coated with CNT. Without wishing to be bound by theory, this can be due to the low concentration of CNT in suspension or the relatively low deposition time. Figure 12(a) illustrates qualitatively that the scaffold pore structure remained invariant after electrophoretic deposition of CNT, indicating that carbon nanotubes or agglomeration of CNT did not block the pores.

25

In order to enhance the homogeneity of the CNT coating, a concentrated suspension was prepared (suspension no. 2 in Table 6). EPD using the concentrated suspension was conducted at constant voltage values in the range 15-30 V, with deposition times ranging from 4 to 20 minutes and electrode separation of 1 cm. When the applied voltage was below 15V, CNTs could not be deposited successfully. Without wishing

30

to be bound by theory, this behavior may be due to the insufficient electric field force acting on the CNTs to overcome the electrostatic repulsive force, which depends on the degree of electric double layer overlap. It was also found that if the voltage applied was above 30 V gas evolution occurred, due to water electrolysis.

- 5 The evolved gas interferes during the coating deposition producing an heterogeneous film.

The best results in terms of homogeneity of the CNT deposit microstructure and uniform CNT coating thickness were achieved using the concentrated suspension.

- 10 The images in Figures 12(a) and 12(b) reveal a uniformly coated scaffold as well as a homogeneous coating of the pore walls (struts) in the micro-scale. According to SEM observations, the optimal experimental conditions for EPD were found to be: applied electric field of 15 V/cm and deposition time of 20 minutes.

- 15 Preferably, the bioactive glass scaffold is surrounded by a copper wire cage between the two electrodes next to the anode where the electrophoretic deposition takes place, given that CNTs would acquire a net negative surface charge in suspension.

- 20 In another preferred embodiment, the coated bioactive glass scaffolds are dried after electrophoretic deposition.

In one particularly preferred embodiment, the coated bioactive glass scaffolds are dried at room temperature.

- 25 In an especially preferred embodiment, the coated bioactive glass scaffolds are dried in normal air.

A further aspect of the invention relates to a composite comprising a bioactive glass scaffold having carbon nanotubes deposited on a surface thereof.

30

Preferably, the bioactive glass contains 45 % SiO₂, 24.5 % Na₂O, 24.4 % CaO and 6 % P₂O₅ by weight.

More preferably, the bioactive glass scaffold is prepared by a method according to the invention as described above.

5 Preferably, the carbon nanotubes are deposited on the surface of the bioactive glass scaffold by electrophoretic deposition.

Surface Functionalisation of Bioactive Scaffolds

10 In one preferred embodiment, the bioactive scaffolds prepared by the present method are chemically treated to couple proteins in a process called surface functionalisation.

15 Preferably, surface functionalisation is achieved by treating the bioactive glass scaffold with 3-aminopropyl-triethoxysilane (APTS). The working mechanism of silanization is shown in Figure 19. The efficiency and stability of the surface modification were satisfactory as quantitatively assessed by X-ray photoemission spectroscopy (XPS), as shown in Figure 20.

20 It was found that treatment in buffered (pH = 8) water solution at 80°C for 4 hrs, applied during the surface functionalisation procedure, accelerated the bioreactive kinetics of the scaffolds: i.e. the transition of the relatively bioinert but mechanically competent crystalline structure of the struts to a biodegradable but mechanically weak amorphous network during immersion in simulated body fluid. Thus the aqueous heat treatment is an important factor that must be considered in the design of these Bioglass®-derived glass-ceramic scaffolds.

25 A further aspect of the invention relates to a surface-functionalised bioactive glass scaffold obtainable by reacting a bioactive glass scaffold with a surface functionalisation reagent.

30 Preferably, the bioactive glass contains 45 % SiO₂, 24.5 % Na₂O, 24.4 % CaO and 6 % P₂O₅ by weight.

More preferably, the bioactive glass scaffold is prepared by a method according to the invention as described above.

5 Preferably, the surface functionalisation reagent is 3-aminopropyl-triethoxysilane (APTS).

Yet another aspect of the invention relates to a bioactive glass scaffold obtained by the method set forth above.

10 Yet another aspect of the invention relates to medical article comprising a bioactive glass scaffold obtainable by the methods set forth above. In one preferred embodiment, the medical article is an implantable device that is capable of inducing tissue formation in autogenic, allogenic and xenogeneic implants. Examples of such devices include prosthetic implants, hip devices, sutures, stents, screws, rods, cages
15 for spine fusion, pins, valves, sheets, plates, tubes, and the like.

Yet another aspect of the invention relates to the use of a bioactive glass scaffold obtainable by the method set forth above in tissue engineering and more preferably, bone tissue engineering.

20 Another aspect of the invention relates to a method of preparing a bioactive and biodegradable glass scaffold comprising $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$, said method comprising the steps of:

- (i) preparing a slurry comprising bioactive glass powder and a binder, wherein the bioactive glass powder contains 45 % SiO_2 , 24.5 % Na_2O , 24.4 % CaO
25 and 6 % P_2O_5 by weight;
- (ii) contacting a polymer template with said slurry so as to form a coated polymer template;
- (iii) allowing the coated polymer template obtained in step (ii) to dry at ambient temperature;

- (iv) forming a bioactive glass scaffold by heat treating the coated polymer template obtained in step (iii) to remove the polymer template; and
- (v) sintering the bioactive glass scaffold obtained in step (iv).

In one preferred embodiment, the method further comprises depositing carbon
5 nanotubes onto the bioactive glass scaffold obtained in step (v) and/or
functionalising the surface of the bioactive glass, for example by reacting with
APTS.

Another aspect of the invention relates to a method of preparing a bioactive glass scaffold, said method comprising the steps of:

- 10 (i) preparing a slurry comprising bioactive glass powder and a binder;
- (ii) contacting a polymer template with said slurry so as to form a coated polymer template;
- (iii) allowing the coated polymer template obtained in step (ii) to dry at ambient temperature;
- 15 (vi) forming a bioactive glass scaffold by heat treating the coated polymer template obtained in step (iii) to remove the polymer template.

By way of summary, the present invention provides highly porous, mechanically competent, bioactive and biodegradable 45S5 Bioglass[®]-based scaffolds using the
20 replication technique followed by tailored/controlled high temperature sintering. Under this condition, nearly full densification of the struts of the foam-like scaffolds occurred and fine crystals of $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$ form, which confer the scaffolds with the maximum possible compressive and flexural strength. The key findings are that the mechanically stiff phase $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$ can transform into a biodegradable amorphous
25 calcium phosphate after immersion of the scaffold in simulated body fluid (SBF) for up to 4 weeks, and that the kinetics of this transformation, and thus the degradation of the scaffold, are controllable. This investigation has shown that the goal of an ideal bone engineering scaffold that provides mechanical support temporarily while maintaining bioactivity and that can biodegrade at a later stage is achievable with the
30 presently developed bioactive glass-based foams.

The inventors have also demonstrated the high versatility of EPD to develop CNTs coatings on highly porous Bioglass[®] scaffolds. This work has successfully produced uniform CNTs deposits on highly porous bioactive and biodegradable 45S5 Bioglass[®]-derived glass-ceramic scaffolds, intended for bone tissue engineering, by the electrophoretic deposition technique (Figures 12 and 13). The optimal experimental conditions were determined to be: applied electric field of 15V/cm and deposition time 20 minutes, utilizing a concentrated CNT suspension in water. The scaffolds pore structure remained invariant after the CNT coating, as assessed by SEM and the incorporation of CNTs induced a nanostructured internal surface of the pores.

The present invention is further described by way of the following examples.

EXAMPLES

15 Materials

The starting material was melt-derived 45S5 Bioglass[®] powder (particle size ~5µm) provided by a collaborator as a gift (Dr. I. Thompson, Imperial College London). A fully reticulated polyester-based polyurethane foam with 60ppi (pores per inch) from Recticel UK (Corby) was used in this study. The details of the polyurethane foam used have been reported by other authors (Haugen H, Will J, Kohler A, Hopfner U, Aigner J, Wintermantel E. Ceramic TiO₂-foams: characterisation of a potential scaffold. J Euro Ceram Soc 2004; 24: 661-668). The foam was supplied in large samples of 20mm in thickness and was cut to size 10 mm×10 mm×20 mm for compression strength tests and 10 mm×10 mm×60 mm for bending strength tests.

25

30

Example 1

Scaffold fabrication

The replication method involves preparation of green bodies of ceramic (or glass) foams by coating a polymer (e.g. polyurethane) foam with a ceramic (or glass) slurry. The polymer, having the desired pore structure, serves as a sacrificial template for the ceramic coating. The polymer template is immersed in the slurry, which subsequently infiltrates the structure and ceramic (glass) particles adhere to the surfaces of the polymer. Excess slurry is squeezed out leaving a more or less homogeneous coating on the foam struts. After drying, the polymer is slowly burned out in order to minimise damage to the ceramic (glass) coating. Once the polymer has been removed, the ceramic (or glass) network is sintered to a desired density. The process replicates the macrostructure of the starting sacrificial polymer foam, and results in a rather distinctive microstructure within the struts. A flowchart of the process is given in Figure 7.

(a) Preparation of a slurry

The slurry for the impregnation of the polyurethane foam was prepared using the following recipe.

Polyvinyl alcohol (PVA) (60g) was dissolved in water, the ratio being 0.01mol/L. Then 45S5 Bioglass[®] powder (40g) was added to 100ml PVA-water solution up to 40 wt.%. Each procedure was carried out under vigorous stirring using a magnetic stirrer for one hour.

Alternatively, the slurry can be prepared by adding 45S5 Bioglass[®] powder (40g) and PDLLA (60g) in DMC solution. The ratio of PDLLA to DMC was 1.5g/60ml. Once again, the slurry was prepared by vigorous stirring using a magnetic stirrer for 1h.

The second slurry recipe produces a more homogeneous and thicker coating on a green body than the first slurry recipe.

(b) Preparation of a green body

A polymer template is immersed in the slurry for 15min. After taking out the foam, the excess slurry is completely squeezed out. Then the coated foam is rubbed manually until the coating appearance is homogeneous by visual inspection. Finally
5 the foam is placed on a smooth surface and let to dry at ambient temperature for at least 12 hours, turning the foam at a frequency of once per min during the initial 15 minutes for homogeneous drying.

Preparation of a green body by this method results in a homogeneous porous structure, which in turn ensures reliable mechanical properties of the scaffold.

10 (c) Heat treatment

After drying, the polymer template is slowly burned out at 400° for 1 hr. Then the glass foam (green body) is sintered at 1000°C for 1 hr.

Post-forming treatments for the burnout of the polymer and sintering of the 45S5 Bioglass® structure were programmed as shown in Figure 8. The burning condition
15 of the polymer templates was the same for all samples: 400°C/1hr. Sintering conditions were designed to be 900°C/5hrs; 950°C/0-5hrs; and 1000°C/0-2hrs. The heating and cooling rates were 2°C/min and 5°C/min, respectively.

As can be seen from Figure 9, the replication technique and the discovery of proper sintering conditions (temperature, time, heating and cooling rates) are important in
20 achieving mechanically strong and still biodegradable sintered 45S5 Bioglass®-based highly porous scaffolds.

Example 2

Characterisation

The density ρ_{foam} of the scaffolds was determined from the mass and dimensions of
25 the sintered bodies. The porosity p was then calculated by

38

$$p = 1 - \frac{\rho_{\text{foam}}}{\rho_{\text{solid}}} = 1 - \rho_{\text{relative}}, \quad (2)$$

where $\rho_{\text{solid}} = 2.7 \text{ g/cm}^3$ is the density of solid 45S5 Bioglass[®].

The pore size of sintered scaffolds was estimated as follows. The cell size of the as-received polymer foam was 740-1040 μm . The volume shrinkage from a polymer
 5 template to a sintered 45S5 Bioglass[®]-based scaffold was determined to be 33 % on average for the sintering condition of 1000°C/1hr, through measuring the volumes of the starting polymer and sintered 45S5 Bioglass[®]-based foams. Therefore, the linear shrinkage would be ~70%. Finally, the range of pore sizes of the foams sintered at 1000°C for 1hr was calculated to be $0.70 \times (740-1040) \mu\text{m} = 510-720\mu\text{m}$.

10 The microstructure of the foams was characterised in a JEOL 5610LV scanning electron microscope (SEM), before and after immersion in simulated body fluid (SBF). Samples were gold coated and observed at an accelerating voltage of 15 kV.

Selected foams were also characterised using X-ray diffraction (XRD) analysis with the aim to assess the crystallinity after sintering and formation of HA crystals on
 15 strut surfaces after different times of immersion in SBF. The foams were first ground into a powder. Then 0.1 g of the powder was collected for XRD analysis. A Philips PW 1700 Series automated powder diffractometer was used, employing Cu $\text{K}\alpha$ radiation (at 40kV and 40mA) with a secondary crystal monochromator. Data were collected over the range of $2\theta = 5-100^\circ$ using a step size of 0.04° and a counting
 20 time of 25s per step.

Figure 3 shows the X-ray diffraction spectra of 45S5 Bioglass[®] powder as received, sintered at 900°C for 5 h and sintered at 1000°C for 1 h.

The crystalline phase in the foams was identified as being $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$ as the angle and intensity of the peaks matched the standard PDF #22.1455. The major peaks of
 25 the phase $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$ are marked in Figure 3 with the symbol ∇ .

The components of 45S5 Bioglass[®] and the crystalline Na₂Ca₂Si₃O₉ phase of the present invention are given as set forth in Table 1.

Example 3

Mechanical testing

- 5 The compression strength of foams was measured using a Zwick/Roell Z010 mechanical tester at a crosshead speed of 0.5 mm/min. The samples were rectangular in shape, with dimensions: 10 mm in height and 5 mm × 5 mm in cross-section. During compression test, the load was applied until densification of the porous samples started to occur.
- 10 Table 2 shows the raw data of compressive strength of foams sintered at 1000°C for 1hr.

- 15 Three-point bending strength tests were carried out using a Hounsfield testing machine. The size of the specimens was ~3 mm × 4 mm × 40 mm. The load was applied over a 30mm span and at the mid-point of the 4 mm × 40 mm surface. All tests were performed using a cross-head speed of 0.5 mm/min. The bending strength was calculated according to equation 3 (Dowling NE. Mechanical behaviour of materials: Engineering materials for deformation, fracture, and fatigue. 2nd edition. Englewood Cliffs, NJ: Prentice-Hall Inc.; 1998. pp. 603-648 [Chapter 13]):

$$\sigma_f = \frac{3P_f L}{2BW^2}, \quad (3)$$

- 20 where P_f is the load at fracture, $L = 30$ mm is the sample length over which the load is applied, $B \approx 4$ mm is the sample breadth, and $W \approx 3$ mm is the sample height.

Table 3 shows the raw data of bending strength of foams sintered at 1000°C for 1 hr.

Figure 4 shows the experimental and theoretical compressive strength and three-point bending strength of scaffolds sintered at 1000°C for 1h.

Example 4

Assessment of bioactivity in simulated body fluid (SBF)

This part of the study was carried out using the standard *in vitro* procedure described
5 by Kokubo et al (Kokubo T, Hata K, Nakamura T, Yamamura T. Apatite formation
on ceramics, metals, and polymers induced by a CaO-SiO₂-Based glass in simulated
body fluid. In: Bonfield W, Hastings GW, Tanner KE, editors. Bioceramics, vol.4.
London: Guildford, Butterworth-Heinemann; 1991, pages 113-120). The foams
were immersed in 75 ml of acellular SBF in clean conical flasks, which had
10 previously been washed using HCl and deionised water. The conical flasks were
placed inside an incubator at controlled temperature of 37 °C. The pH of the solution
was maintained constant at 7.25. The size of all samples for these tests was 10 mm ×
10 mm × 10 mm. Two samples were extracted from the SBF solution after given
times of 3, 7, 14, and 28 days. The SBF was replaced twice a week because the
15 cation concentration decreased during the course of the experiments, as a result of
the changes in the chemistry of the samples. Once removed from the incubation, the
samples were rinsed gently, firstly in pure ethanol and then using deionised water,
and left to dry at ambient temperature in a desiccator.

20 Table 4 shows the summary of characteristics of 45S5 Bioglass®-based foams after
immersion in SBF.

Example 5

Table 5 shows the structural characteristics and mechanical properties of the highly
porous scaffold of the present invention and ceramic or glass foams for bone tissue
25 engineering produced by prior art methods. As can be seen, the scaffolds of the
present invention have excellent porosity and pore sizes, along with open and
interconnected pores and excellent compressive strengths.

Example 6(a) Scaffold fabrication in preparation for carbon nanotube deposition

Highly porous foam-like Bioglass® based scaffolds were prepared by using the replication technique, as described above. The method involves coating a polymer
5 (e.g. polyurethane) foam with a glass slurry. The polymer, having the desired pore structure, simply served as a sacrificial template for the glass coating. The polymer template was immersed in the slurry, which subsequently infiltrated the structure and glass particles adhered to the surfaces of the polymer. Excess slurry was squeezed out leaving a more or less homogeneous coating on the foam struts. After drying, the
10 polymer was slowly burned out in order to minimise damage to the glass coating. Once the polymer had been removed, the glass structure was sintered to a desired density. The process replicates the macrostructure of the starting sacrificial polymer foam, and results in a rather distinctive and well-defined microstructure within the struts.

15 The slurry was prepared by mixing 3g of poly(D,L lactic acid) (PDLLA), 100 mL of dimethylcarbonate (DMC) and 40 wt% of 45S5 Bioglass® powder in distilled water. This procedure was carried out under vigorous stirring using a magnetic stirrer for 1h. A 45S5 Bioglass® powder was used in as received condition (mean particle size
20 of $< 5 \mu\text{m}$). Two different kinds of polyester-based polyurethane foams with 45 ppi and 60 ppi (pores per inch) obtained from Recticel UK (Corby), were used as the sacrificial templates. Polyurethane foams cut in prismatic shape ($10 \times 10 \times 10 \text{ mm}^3$) were used as sacrificial templates. They were immersed in the slurry for 15 minutes. After coating with Bioglass® the scaffolds were dried at room temperature for ~12h.
25 Finally, the polymer was slowly burned out by heating the samples at 550°C for 1 hour (heating rate: $2^\circ\text{C}/\text{min}$). Subsequently, the foams were sintered at 1100°C for 3 h using a heating rate of 2°C min^{-1} .

30 SEM images in Figure 11 show the interconnected, macroporous structures of the scaffolds prepared with two different pore sizes of polyurethane foams. The foams produced are very similar to spongy bone (also called cancellous bone) in terms of their pore structure. The highly porous (porosity: $>90\%$, cell diameter: $510\text{-}720\mu\text{m}$)

scaffolds are in fact made of a partial crystallized glass, e.g. a glass-ceramic microstructure, which confers some mechanical competence to the scaffolds despite the high porosity. When sintered at a temperature $> 1000\text{ }^{\circ}\text{C}$, the nearly full densification achieved and the fine crystals of $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$ present are responsible for the adequate compression strength of the scaffold.

(b) Carbon nanotube suspension preparation

The CNTs suspension was prepared by adding to an aqueous solution of multi-walled carbon nanotubes of commercial origin (Yorkpoint New Energy Sci. & Tech. Department Co. Ltd., Guangzhon, China), Triton X-100 as a surfactant and iodine 99,999% (Aldrich Chemical Company Inc) as a charger. Triton X-100 was added as an anionic surfactant. The high dispersion efficiency of Triton X-100 is due to its chemical structure, where the aromatic group is responsible for the strong hydrophobic interaction between Triton X-100 and the CNTs. It is believed that the π -like stacking of the benzene rings of the surfactant onto the surface of graphite increases the binding and surface coating of surfactant molecules onto graphite significantly. In order to enhance particle charging in the solution, iodine was added to the suspension.

The resulting suspension was sonicated for 4-5 h to help the particles to overcome the attractive van der Waals forces. Finally, the suspension was centrifuged for 15 minutes at 3000 rpm to remove the large CNT agglomerations and avoid their deposition during EPD. After centrifugation, the supernatant of the suspension was carefully extracted from the centrifuge tube and the suspension was placed in a glass recipient for EPD.

(c) EPD of CNTs on Bioglass[®] scaffolds

A schematic diagram of the EPD cell used in this investigation is shown in Figure 10. The electrodes used were made of stainless steel 316L foil with dimensions of 1.5 cm x 1.5 cm x 0.02 cm. In order to achieve a uniform CNT coating throughout the 3D porous structure, the Bioglass[®] scaffolds were placed inside a copper wire cage between the two electrodes next to the anode where the deposition should take

place, knowing that CNT would acquire a net negative surface charge in suspension. The electrodes were then connected to a DC power supply. Two different CNT suspensions were used in order to achieve CNT deposition on the scaffolds. Table 6 shows the compositions of the suspensions used. EPD was carried out by setting a
5 constant voltage in the range 10-55V, with deposition time ranging between 4 and 20 min, and electrode separation of 2 cm. After the EPD process, the copper wire frame was carefully and slowly withdrawn from the EPD cell in order to avoid any influence of a drag force between the suspension and the deposited wet CNT film. Finally, the samples were dried slowly at room temperature in normal air.

10

(d) Characterisation techniques

Scanning electron microscopy (SEM) (LEO1535) was used to examine the morphological and textural features of the scaffolds, before and after the electrophoretic deposition. Comparison of the results of the different tests led to the
15 determination of the optimal experimental conditions to produce the electrophoretic deposited CNT of best quality in terms of homogeneity of the microstructure, uniform coating thickness and adherence to the scaffold.

The above method successfully produced uniform CNTs deposits on highly porous
20 bioactive and biodegradable 45S5 Bioglass[®] -derived glass-ceramic scaffolds, intended for bone tissue engineering, by the electrophoretic deposition technique. The optimal experimental conditions were determined to be: applied electric field of 15V/cm and deposition time 20 minutes, utilizing a concentrated CNT suspension in water. The scaffolds pore structure remained invariant after the CNT coating, as
25 assessed by SEM.

Example 7

Preparation of 45S5 Bioglass[®]-derived glass-ceramic scaffolds with gradient porosity

30 The main steps for the preparation of the scaffolds with a gradient of porosity are as follows:

1. Fabrication of the metallic mould (e.g Al);
2. Compressing the polyurethane sponge (PU) in the mould;
3. Preparation of the Bioglass[®] slurry;
4. Immersion of the polyurethane preform in the Bioglass[®] slurry (to achieve
5 homogeneous Bioglass[®] coating);
5. Drying the green body at ambient temperature;
6. Heat treatment of the green body for burning out the organic phases and
sintering the Bioglass[®] coating.

10 (a) Realisation of the mould

The mould can be made of aluminium foil with 1mm thickness. Rectangular pieces were cut (using a pair of scissors) and then bent to obtain, for example, a trapezoidal form (see Figure 14). The dimensions of the mould can be tailored to obtain different compression degrees of the polyurethane sponge.

15

(b) Compressing the polyurethane sponge in the mould

This step is effective in forming the polymer sponge. The polymer foam was compressed in the mould. To maintain its compressed shape, a low heat treatment was necessary.

20

Reticulated polyurethane sponge was cut to various thicknesses and shapes (Figure 15a). The PU pieces were subsequently compressed in the mould (Figure 15b) at temperatures between 150-200°C for 30-60 minutes. A continuous porosity gradient can be thus obtained (Figure 15c) in the length direction.

25

An image of the internal structure of the sponge preform is presented in Figure 16. This picture was obtained by overlapping SEM images taken of the same sample. As can be seen, the pores size varies between 0.2-1mm and the foam is characterised by a highly interconnected porous network. The pores size increases from left to
30 right in Figure 16.

(c) Preparation of the slurry

For the preparation of the slurry, 3wt% of PDLLA (Purac Biochem, Gorinchem, Holland) was dissolved in dimethylcarbonate (DMC) (Sigma Aldrich), using a magnetic stirrer for 1h. Then, 40wt% 45S5 Bioglass[®] powder (with the mean
5 particle size <5µm) was added. The obtained suspension was stirred for another 1h.

(d) Immersion of the PU preform in the slurry

Small pieces of pre-moulded polyurethane foam were immersed in the above prepared slurry and manually rotated to assure the homogenous slurry filtration to
10 the inner structure. Then, the foams were extracted from the suspension and squeezed out to remove the slurry excess.

(e) Drying the green body at ambient temperature

The obtained samples (green bodies) were placed on a smooth surface and dried at
15 ambient temperature for 1 day.

(f) Heat treatment of the green body

The dried green bodies were heat treated in a chamber furnace, up to a temperature of 1100°C for 3h. During the heat treatment, the organic phases (PDLLA, DMC and
20 the polyurethane foam) were burnt out, leaving a Bioglass[®]-based porous structure instead. At the same time, the glass phase crystallised to obtain a glass-ceramic structure with enhanced mechanical properties.

An image of the scaffold after sintering at 1100°C/3h is presented in Figure 17. It
25 can be observed that the trapezoidal shape is still maintained. The highest degree of compression is obtained in the bottom part of this image. Therefore, the gradient of porosity is from the bottom to the top, in the length direction.

The internal structure of the sintered scaffold is presented in Figure 18. This picture
30 was obtained by overlapping SEM images taken from the same sample. The interconnected porous network is still maintained and a continuous gradient of

porosity is observed. Most of the inner pores are open. The pore size varies from left (smaller size) to right (larger size). The highly compressed part (on the left hand side) contains some closed porosity, probably due to the broken struts of the polyurethane preform.

5

This process allows the preparation of complex shapes with tailored porosity gradients. The flexibility in the gradient design, as well as scaffold shape are provided by the possibility of preforming the polymer sponges.

10 Surface Functionalization

The surfaces of the above-described 45S5 Bioglass®-derived scaffolds were modified by applying 3-AminoPropyl-TriethoxySilane (APTS). APTS was purchased from Sigma Aldrich (440140). The working mechanism of silanisation is shown in Figure 19. An aqueous APTS solution of 0.45 mol / l was prepared and the pH value adjusted to 8 by addition of 1 N HCl, resulting in a total volume of 70 ml. The samples to be functionalized were immersed into the obtained aqueous solution, contained in a glass bottle with the lid fastened. The solution was heated up to 80 °C in an oil bath under stirring conditions. After 4 hrs the samples were taken out and cleaned for 5 min in 300 ml of deionised water. Finally, the surface functionalized samples were cleaned again in deionised water and dried at ambient conditions.

20

The efficiency and stability of the surface modification were satisfactory as quantitatively assessed by x-ray photoemission spectroscopy (XPS), as shown in Figure 20.

25

Cell Culture

A type of human osteoblasts, MG63, was used for the *in vitro* assessment on the cell delivery ability of the scaffolds, and the viability and proliferation of cells on the scaffolds. To do a quantitative analysis, pellets were used to replace the scaffolds, as pellets provide a flat surface, the area of which can be determined accurately. The pellets were prepared by the same treatments as the scaffolds. Cell proliferation on pure Bioglass®, sintered glass-ceramic, and functionalised samples was

30

- quantitatively measured using AlamarBlue™. The data are illustrated in Figure 21. Firstly, the three types of pellets all exhibited a better ability to foster cells than the Thermanox® control. Secondly, pure Bioglass® pellets were the best among the three groups of pellets. However, the advantage of Bioglass® pellets in supporting cell proliferation was only slightly better than the other two types of pellets, and the slight advantage of Bioglass® pellets was even reduced with the increase of culture time (see the data of day 6). Finally, the functionalised pellets showed a better ability to foster cells than sintered pellets after day 6.
- 10 SEM observation showed that the sintered glass-ceramic could foster cells, as shown in Figure 22. A comparison of Figures 22 and 23 reveals that the proliferation of cells has been enhanced by surface functionalisation, which is in agreement with the data in Figure 21.
- 15 Various modifications and variations of the described aspects of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications
- 20 of the described modes of carrying out the invention which are obvious to those skilled in the relevant fields are intended to be within the scope of the following claims.

Table 1: Components of 45S5 Bioglass[®] and crystalline phase Na₂Ca₂Si₃O₉ (mol.%)

	45S5 Bioglass [®]	Na ₂ Ca ₂ Si ₃ O ₉
SiO ₂	46.134	50
Na ₂ O	24.35	16.667
CaO	26.912	33.333
P ₂ O ₅	2.6038	0

Table 2: Raw data of compressive strength of foams sintered at 1000°C for 1hr

Porosity	0.895	0.921	0.915	0.918	0.910	0.913
Compressive strength σ_{exp} (MPa)	0.425	0.272	0.314	0.327	0.330	0.360
Theoretical strength σ_{theo} (MPa)	0.710	0.469	0.519	0.497	0.566	0.538
Ratio of $\sigma_{\text{theo}} / \sigma_{\text{exp}}$	1.67	1.72	1.65	1.52	1.72	1.50

Table 3: Raw data of bending strength of foams sintered at 1000°C for 1 hr

Porosity	0.947	0.943	0.921	0.916	0.911
Bending Strength σ_{3b} (MPa)	0.446	0.427	0.458	0.481	0.470

5

Table 4: Summary of characteristics of 45S5 foams after immersion in SBF

Immersion time in SBF	1000°C / 30min	1000°C / 1hr
3 days	Sparsely distributed hydroxyapatite* precipitates	Very few of hydroxyapatite* precipitates
1 week	Strut surface was unevenly covered by aggregated hydroxyapatite* spheres	Sparsely distributed hydroxyapatite* precipitates
2 weeks	Hydroxyapatite* spheres were fused together.	Strut surface was fully covered by a large amount of hydroxyapatite* spheres, size being ~1µm
4 weeks	The amorphous apatite phase transformed into apatite crystallites	Hydroxyapatite* spheres grew, size being ~2.5µm.

*The hydroxyapatite could be a mixture of amorphous and crystalline calcium phosphates.

Table 5: Structural characteristics and mechanical properties of the highly porous scaffold of the present invention and ceramic or glass foams for bone tissue engineering produced by prior art methods.

Technique	Material	Porosity (%)	Pore Size (µm)	Closed (C) or Open (O)	Compressive Strength (MPa)
Polymer-sponge Present work	45S5 Bioglass®	89-92	510-720	O	0.27-0.42
	Glass-reinforced HA	85-97.5	420-560	O	0.01-0.175
	HA	69-86	490-1130	O	0.03-0.29
Gel-casting/foamed by vigorous stirring	HA	76.7-80.2	20-1000	Partly O/C	4.4-7.4
	HA		Cell: 100-500 Window: 30-120	Partly O/C	1.6-5.8
polymer-sponge	HA	70-77	200-400	O	0.55-5
	β-TCP + HA	73	200-400	O	9.8
Sol-gel /foamed by vigorous stirring	Bioactive glasses	70-95	Cell: up to 600 Windows: 80-120	Partially O/C	Not Available

Table 6: Compositions of the aqueous suspensions for EPD.

Suspension no.	Distilled water (mL)	Triton X-100 (mL)	Iodine (g)	Carbon nanotubes (g)
1	200	0.3	0.0100	0.1200
2	40	0.5	0.0267	0.2052

CLAIMS

1. A method of preparing a bioactive glass scaffold, said method comprising the steps of:
 - (i) preparing a slurry comprising bioactive glass powder and a binder;
 - (ii) contacting a polymer template with said slurry so as to form a coated polymer template;
 - (iii) allowing the coated polymer template obtained in step (ii) to dry at ambient temperature;
 - (iv) forming a bioactive glass scaffold by heat treating the coated polymer template obtained in step (iii) to remove the polymer template; and
 - (v) sintering the bioactive glass scaffold obtained in step (iv).
2. A method according to claim 1 wherein the polymer template is a foam.
3. A method according to claim 1 wherein the bioactive glass scaffold has a porosity gradient.
4. A method according to claim 4 wherein the porosity gradient is continuous.
5. A method according to claim 3 or claim 4 wherein the polymer template is pre-treated by:
 - (a') compressing the polymer template into a mould; and
 - (b') heating the compressed polymer template in the mould.
6. A method according to claim 5 wherein the mould is a metallic mould.
7. A method according to claim 5 or claim 6 wherein the mould comprises aluminium.
8. A method according to any one of claim 5 to 7 where step (b') comprises heating the compressed polymer template in the mould at a temperature of at least 150°C.

9. A method according to claim 8 where step (b') comprises heating the compressed polymer template in the mould at a temperature of about 150°C to about 200°C.
10. A method according to claim 8 or claim 9 wherein the temperature is about 200°C.
11. A method according to claim 10 where step (b') comprises heating the compressed polymer template in the mould at a temperature of about 150°C to about 200°C for a period up to about 60 minutes.
12. A method according to claim 11 where step (b') comprises heating the compressed polymer template in the mould at a temperature of about 150°C to about 200°C for a period up to about 30 minutes.
13. A method according to claim 11 or claim 12 where step (b') comprises heating the compressed polymer template in the mould at a temperature of about 200°C for a period of about 30 minutes.
14. A method according to any preceding claim wherein the polymer template is a polyurethane foam.
15. A method according to claim 14 wherein the polyurethane foam is a fully opened foam having at least 45 pores per inch.
16. A method according to claim 14 wherein the polyurethane foam is a fully opened foam having at least 60 pores per inch.
17. A method according to any preceding claim wherein step (ii) comprises immersing the polymer template in the slurry.

18. A method according to any preceding claim wherein excess slurry is removed from the polymer template by squeezing the polymer template.
19. A method according to any preceding claim wherein the bioactive glass contains 45 % SiO₂, 24.5 % Na₂O, 24.4 % CaO and 6 % P₂O₅ by weight.
20. A method according to any preceding claim wherein the ratio of bioactive glass powder to binder is from about 20:80 to 50:50 w/w.
21. A method according to claim 20 wherein the ratio is about 40:60 w/w.
22. A method according to any preceding claim wherein the binder is an aqueous solution of polyvinyl alcohol.
23. A method according to claim 22 wherein the concentration of polyvinyl alcohol is from about 0.005 mol/L to about 0.1 mol/L.
24. A method according to claim 23 wherein the concentration of polyvinyl alcohol is about 0.01 mol/L.
25. A method according to claim 21 wherein the slurry consists essentially of about 40 % of the bioactive glass of claim 19 and about 60 % of an aqueous solution of polyvinyl alcohol.
26. A method according to any one of claims 1 to 21 wherein the binder is PDLLA in DMC solution.
27. A method according to claim 26 wherein the ratio of PDLLA:DMC solution is from about 0.5 g/60ml to about 2 g/60ml.
28. A method according to claim 27 wherein the ratio of PDLLA:DMC solution is about 1.0 g/60 ml to 2.0 g/60 ml.

29. A method according to claim 28 wherein the PDLLA:DMC solution is about 1.5 g/60 ml to about 1.8 g/60 ml.
30. A method according to claim 21 wherein the slurry consists essentially of about 40 % of the bioactive glass of claim 19 and about 60 % of PDLLA in DMC solution.
31. A method according to any preceding claim wherein step (iii) comprises turning the coated polymer template to allow homogenous drying.
32. A method according to claim 31 wherein the coated polymer template is turned about once a minute for about 15 minutes.
33. A method according to any preceding claim wherein step (iv) comprises burning out the polymer template.
34. A method according to claim 33 where step (iv) comprises heating the coated polymer template to a temperature of at least 200°C.
35. A method according to claim 34 wherein step (iv) comprises heating the coated polymer template to a temperature of about 400°C at a rate of about 2°C/minute.
36. A method according to claim 35 wherein the coated polymer template is maintained at a temperature of 400°C for about an hour.
37. A method according to claim 34 wherein step (iv) comprises heating the coated polymer template to a temperature of about 550°C at a rate of about 2°C/minute.
38. A method according to claim 37 wherein the coated polymer template is maintained at a temperature of 550°C for about an hour.

39. A method according to any preceding claim wherein step (v) comprises sintering the bioactive glass scaffold at a temperature of from about 900°C to about 1200°C.
40. A method according to claim 39 wherein step (v) comprises sintering the bioactive glass scaffold at a temperature of from about 950°C to about 1100°C.
41. A method according to claim 40 wherein step (v) comprises sintering the bioactive glass scaffold at a temperature of about 1100°C.
42. A method according to claim 41 wherein step (v) comprises sintering the bioactive glass scaffold at a temperature of from about 900°C to about 1100°C for a period of up to about 5 hours.
43. A method according to claim 42 wherein step (v) comprises sintering the bioactive glass scaffold at a temperature of from about 900°C to about 1100°C for a period of about 3 hours.
44. A method according to claim 43 wherein step (v) comprises sintering the bioactive glass scaffold at a temperature of about 1100°C for about 3 hours.
45. A method according to any one of claims 1 to 44 wherein step (v) comprises sintering the bioactive glass scaffold at a temperature of from about 950°C to about 1000°C.
46. A method according to claim 45 wherein step (v) comprises sintering the bioactive glass scaffold at a temperature of about 1000°C.
47. A method according to claim 45 wherein step (v) comprises sintering the bioactive glass scaffold at a temperature of from about 900°C to about 1000°C for a period of up to about 5 hours.

48. A method according to claim 47 wherein step (v) comprises sintering the bioactive glass scaffold at a temperature of from about 900°C to about 1000°C for a period of about 1 hour.
49. A method according to claim 48 wherein step (v) comprises sintering the bioactive glass scaffold at a temperature of about 1000°C for about 1 hour.
50. A method according to any preceding claim wherein after sintering, the bioactive glass scaffold is cooled at a rate of about 5°C/minute.
51. A method of preparing a bioactive glass scaffold, said method comprising the steps of:
- (i) preparing a slurry comprising bioactive glass powder and a binder, wherein the bioactive glass powder contains 45 % SiO₂, 24.5 % Na₂O, 24.4 % CaO and 6 % P₂O₅ by weight;
 - (ii) contacting a polymer template with said slurry so as to form a coated polymer template;
 - (iii) allowing the coated polymer template obtained in step (ii) to dry at ambient temperature;
 - (iv) forming a bioactive glass scaffold by heat treating the coated polymer template obtained in step (iii) to remove the polymer template; and
 - (v) sintering the bioactive glass scaffold obtained in step (iv) at a temperature of from about 900°C to about 1100°C;
52. A method according to any preceding claim which further comprises the step of depositing carbon nanotubes onto the bioactive glass scaffold obtained in step (v).
53. A method according to any preceding claim wherein the carbon nanotubes are deposited by electrophoretic deposition.

56

54. A method according to any preceding claim wherein the carbon nanotubes are deposited by electrophoretic deposition using an aqueous suspension comprising carbon nanotubes.

55. A method according to claim 54 wherein the ratio of carbon nanotubes : water is about 0.1 mg/ 1 ml to about 6 mg/ 1 ml.

56. A method according to claim 54 or claim 55 wherein the ratio of carbon nanotubes : water is about 0.6 mg/ 1 ml to about 5.1 mg/ 1 ml.

57. A method according to any one of claims 54 to 56 wherein the aqueous suspension further comprises a surfactant.

58. A method according to claim 57 wherein the surfactant is an anionic surfactant.

59. A method according to claim 58 wherein the surfactant is polyethylene glycol *tert*-octylphenyl ether (Triton X-100).

60. A method according to any of claims 54 to 59 wherein the aqueous suspension further comprises a charger.

61. A method according to claim 60 wherein the charger is iodine.

62. A method according to any one of claims 54 to 61 wherein the aqueous suspension is sonicated before electrophoretic deposition.

63. A method according to any one of claims 54 to 62 wherein the aqueous suspension is centrifuged before electrophoretic deposition.

64. A method according to any one of claims 53 to 63 wherein electrophoretic deposition is carried out at an applied electric field in the range of about 10V/cm to about 55V/cm.
65. A method according to any one of claims 53 to 64 wherein the deposition time is in the range of about 4 minutes to about 20 minutes.
66. A method according to any one of claims 54 to 65 wherein the aqueous suspension comprises carbon nanotubes : water in a ratio of about 5.1 mg/1 ml, polyethylene glycol *tert*-octylphenyl ether (Triton X-100) and iodine, the applied electric field is about 15V/cm and the deposition time is about 20 minutes.
67. A method according to any preceding claim which comprises the additional step of drying the coated bioactive glass scaffolds.
68. A method according to any preceding claim which further comprises the step of functionalising the surface of the bioactive glass scaffold.
69. A method according to claim 68 which comprises treating the bioactive glass scaffold with 3-aminopropyl-triethoxysilane (APTS).
70. A bioactive glass scaffold obtainable by the method of any one of claims 1 to 69.
71. A medical article comprising a bioactive glass scaffold according to claim 70.
72. Use of a bioactive glass scaffold according to claim 70 in tissue engineering.
73. A method of preparing a bioactive glass scaffold, said method comprising the steps of:
- (i) preparing a slurry comprising bioactive glass powder and a binder;

- (ii) contacting a polymer template with said slurry so as to form a coated polymer template;
- (iii) allowing the coated polymer template obtained in step (ii) to dry at ambient temperature; and
- (iv) forming a bioactive glass scaffold by heat treating the coated polymer template obtained in step (iii) to remove the polymer template.

74. A method of preparing a bioactive and biodegradable glass scaffold comprising $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$, said method comprising the steps of:

- (i) preparing a slurry comprising bioactive glass powder and a binder, wherein the bioactive glass powder contains 45 % SiO_2 , 24.5 % Na_2O , 24.4 % CaO and 6 % P_2O_5 by weight;
- (ii) contacting a polymer template with said slurry so as to form a coated polymer template;
- (iii) allowing the coated polymer template obtained in step (ii) to dry at ambient temperature;
- (iv) forming a bioactive glass scaffold by heat treating the coated polymer template obtained in step (iii) to remove the polymer template; and
- (v) sintering the bioactive glass scaffold obtained in step (iv).

75. A method according to claim 74 which further comprises the step of depositing carbon nanotubes onto the bioactive glass scaffold obtained in step (v).

76. A method according to claim 74 which further comprises the step of functionalising the surface of the bioactive glass scaffold obtained in step (v).

77. A method according to any one of claims 51 to 69 or 73 to 76 wherein the polymer template is a foam as defined in any one of claims 2 to 16.

78. A surface-functionalised bioactive glass scaffold obtainable by reacting a bioactive glass scaffold with a surface functionalisation reagent.

79. A surface-functionalised bioactive glass scaffold according to claim 78 wherein the bioactive glass contains 45 % SiO₂, 24.5 % Na₂O, 24.4 % CaO and 6 % P₂O₅ by weight.

80. A surface-functionalised bioactive glass scaffold according to claim 78 or claim 79 wherein the bioactive glass scaffold is prepared by a method according to any one of claims 1 to 69 or 73 to 77.

81. A surface-functionalised bioactive glass scaffold according to any one of claims 78 to 80 wherein the surface functionalisation reagent is 3-aminopropyltriethoxysilane (APTS).

82. A composite comprising a bioactive glass scaffold having carbon nanotubes deposited on a surface thereof.

83. A composite according to claim 82 wherein the bioactive glass contains 45 % SiO₂, 24.5 % Na₂O, 24.4 % CaO and 6 % P₂O₅ by weight.

84. A composite according to claim 82 or claim 83 wherein the bioactive glass scaffold is prepared by a method according to any one of claims 1 to 69 or 73 to 77.

85. A composite according to any one of claims 82 to 84 wherein the carbon nanotubes are deposited on the surface of the bioactive glass scaffold by electrophoretic deposition.

86. A method, bioactive glass scaffold, surface-functionalised bioactive glass scaffold, use, article or composite substantially as described herein with reference to the accompanying examples and figures.

(1/14)

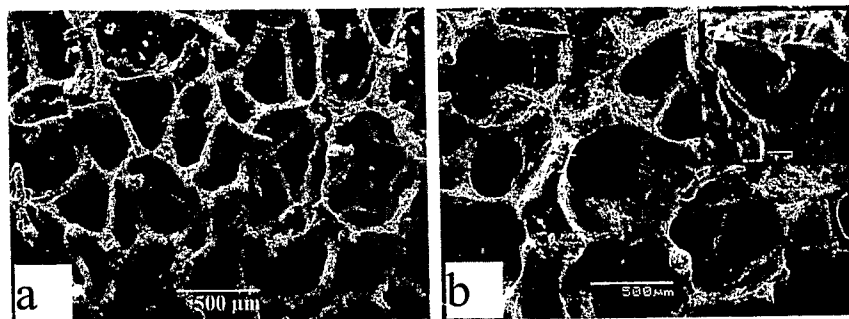


FIGURE 1

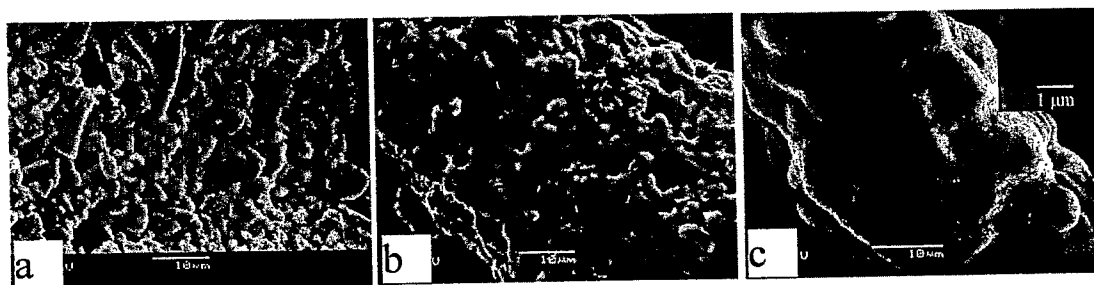


FIGURE 2

(2/14)

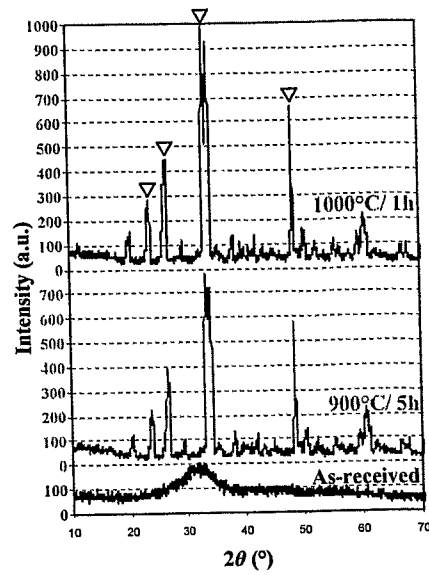


FIGURE 3

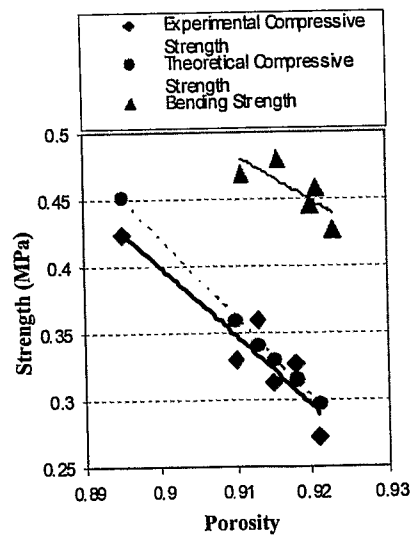


FIGURE 4

(3/14)

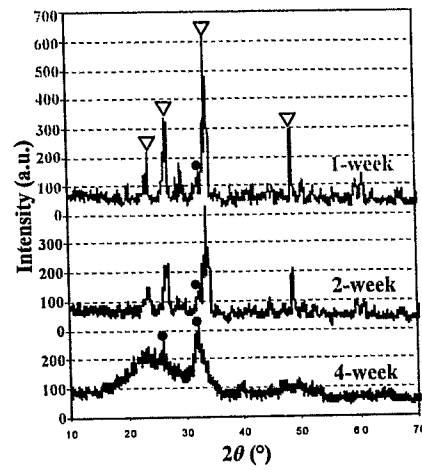


FIGURE 5

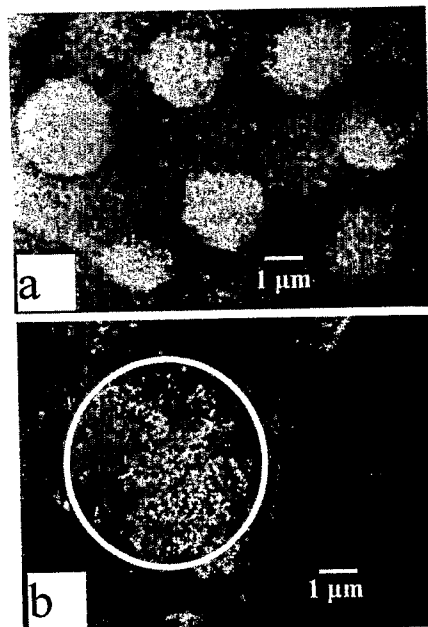


FIGURE 6

(4/14)

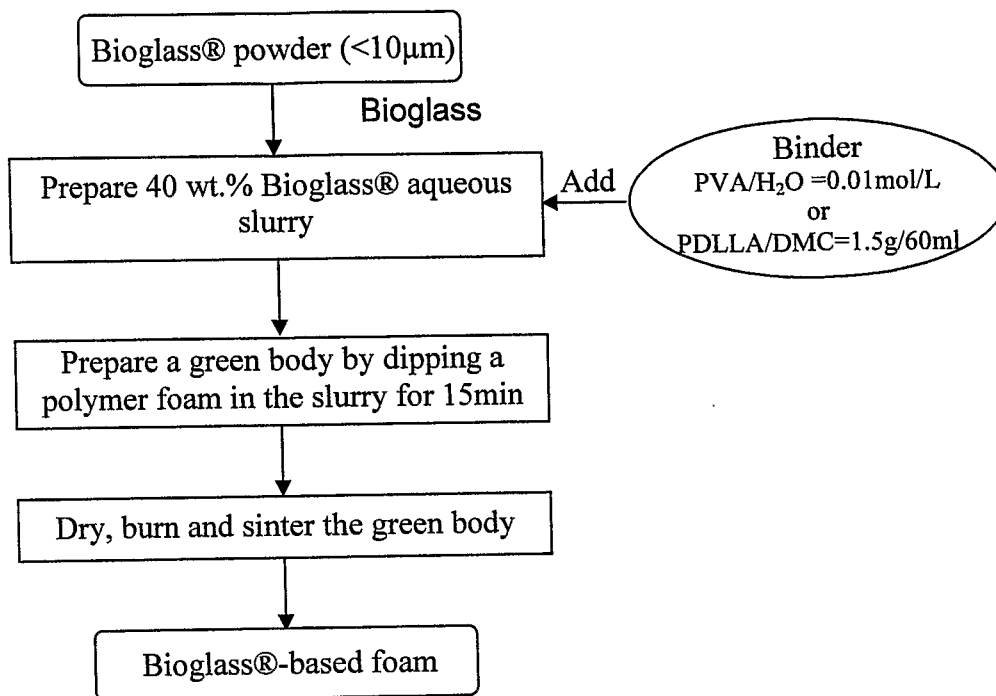


FIGURE 7

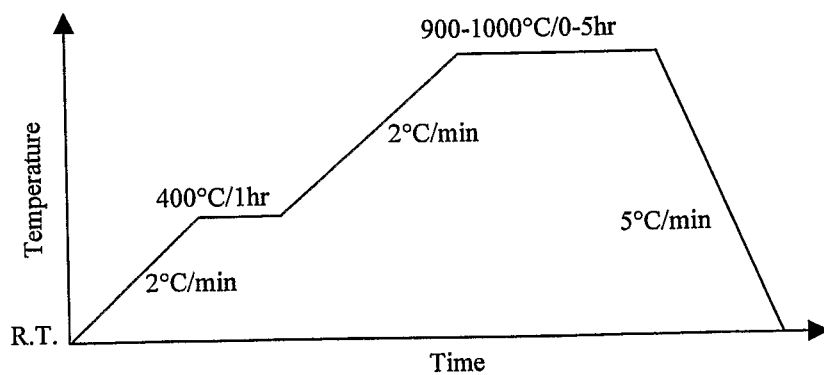


FIGURE 8

(5/14)

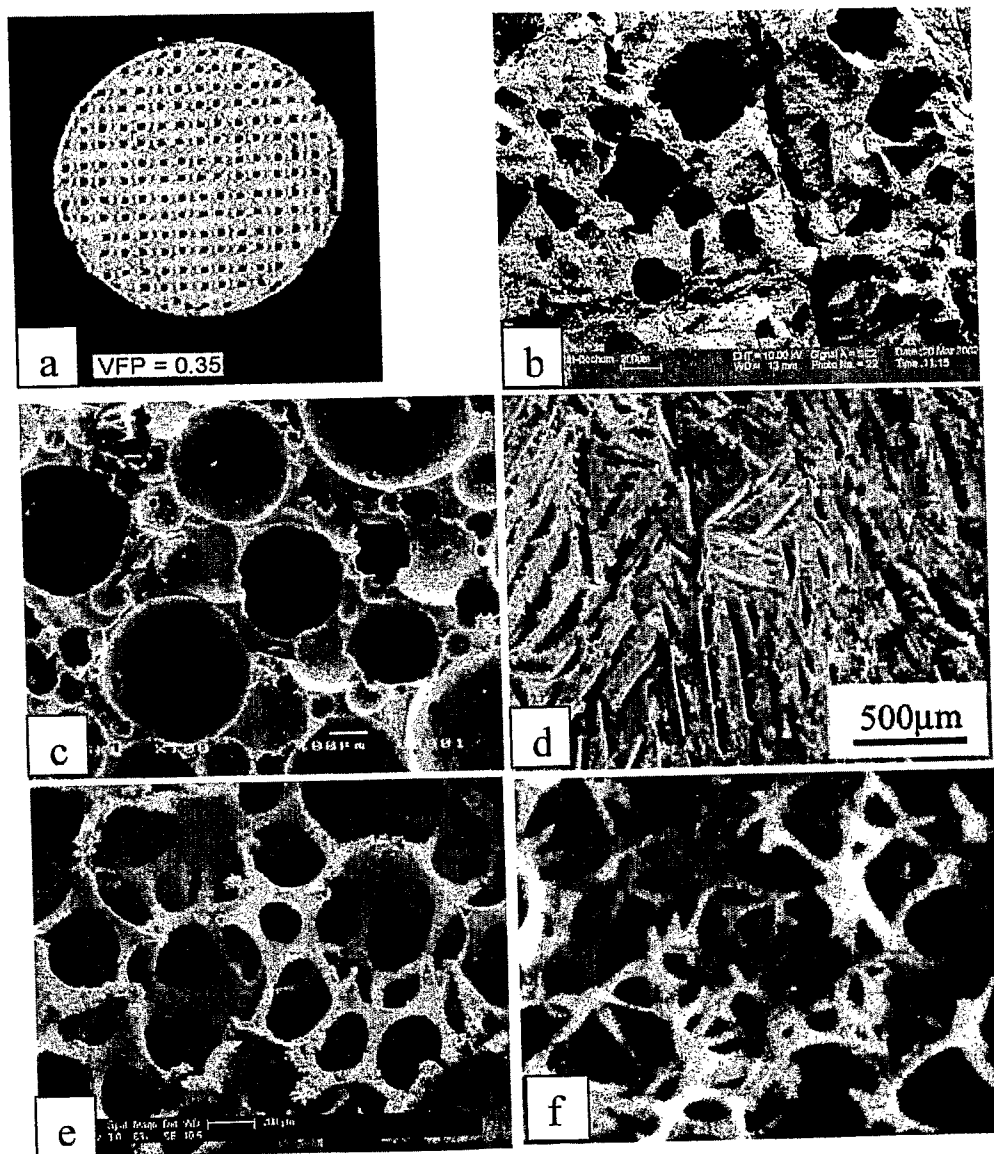


FIGURE 9

(6/14)

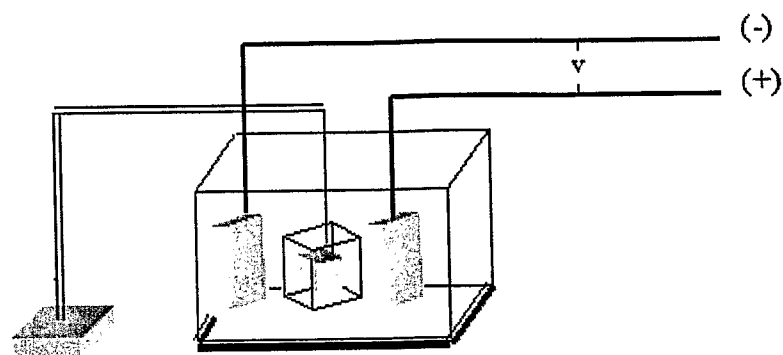


FIGURE 10

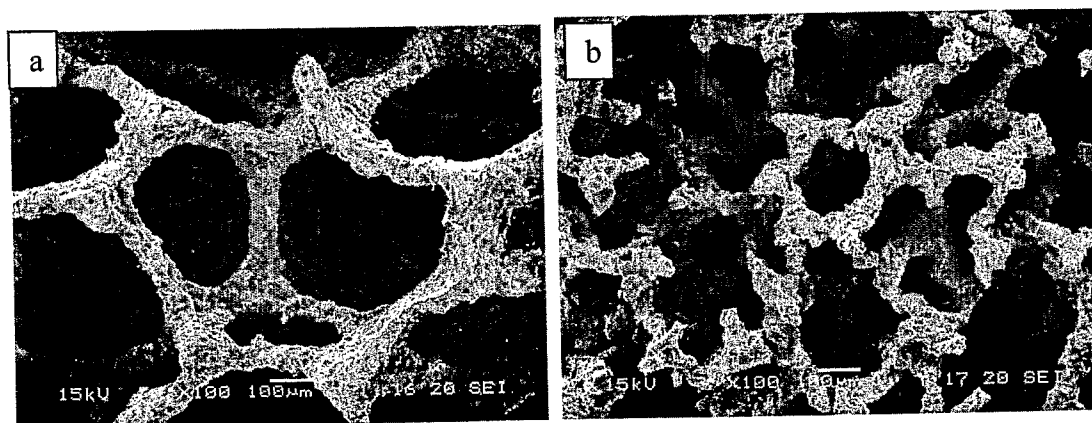


FIGURE 11

(7/14)

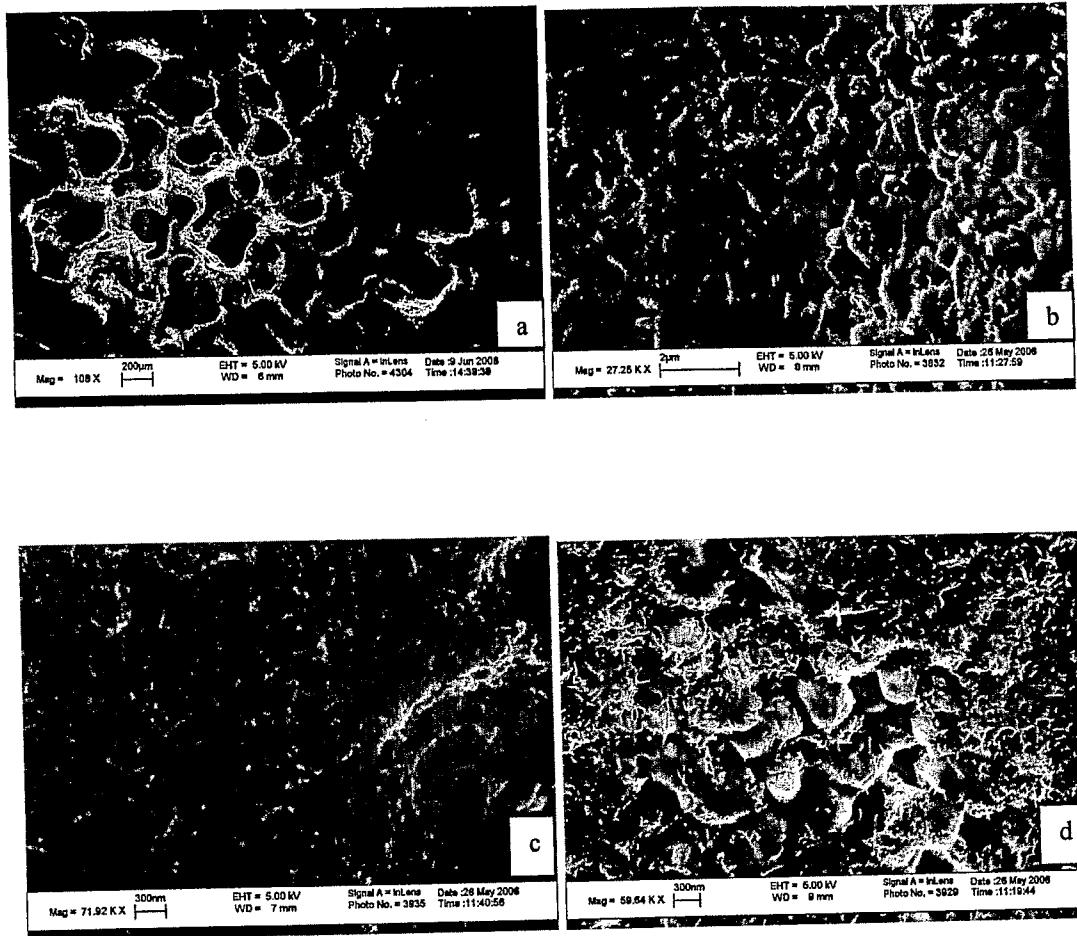


FIGURE 12

(8/14)

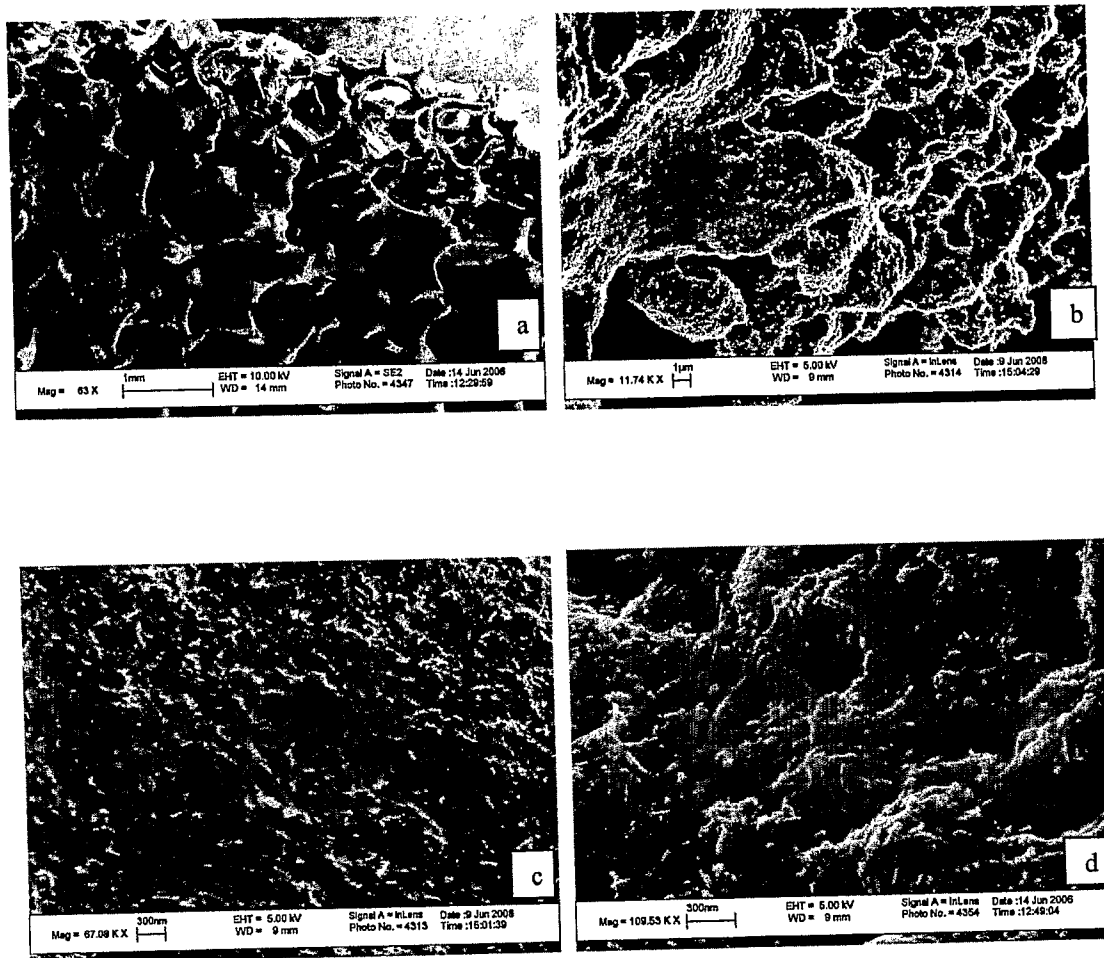


FIGURE 13



FIGURE 14

(9/14)

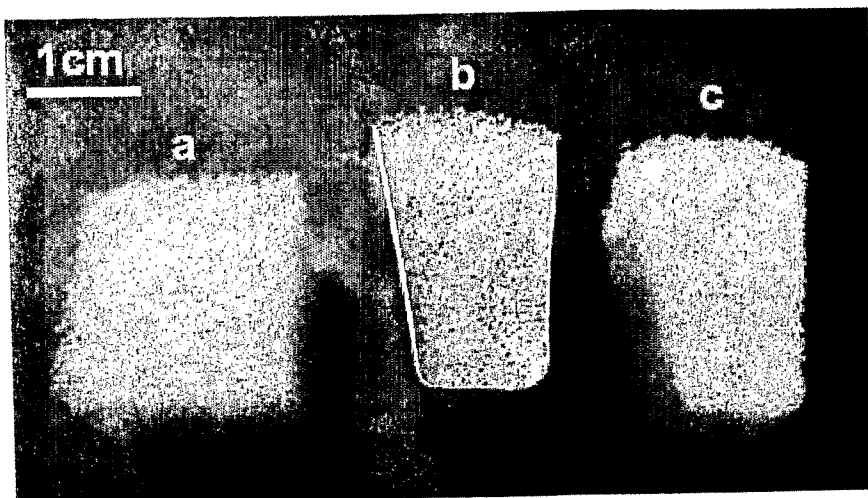


FIGURE 15



FIGURE 16

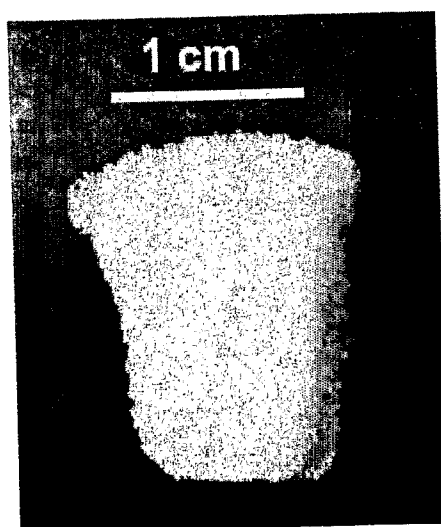


FIGURE 17

(10/14)

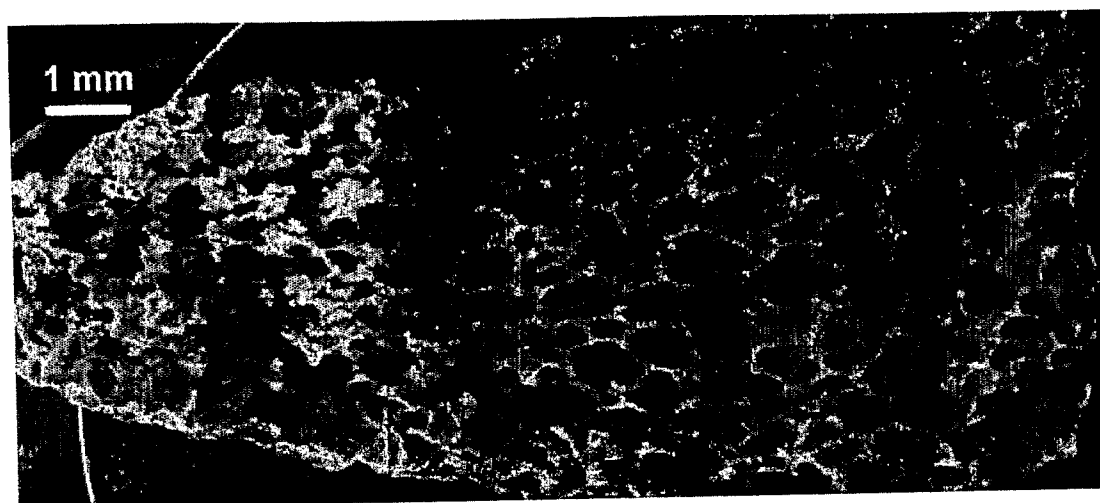


FIGURE 18

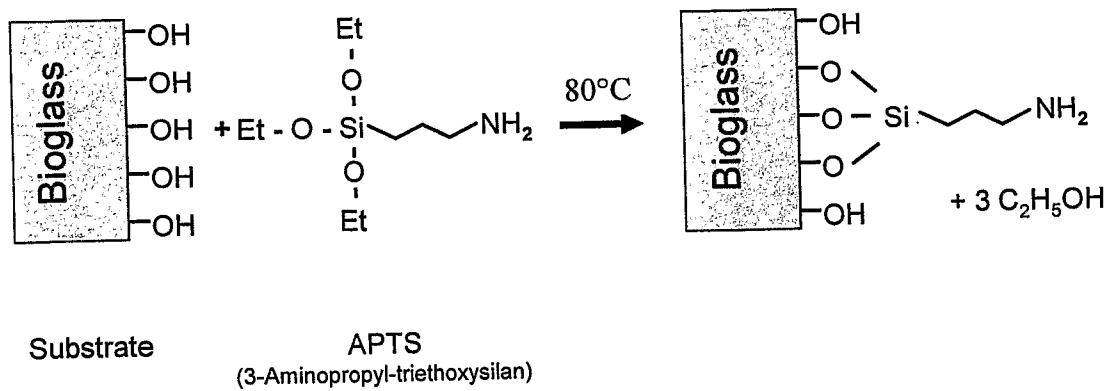
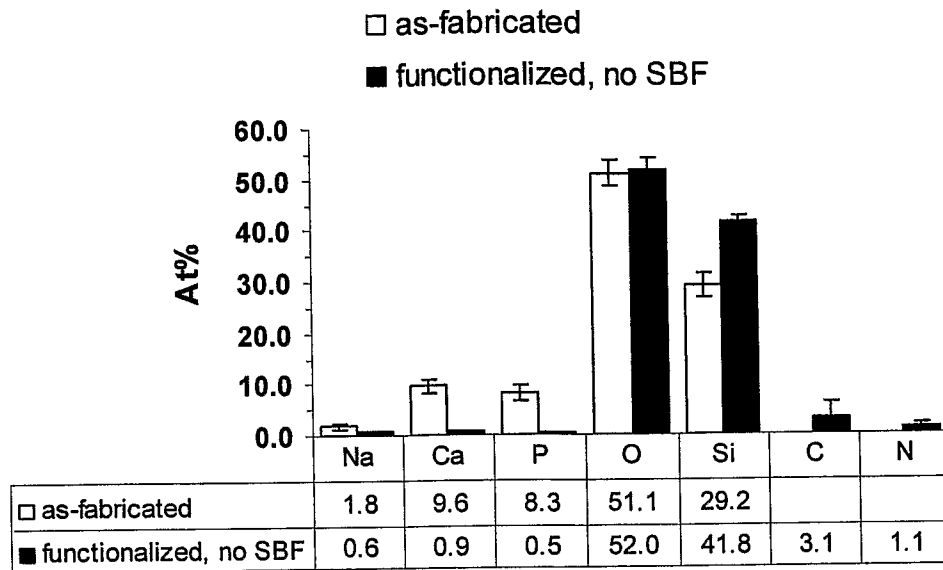
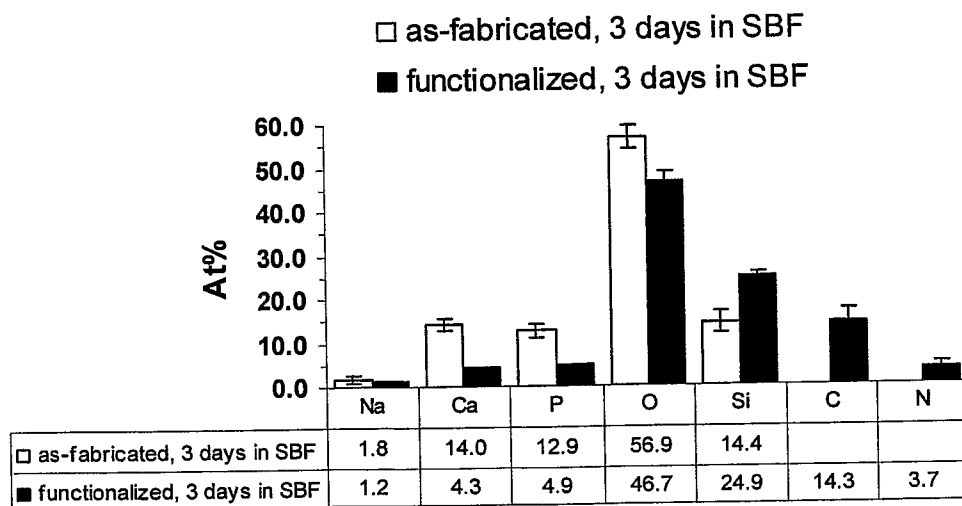


FIGURE 19

(11/14)



(a)



(b)

FIGURE 20

(12/14)

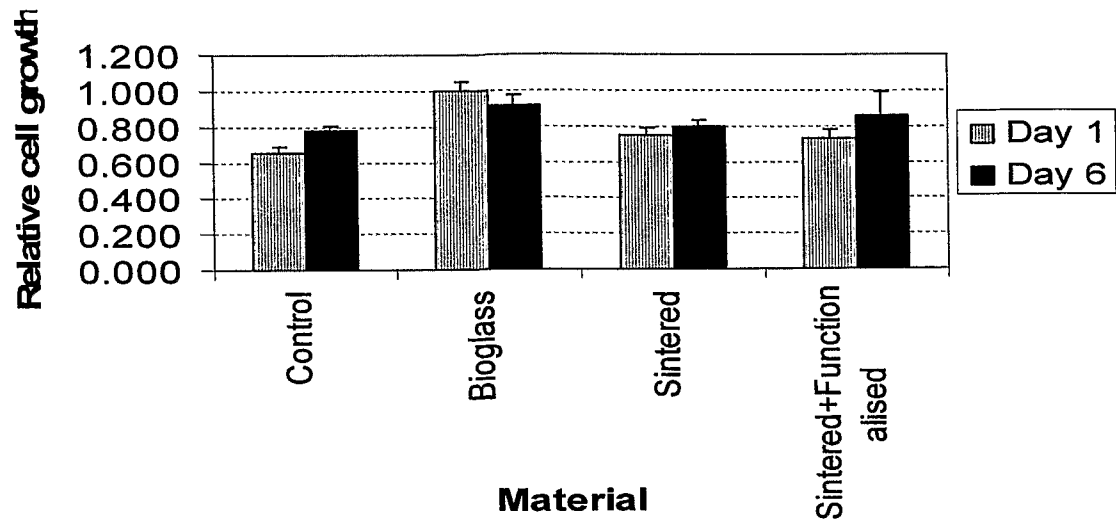


FIGURE 21

(13/14)

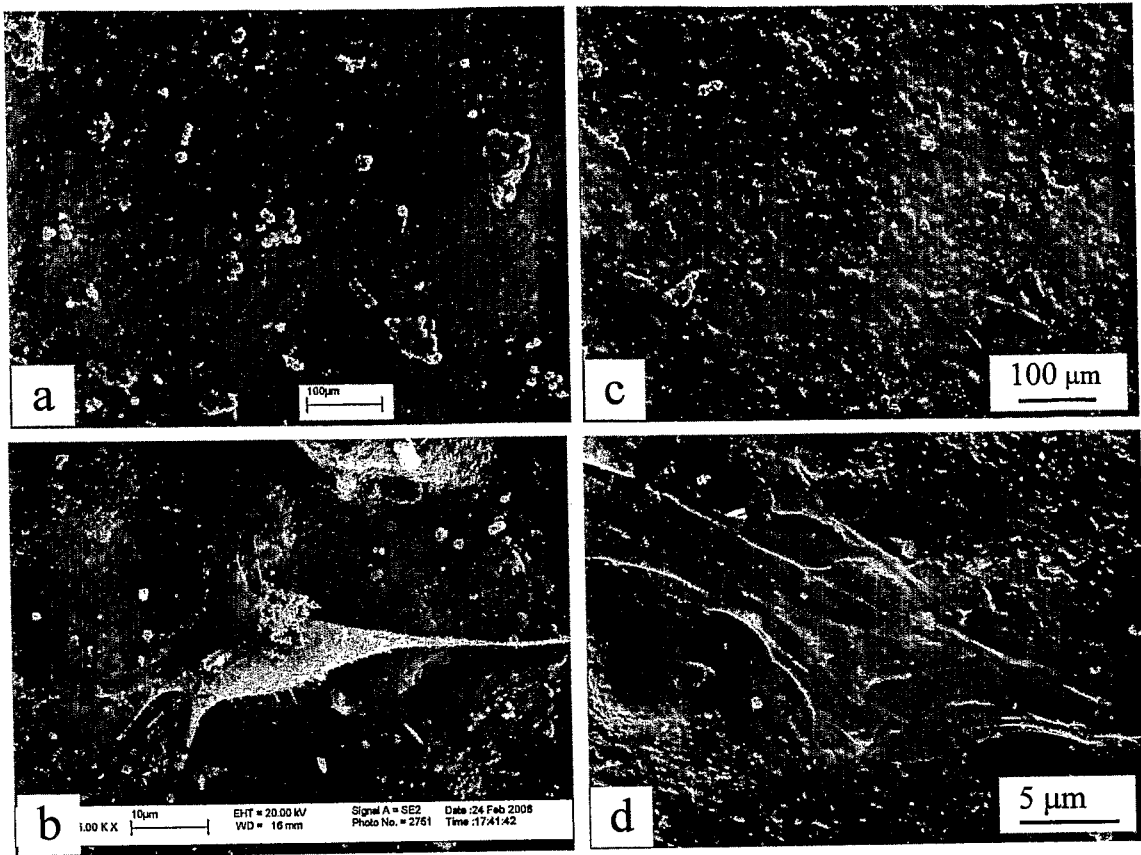


FIGURE 22

(14/14)

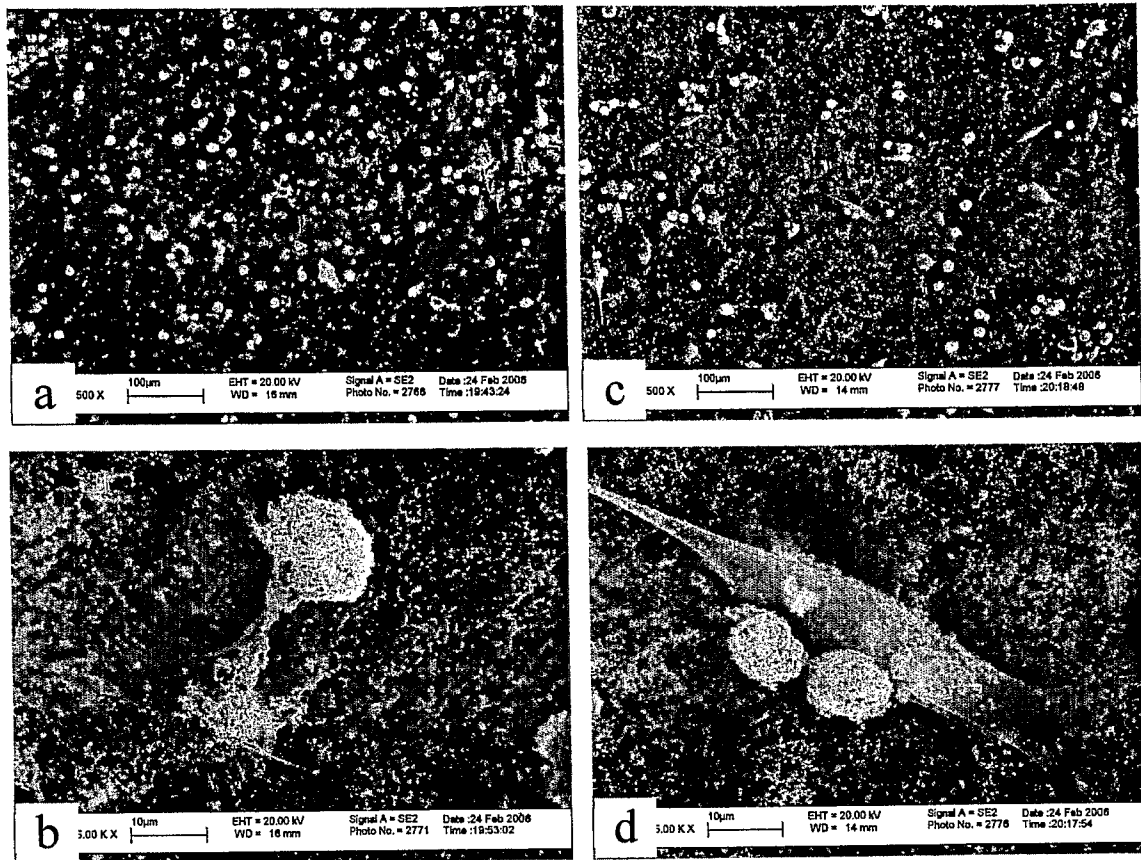


FIGURE 23