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Sodium silicate bonded borate glass scaffolds for tissue engineering

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Abstract Borate and silicate glass particles and microspheres with size distributions in the range of approximately 100–400 micron were loosely compacted and bonded by sodium silicate solution to prepare resorbable, porous glass constructs with porosity 30–50%. Conversion of the binding borate glass to hydroxyapatite was investigated by measuring the weight loss of the constructs in a solution of 0.25 M K_2HPO_4 with a pH value of 9.0 at 37 °C, as a function of time. Almost full conversion of the borate glass to hydroxyapatite was achieved in less than 6 days. X-ray diffraction revealed an initially amorphous product that subsequently crystallized to hydroxyapatite.

Introduction

Nowadays, in modern medicine, tissue engineering has emerged as a revolutionary approach to the reconstruction and regeneration of lost or damaged tissue, which involves the use of biomaterials to repair or replace damaged or diseased tissue to using three-dimensional scaffolds with controlled structure, in which cells are seeded prior to implantation. The scaffold acts to deliver cells to the appropriate site, define a space for tissue development, and direct the shape and size of the engineered tissue [1–6]. The clinical success of the tissue-engineered construct is critically dependent on the biomaterials and three-dimensional scaffolds, which would guide the growth of new tissue in vitro and in vivo, and on a suitable supply of cells [7–10].

Bioactive glasses and ceramics have been used as scaffold materials for bone tissue engineering [11–15]. They have the ability to convert to hydroxyapatite in body fluids and in aqueous solutions containing calcium and phosphate ions, and the ability to bond directly to bone. Since the its bone bonding properties were reported in 1971 by Hench et al. [12], the bioactive glass codenamed 45S5 and referred to as Bioglass® (composition: 45 wt% SiO_2; 24.5 wt% Na_2O; 24.5 wt% CaO; 6 wt% P_2O_5) has formed the primary system of interest. Recently, researchers at the University of Missouri-Rolla have shown that some borate glasses can convert to hydroxyapatite and bond directly to bone in a manner comparable to the silicate-based 45S5 glass [16–18]. The fabrication of the borate glasses into porous, three-dimensional scaffolds suitable for tissue engineering applications have been tried by pure sintering and salt-sintering processes [19, 20]. However, these two processes require high temperature routes for the fabrication of the scaffold.

In this article, porous-borate glass scaffolds were fabricated by a sodium silicate bonding process at low temperatures. The bioactivity of the scaffolds was evaluated by the transformation of the scaffolds to hydroxyapatite in 0.25 M K_2HPO_4 solutions with pH 9.0 at 37 °C. The effect of pore size on dissolving rate was

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studied and the surface morphologies, microstructures, and chemical compositions were analysed.

Experiments

A sodium-calcium borate glass from the system $Na_2O \cdot CaO \cdot B_2O_3$ was prepared by melting reagent-grade chemicals for 1 h at 1,000 °C in a platinum crucible. After quenching, the glass was crushed in a hardened steel mortar and pestle, purified by removing the metallic impurities magnetically, and sieved through stainless-steel sieves to produce particles with the following size ranges: 90–150, 150–212, 212–355 and 355–425 μm. The particles were spheroidized by allowing them to fall freely through a vertical tube furnace at ~940 °C.

Porous cylindrical scaffolds with the required diameter and height (typically 5 mm by 6.25 mm) were fabricated by pouring the glass particles or microspheres wetted by 2% sodium silicate solution into a plastic mold, and molding the system for a given time at a given press. Then the scaffolds were wetted by 5% sodium silicate solution drops and dried at 37 °C by turns, then dried at 90 °C overnight.

The porous scaffolds were observed by optical microscopy (Nikon Optiphot) and their pore characteristics (porosity and average pore size) were estimated using image analysis (NIH Imaging) and density measurements. Using image analysis, the porosity was estimated from the area of the planar surface occupied by pores divided by the total cross-sectional area. The density of the scaffolds was determined from its mass and external dimensions, and the relative density ρ was found by dividing by the theoretical density of the glass (2.58 g/cm^3). The porosity P was fond from the relation $P = 1 - \rho$.

In order to study the conversion of the porous borate glass scaffolds to that of hydroxyapatite, the scaffolds were immersed for given times (~1 week) in an unstirred K_2HPO_4 solution (concentration = 0.25 M) at a constant temperature 37 °C and a starting pH value of 9.0. This solution was chosen to save time, because two necessary ions including HPO_4^{2-} and OH^- were supplied to react with the glasses [17–21]. Each scaffold was immersed in 100 cm^3 of the phosphate solution. After a given immersion time, the scaffold was dried and its mass was measured. The pH variations of phosphate solution were also measured by AR25 Dual Channel pH/Ion Meter. The structural characteristics of the converted material were observed using scanning electron microscopy (SEM; Hitachi S-4700). The crystal structure was analyzed using X-ray diffraction (XRD; Scintag 2000) in the step-scan mode at a rate 0.05°/min in the range of 20–70° 2θ.

Results and discussions

Characterization of porous bonded borate glass scaffolds

Pore structure of the bonded scaffold prepared from borate glass microspheres with sizes in the range of 90–150 μm is shown in Fig. 1. The microspheres have smooth surfaces and uniform shapes. Binding at the contact points of spheres by sodium silicate are clearly visualized. From this figure, the interconnectivity and three-dimensional nature of the bonded structure can be observed.

The porosity of the scaffolds prepared from particles with the four size ranges is shown in Fig. 2. Scaffolds prepared from larger particles (sizes in the range of 212–355 μm and 355–425 μm) have an higher porosity (~50%), compared to scaffolds prepared from smaller particles (sizes in the range of 90–150 μm and 150–212 μm) for which the porosity is 30–40%. It is possible that the smaller particles have a distribution of sizes that may lead to more efficient packing.

A general trend of increasing compressive modulus with decreasing particle size has been testified [21]. While at smaller particle size, the scaffold has smaller pore diameter.

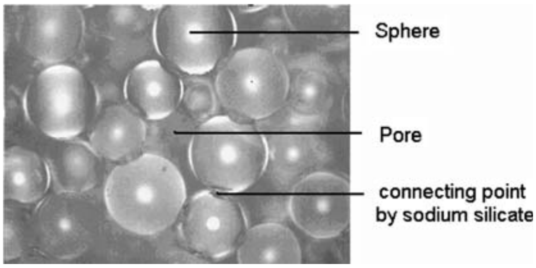


Fig. 1 Optical micrograph of the surface of a borate glass scaffold formed from microspheres with sizes in the range of 90–150 μm bonded by sodium silicate

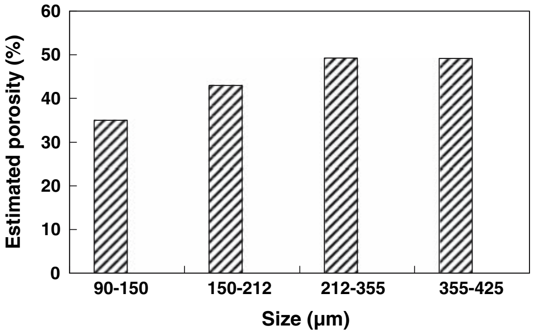


Fig. 2 Estimated porosity of borate glass scaffolds prepared from particles with sizes in the indicated ranges

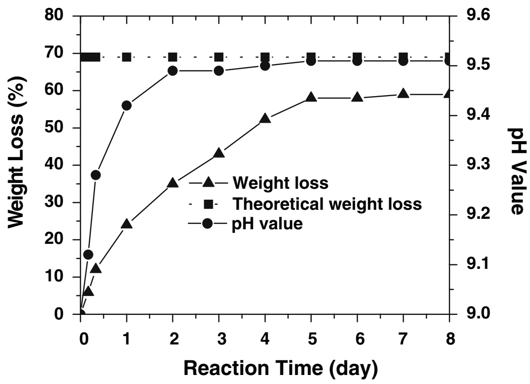


Fig. 3 Weight loss of borate glass scaffolds and evolution of the pH of the phosphate solution as a function of reaction time in a 0.25 M K_2HPO_4 solution at 37 °C and a starting pH value of 9.0

Conversion of borate glass scaffolds to hydroxyapatite

The weight loss of porous-borate glass scaffolds when immersed in 0.25 M K_2HPO_4 solutions is shown in Fig. 3 as a function of reaction time for scaffolds prepared from microspheres with sizes in the range of 212–355 μm. The curves show that the weight loss increases with time but eventually flattens out, becoming approximately constant after ~6 days. The pH variations of phosphate solution with initial value 9 are also shown in Fig. 3. The evolution of the pH of the phosphate solution reacting with the bonded-borate scaffolds appears to undergo a dissolution and precipitation process.

The reaction process of the borate glass is a bulk dissolution in which the highly soluble boron dissolves into the solution, breaking the glass structure and releasing sodium and calcium ions. At the same time, as soon as the calcium ions goes into solution, it reacts with PO_4^- and OH^- to form ACP and then HA.

Hydrogen ions diffuse into the glass-forming hydroxyl groups and substitute for sodium ions. The exchange of hydrogen and sodium ions between glass and solution increase the pH of the solution. The pH increases until eventually the boron present in the solution hydrolyzed into boric acid or combined with the sodium present in the solution and act as a buffer.

If it is assumed that the CaO in the glass reacts completely with the phosphate solution to form hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, whereas the B_2O_3 and alkali oxide in the glass dissolve completely into the solution, then the theoretical weight loss is found to be ~69% (dashed line in Fig. 3). The maximum weight loss observed for the borate glass scaffolds is ~5–10% lower than the theoretical value. This discrepancy may be due to that the hydroxyapatite formed by the conversion of similar borate glasses in phosphate solutions near room temperature indicates that the Ca/P atomic ratio is well below the stoichiometric value.

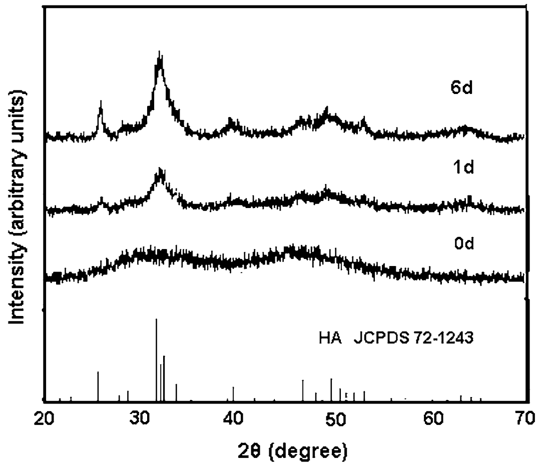
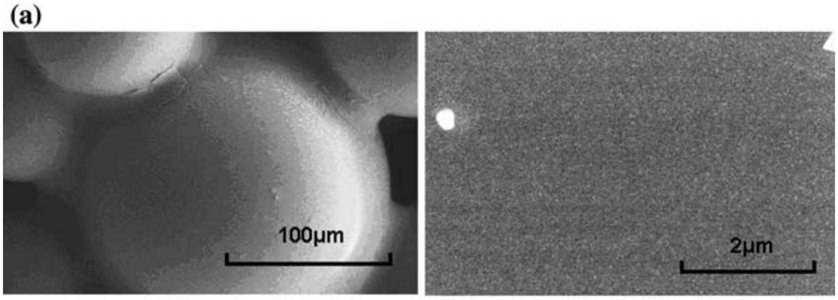


Fig. 4 X-ray diffraction pattern of the borate glass scaffold prepared from 212 to 355 μm microspheres before and after reaction for 1 and 7 days in a solution of 0.25 M K_2HPO_4

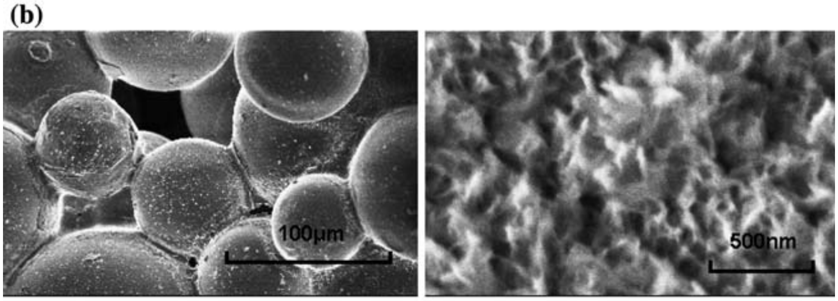
In order to further study the conversion of the scaffolds to hydroxyapatite, the scaffolds from the 212–355 μm particles were immersed in an unstirred 0.25 M K_2HPO_4 solution at a constant temperature 37 °C and a starting pH value of 9.0. Each scaffold (0.25 g) was immersed in 100 cm^3 of the phosphate solution. After a given immersion time, the scaffold was grounded to a powder, which was analyzed using X-ray diffraction, shown in Fig. 4. The XRD data indicate that the conversion of the borate glass to hydroxyapatite occurs initially by the formation of an amorphous calcium phosphate phase that later crystallizes to hydroxyapatite.

SEM micrographs of the unconverted glass scaffold, the partially converted glass scaffold (immersed in K_2HPO_4 solution for 1 day), and the fully converted glass scaffold (immersed in K_2HPO_4 solution for 7 days) are shown in Fig. 5. The unconverted glass scaffold shows the relatively smooth surface features characteristic of a spheroidized glass. Conversion of the glass to hydroxyapatite does not involve a change in dimensions of the microspheres, so the external volume of the scaffold, as well as the volume and the size of the macropores remains constant. However, the converted calcium phosphate material is highly porous, with the pore size being on the order of a few 10s of nanometers. Conversion of the sintered glass scaffolds to hydroxyapatite leads to the presence of two distinctive types of pores in the scaffolds: (i) macropores occurring between the particles which are controlled by the size of the particles and by the processing of the scaffold, and (ii) fine pores with sizes on the order of a few 10s of nanometers, occurring on the surface and within the converted

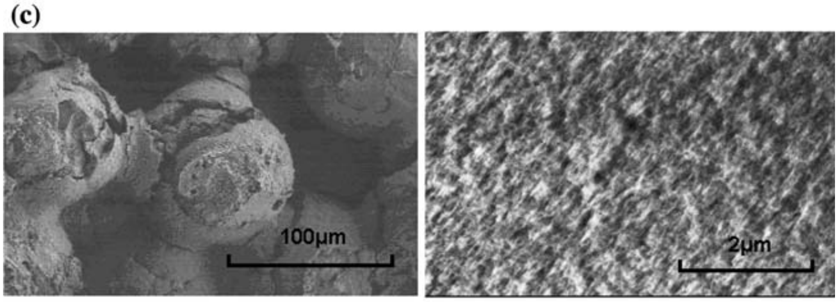
Fig. 5 SEM micrographs of the surface of bonded borate glass scaffolds prepared from 90 to 150 μm microspheres: (**a**) unconverted glass; (**b**) partially converted glass formed by reaction for 1 day in 0.25 M K_2HPO_4 solution; (**c**) fully converted glass reacted for 7 days in 0.25 M K_2HPO_4 solution



Bonded borate glass scaffold



Scaffold immersed for 1 day in K_2HPO_4 solution



Scaffold immersed for 7 days in K_2HPO_4 solution

calcium phosphate material, which are controlled by the conversion reaction.

Conclusion

Porous-borate glass scaffolds were fabricated by a sodium silicate bonding process at low temperature. A sample with 250–315 μm glass particles possessed a structure with ~50% porosity. Borate glass scaffolds were soaked in a 0.25 M K_2HPO_4 solution at 37 °C and $pH = 9.0$ were converted into hydroxyapatite. This reaction increased steadily with time and was completed after approximately 6 days. The conversion reaction involved the initial formation of an amorphous calcium phosphate phase which later crystallised to hydroxyapatite. The weight loss of the samples observed during conversion in hydroxyapatite can quantitatively be explained assuming a dissolution of all Na_2O and B_2O_3 from the glass and a reaction of the CaO of the glass with the phosphate of the solution to hydroxyapatite.

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