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Preparation of bioactive glass-polyvinyl alcohol hybrid foams by the sol-gel method

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A new class of materials based on inorganic and organic species combined at a nanoscale level has received large attention recently. In this work the idea of producing hybrid materials with controllable properties is applied to obtain foams to be used as scaffolds for tissue engineering. Hybrids were synthesized by reacting poly(vinyl alcohol) in acidic solution with tetraethylorthosilicate. The inorganic phase was also modified by incorporating a calcium compound. Hydrated calcium chloride was used as precursor. A surfactant was added and a foam was produced by vigorous agitation, which was cast just before the gel point. Hydrofluoric acid solution was added in order to catalyze the gelation. The foamed hybrids were aged at 40 °C and vacuum dried at 40 °C. The hybrid foams were analyzed by Scanning Electron Microscopy, Mercury Porosimetry, Nitrogen Adsorption, X-ray Diffraction and Infra-red Spectroscopy. The mechanical behavior was evaluated by compression tests. The foams obtained had a high porosity varying from 60 to 90% and the macropore diameter ranged from 30 to 500 μm. The modal macropore diameter varied with the inorganic phase composition and with the polymer content in the hybrid. The surface area and mesopore volume decreased as polymer concentration increased in the hybrids. The strain at fracture of the hybrid foams was substantially greater than pure gel-glass foams.

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1. Introduction

Bone tissue engineering has been investigated in the past few years as an alternative strategy to regenerate bone [1, 2]. The goal is to combine progenitor or mature cells with biocompatible materials or scaffolds, with or without appropriate growth factors, to initiate tissue repair and regeneration. Development of optimal scaffolds is still a challenge in tissue engineering; many materials have been proposed but few have reached clinical efficacy [3].

Biomaterials for bone growing scaffolds need to be biocompatible, ideally osteoinductive, osteoconductive, and mechanically compatible with native bone to fulfil their desired role in bone tissue engineering. Materials for delivery of stem cells that stimulate differentiation and bone conduction include bioactive ceramic materials such as porous hydroxyapatite or other calcium phosphates [4, 5] and bioactive glass foams [6]. Hydroxyapatite is resorbed relatively slowly while degradability may be increased by the use of more soluble phases such as tricalcium phosphate or a combination of two calcium phosphates [7]. Bioactive glass foams have an additional attraction as a scaffold material for bone tissue engineering since there is evidence of genetic control of the cellular response to these materials [8, 9]. Seven families of genes are up-regulated when primary human osteoblasts are exposed to the ionic dissolution products of bioactive glasses. The role of soluble silicon ions in stimulating collagen type I synthesis in humam osteoblast-like cells and promoting osteoblast differentiation has been demonstrated [10] and recently is being applied in producing silicon-substituted hydroyapatite with increased bioactivity [11, 12]. Another advantage of bioactive glasses is the possibility of controlling their degradability through compositional and structural variations. However, the low toughness associated with ceramic materials remains a limitation for their use either as bone replacement or as scaffolds for tissue engineering.

Many biodegradable polymers are also considered for bone tissue engineering [13]. They are biocompatible, can be chemically modified and their degradation rates can be controlled. They can be readily processed into three-dimensional porous structures with desired

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textural features. They can be used either alone or in combination with other osteoconductive materials such as hydroxyapatite or bioactive glass [14, 15]. However, their major disadvantage is their lack of mechanical strength and stability, especially made with large volume fractions of macroporosity. Therefore, despite the availability of scaffolds with appropriate biological and structural characteristics for tissue engineering they still need improvement in their mechanical behaviour.

One approach to enhance the mechanical properties of materials is production of inorganic-organic hybrids [16–18], in which inorganic phases with nanometer dimensions are inserted into a compliant polymer matrix. This type of structural organization of synthetic materials resembles the structure of natural tissues, such as bone, where the inorganic component (hydroxy-carbonate-apatite) and biological entities, such as collagen, interact at a molecular scale by generating phases linked by primary chemical bonds [19]. Thus, the science and technology related to inorganic-organic hybrids can possibly be applied to the preparation of a new class of biomaterials structurally similar to natural tissues for biomedical applications.

In this work hybrid materials are produced with controllable properties as a means to obtain foams to be used as scaffolds for tissue engineering. The processing route is the sol-gel method already used successfully to obtain bioactive glass foams [6, 20, 21]. The inorganic phase is bioactive glasses, either pure silica or the silica-calcia system. A wide variety of types of organic polymers have been employed in the sol-gel syntheses of hybrids in which the inorganic phase is silica [22–24]. The type of polymer employed is one of the main features affecting the structure and properties of hybrids because they depend essentially on chemical interactions established between organic and inorganic moieties. The polymer poly (vinyl alcohol) (PVA) is an interesting choice because, in addition to being easily soluble in the water-alcohol mixtures employed in sol-gel method, its pendant hydroxyl groups lead to the formation of hydrogen bonds and eventual condensation with silanol groups [25–27], thus favoring the production of structurally homogeneous materials within a wide range of compositions. Moreover, PVA has been employed in a wide variety of biomedical applications, and is generally considered to be biocompatible [28].

2. Experimental

Inorganic-organic hybrids based on polyvinyl alcohol (PVA) and sol-gel derived silica and silica-calcia systems were prepared in this work. The nominal compositions of the inorganic glass phase and the polymer and glass contents of the hybrids studied are presented in Table I. TEOS (tetraethylorthosilicate) and hydrated calcium chloride (Aldrich Chemical Co., Milwaukee,WI) were used as starting inorganic precursors. Calcium chloride was chosen to replace the calcium nitrate precursor used in previous studies [6, 21] to avoid possible toxic effects due to the presence of nitrates in the final hybrid material. PVA (Acros Organics, New Jersey, USA) with average molecular weight of 16000 Daltons was used as the organic component.

TABLE I Compositions of glasses and hybrid materials prepared

	Glass composition (mol%)			Hybrid compositions	
	SiO_2	CaO		wt% polymer	wt% glass
G58S	58	42	H 1090Gx	10	90
G70S	70	30	H 2080Gx	20	80
G100S	100	0	H 3090Gx	30	70

x = glass composition.

The starting sol was prepared by hydrolysis of TEOS in presence of hydrochloric acid solution 1N and subsequent addition of a certain amount of calcium chloride depending on the intended composition of the inorganic phase. The H_2O/TEOS molar ratio used was 12. PVA solution with concentration 15 wt% was prepared by dissolving the PVA granules in deionized water at 80 °C for 4 h.

After preparation of the starting sol solution, varying amounts of PVA solution, Teepol® surfactant (Thames Mead Ltd.) and HF 5% v/v solution were added to a 40 ml aliquot of the sol. The mixture was foamed by vigorous agitation. Hydrofluoric acid (HF) was added in order to catalyze the gelation, allowing better control of the foam casting. Just before gelation, which was controlled by visual observation, the foams were cast in poly (methyl propylene) containers and sealed. The foamed gels were aged at 40 °C for 72 h. Drying was initially performed in an oven at 40 °C for 72 h and completed in vacuum at the same temperature.

The pore structure of the samples was analyzed using several techniques: Scanning Electron Microscopy, Mercury Porosimetry, Helium Pcnometry and N_2 gas sorption. The phase composition of the materials and constitution was analyzed by X-ray diffraction (XRD) and Fourier transformed infrared (FTIR) spectroscopy.

An initial assessment of the mechanical behavior of the hybrid materials produced was done by a compression test (parallel plate method) in Dynamic Mechanical Analysis (DMA) equipment. Hybrid samples with the composition H2080G70S were tested in two conditions: a) dried in oven at 40 °C (semi-humid state) and b) dried in vacuum (completely dried state). Five specimens for each state, with dimensions 5 × 5 × 5 mm, were compressed with a 500 mN/min rate.

3. Results and discussion

Foams were obtained for all sol compositions and hybrid mixtures. The foaming behaviour of the polymer containing mixture was different from that of the pure sol. In general, for the same surfactant concentration, the addition of the polymer led to a higher initial foam volume (the maximum foam volume obtained by stirring). However, the foam volume decreased with time as the gelation point was approached. Therefore, the time at which the samples were cast could affect the final porosity of the hybrid samples. Another qualitative difference observed was that the polymer addition decreased the drainage therefore increasing the stability of the foams, allowing a larger window in time for

TABLE II Modal pore diameter (μm) of glass and hybrid foams determined by Mercury Porosimetry

	Glass (G)	H1090Gx	H 2080Gx	H 3070Gx
G58S	34	29	30	–
G70S	101	63	46	–
G100S	97	70	76	94

x = glass composition.

casting. The differences caused by polymer addition were particularly relevant for the pure silica composition for which the foaming of the sol is more difficult, both in terms of foam volume and stability.

The addition of substances that adsorb in the air/water interface is the main tool to stabilize thin films and polymers have been used for this purpose [29]. In the last few years more and more polymers are used as additive to stabilize foams and emulsions. They increase the viscosity of the solution, which leads to a reduced drainage velocity and gas permeability of the thin films.

The modal interconnected pore diameters determined by mercury porosimetry for the glass and hybrid foams obtained are presented in Table II. The modal pore diameter of the glass foam is higher than that of the hybrid foam for the composition G70S of the inorganic phase. This difference is illustrated in Fig. 1 by the pore distribution curves for G70S (70 mol% SiO_2-30 mol% CaO) and hybrid H2080G70S (hybrid 20% polymer, 80% glass of 70S30C composition). A relatively narrower macropore size distribution, approximately in the range of 30–120 μm, is observed for the hybrid material, while for the corresponding glass foam the range is 30–250 μm. The difference in modal pore diameter between the glass foams and hybrid foams is not as high for the other glass compositions, as illustrated for foams of glass G100S and hybrid H2080G100S (Fig. 1).

The vertical axis in Fig. 1 (-dV/dlogD) is a differential of the volume of mercury intruded at each pore diameter (D) and is therefore related to the volume of macropores of each diameter. In the case of the glass foam with inorganic phase G70S and corresponding hybrid (H2080G70S) the difference in pore volume was not significant. However, in the case the pure silica system the macropore volume of the hybrid foam H2080G100S was much higher than that of the glass foam G100S. A higher porosity was also observed for the other hybrids of the pure silica system (H1090G100S and H3070G100S) compared to the glass foam. These results confirm that the polymer has a stabilizing effect on the foam, particularly in the pure silica system. For the other glass compositions, although the initial foam volume was increased by the polymer addition, the foam volume decreased until the actual casting time, and consequently the final pore volume retained in the hybrid foams was not much higher than that obtained for pure glass foams.

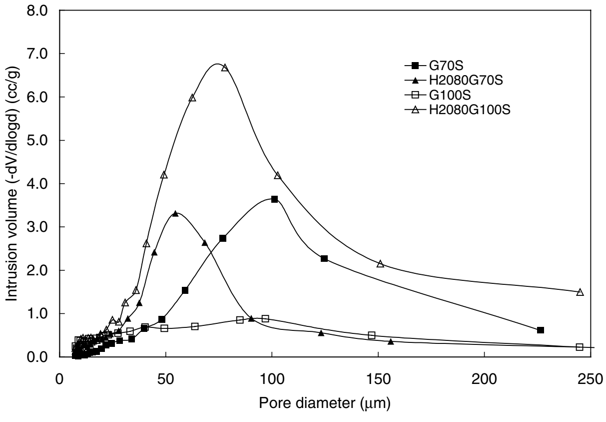


Figure 1 Interconnected macropore size distribution for G70S glass and H2080G70S hybrid foams and 100S glass and H2080G100S hybrid foams obtained from mercury porosimetry.

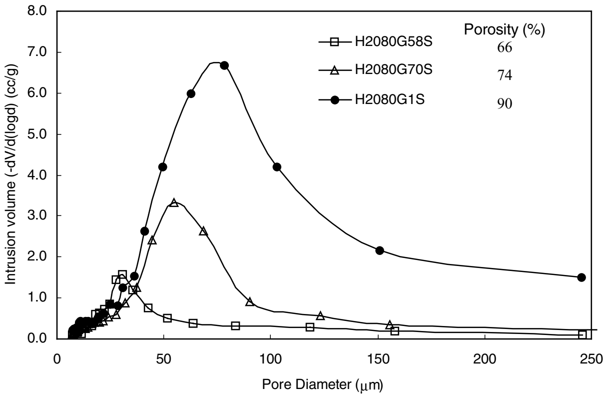
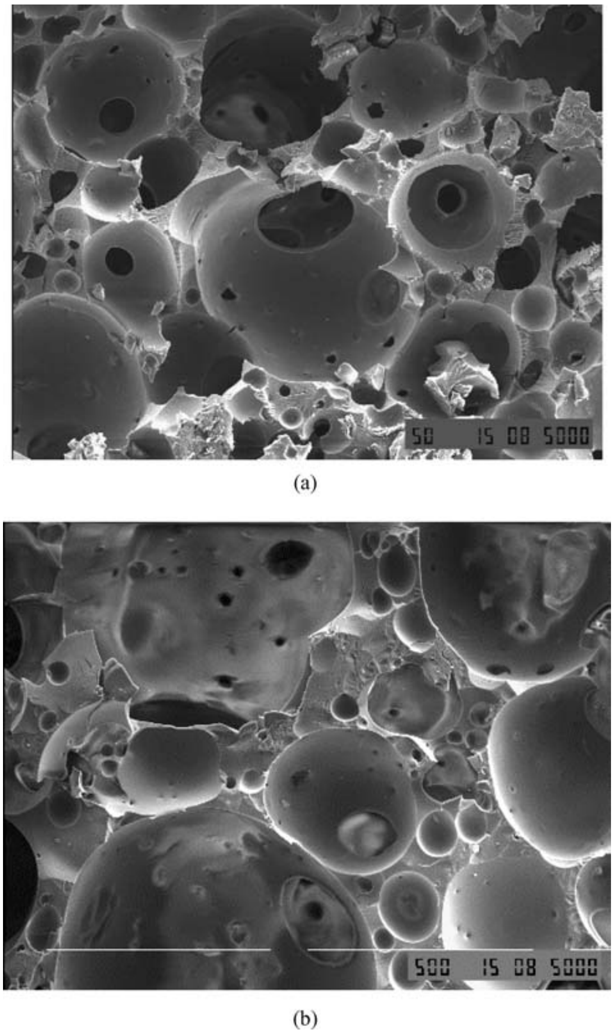


Figure 2 Interconnected macropore size distribution for hybrid foams containing 20 wt% polymer and different glass phase composition obtained from mercury porosimetry.

Fig. 2 shows the pore distribution curve for hybrids containing 20% polymer with different compositions of the glass phase. There was an increase in both modal pore diameter and the range of pore size distribution as the SiO_2content of the glass phase increased. The total porosity of the materials (shown in Fig. 2) was calculated as the relative difference in measured bulk density and true density, determined by measuring the weight and dimensions of samples and by helium picnometry respectively. The estimated total porosity of the samples increased with silica content, as shown by both the height of the distribution curves and by the estimated value of total porosity of the samples.

The above results show that the foam volume and therefore the final total porosity of the samples and the modal macropore diameters were lower for hybrids with higher calcium content in the inorganic phase. Apparently the stabilizing effect of the polymer was not as effective in the presence of large concentrations of calcium cations. One possible explanation for this observation is that the calcium ions interact with the hydrophilic end of the anionic surfactant hindering the interaction this molecule could have with the polymer responsible for the stabilization mechanism. The final pore structure of the obtained hybrids depends on the initial liquid foam structure. It will therefore be affected by physical-chemical factors that affect foam stability, in the case of the present work the calcium concentration in the solution, which varies with the glass phase composition.

The pore morphology and distribution can be visualized by the SEM micrographs presented in Fig. 3. In this



(a)

(b)

Figure 3 SEM micrographs of hybrid foams H2080G70S (100×) and H3070G100S (50×).

TABLE III Surface area, mesopore average diameter and mesopore volume of glass and hybrid foams determined by Nitrogen Adsorption

	Surface area (m^2/g)	Pore diameter (Å)	Pore volume (cc/g)
G58S	21	113	0.06
H1090G58S	11	101	0.035
H2080G58S	1	80	0.002
G70S	82	179	0.37
H1090G70S	76	140	0.27
H2080G70S	2	126	0.004
G100S	227	259	1.47
H2080G100S	169	222	0.90
H3070G100S	168	203	0.85

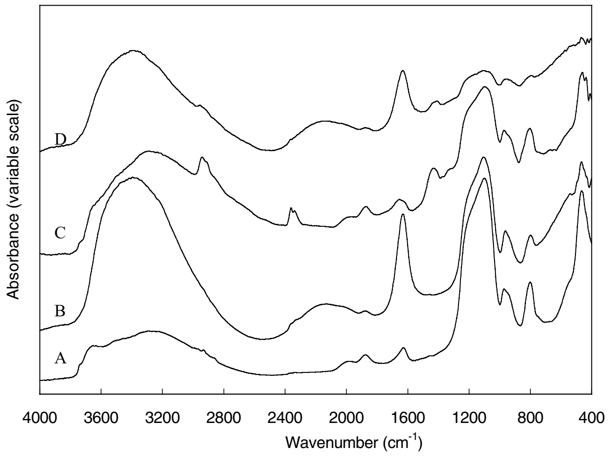


Figure 4 FTIR spectra of glass foams G100S (A), G70S (B) and corresponding hybrid foams H2080G100S (C), H2080G70S (D).

figure pores with higher diameters than the detection limit of mercury porosimetry are present. Although the data obtained by this technique are useful in comparing the macropore structure of the different materials, and how it is affected by processing and composition, the values obtained do not correspond to the real pore structure of the material. The modal interconnected pore diameter is however an important parameter of the pore network since it indicates the largest number of pores of that diameter in the pore network.

The mesoporosity textural characteristics of the glasses and hybrids, determined by Nitrogen Adsorption analysis are presented in Table III. There is generally a decrease in the surface area, mesopore volume and pore average diameter of the hybrids compared to the glass foams. The decrease in the three structural parameters was higher for systems with higher calcium content in the glass phase and higher polymer content in the hybrid. The large decrease observed in the surface area, especially for the higher calcium content materials, is mainly due to a pronounced decrease in the mesopore volume.

FTIR difusive reflection spectra of the glass and hybrid foams are illustrated in Fig. 4. In the spectra of the glass foams G1S and G7S (Fig. 4(A) and (B)) absorption bands corresponding to the inorganic silica phase are observed at 470, 800, 960 and 1100 cm^{-1} and are assigned to, respectively, Si—O—Si bending vibration, Si—O—Si symmetric stretching vibration, Si—OH stretching vibration and Si—O—Si asymmetric stretching vibration [30]. The same bands are observed in the hybrids (Fig. 4(C) and (D)) although the intensity of the Si—O—Si bands was lower in the hybrids, even more so in the hybrid with lower silica content in the glass phase H2080G7S (Fig. 4(D)). The PVA component in the spectra of the hybrids is clearly identified by the band at 2880–2950 cm^{-1} assigned to C—H [31]. A broad band at 3035–3680 cm^{-1} observed both in the glass foams and hybrids is attributed to O—H vibrations of side groups [32]. XRD analysis shows that the hybrid materials obtained are amorphous.

In Fig. 5 representative stress-strain curves for the hybrid H2080G70S in the semi-humid and vacuum dried conditions are shown. For comparison, a curve for pure glass foam with same composition (G70S) is also included. The glass foams used in the mechanical tests were produced with the same procedure used for hybrids except that the calcium precursor was calcium nitrate and after drying the samples were heat treated at 600 °C [6, 21]. This sample was used for comparison with the hybrid samples because the handling strength of the pure glass foams produced with calcium chloride as precursor was too low, and therefore not considered suitable for comparison. It can be seen that larger deformation occurs in the hybrid samples than in the pure

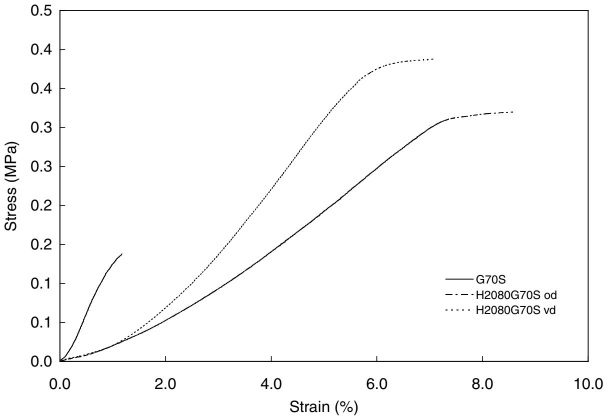


Figure 5 Stress-strain curves obtained by compression test for hybrid H2080G70S in the conditions semi-humid (od) and vaccuum dried (vd). For comparison a curve for glass foam G70S is presented.

glass foams. The polymer addition to the material improves the strain at failure, which is a measure of capability for deformation of material. The ultimate fracture strength is higher and the Young Modulus is lower for the hybrids compared to the pure glass foams. The semi-humid hybrid samples presented slightly lower strengths and Young moduli than the completely dried samples and slightly larger strains at failure. The effect of humidity in increasing the deformation capacity of the material is expected since the role of small molecules, particularly water, as a plasticizer agent for PVA is well known [33]. It is particularly interesting to note that these bioactive hybrids show a certain degree of extensibility.

The results of this work show that hybrids with glass phase containing 70% and 100% SiO_2 present a macropore structure adequate for tissue engineering applications. Hybrids with the higher calcium content studied (50%) are not indicated because of their low porosity and modal pore diameter. It is expected that the degradability rate of the hybrids will increase with the calcium content in the glass phase. The stability and degradability rate of the hybrids in body fluids, not evaluated in this work, is an important aspect of the materials behavior for tissue engeineering applications and will be studied in the future.

Differently from sol-gel derived glasses the last processing step of the hybrids is a drying stage at low temperatures since they can not be treated at high temperatures that would decompose the polymer phase. Therefore hybrids produced by this route may contain residual organics and present a high acidic character due to the catalysts added during processing. In the case of the hybrid foams HF is added as an extra catalyst for the gelation step, which can increase the acidity of the foams still further. Consequently an additional cleaning step is necessary to produce biocompatible hybrid materials produced by the sol-gel route. This issue was evaluated in another study in which hybrid PVA/bioactive glass foams were cleaned using various procedures and a cytotoxicity evaluation was conducted [34]. Hybrids cleaned in NH_4OH solution presented mild toxicity, similar to the levels observed for sol-gel derived glasses. Further studies must be conducted on the hybrid foams to investigate if they maintain the ability to support cell attachment, proliferation and mineralised nodule formation.

The formation of the hybrid foams also leaded to an increase in strength compared to glass foams with the additional advantage that this approach also leaded to an increase in the strain at failure and toughness. To improve further the mechanical properties of hybrid bioactive glass/polymer foams the procedure described must be adjusted to obtain hybrids with larger polymer contents while still maintaing a high porosity.

4. Conclusions

Hybrid PVA-bioactive glass foams were succesfully obtained using the sol-gel method. The foams obtained had a high porosity varying from 60 to 90% and the macropore diameters ranged from 30 to 500 μm. The modal macropore diameters decreased with an increase in calcium content in the inorganic phase and with the polymer content in the hybrid. The surface area and mesopore volume decreased with polymer concentration in the hybrids. The strain at failure and ultimate strength of the hybrid material is higher than those of the pure glass foams.

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