Bioactive glasses for bone tissue engineering application

Furqan Ali Shah1,2,3

1School of Engineering and Materials Science, Queen Mary, University of London, UK
2Department of Biomaterials, Sahlgrenska Academy at University of Gothenburg, Sweden
3BIOMATCELL VINN Excellence Center of Biomaterials and Cell Therapy, Sweden

Corresponding Author
Furqan Ali Shah
Department of Biomaterials
Institute of Clinical Sciences
Sahlgrenska Academy at University of Gothenburg
P.O. Box 412, SE-405 30, Göteborg, Sweden

Abstract
Bioactive glasses are useful materials as synthetic bone graft substitutes due to their potential for osseoinduction and osseoconduction. The ability of these materials to chemically bond to bone is characterized by surface apatite formation. In this paper, the bioactivity of two bioactive glasses has been evaluated in serum supplemented cell culture medium simulating near-physiological conditions and analysed by pH measurements, ICP-ES, FT-IR and XRD. The results show how Bioglass 45S5 that is considered maximally bioactive by many other studies, failed to form detectable crystalline apatite in such conditions, while a slightly different glass composition was able to form detectable crystalline apatite in the same conditions, indicating the effect of minor changes in chemical composition on the bioactivity of such materials.

Introduction
Bioceramics such as hydroxyapatite (Actifuse™) and bioactive glass (PerioGlas®) represent synthetic alternatives to autografts and fresh cancellous bone allografts for augmentation of severely resorbed regions in the dental alveolar ridge, preservation of ridge dimensions following dental extractions and to facilitate implant placement in deficient ridges. Ambiguity exists regarding the efficacy of hydroxyapatite monoliths and scaffolds for alveolar ridge preservation1-3, particularly when placed in fresh extraction sockets. Their major disadvantage is slow rate of degradation and the life-long presence of the synthetic material within a bone defect. Placement of bioactive glass monoliths in artificial sockets created by splitting the alveolar bone prior to definitive restorative treatment3 and immediately following tooth extraction results in circumferential osseous tissue formation, minimal implant dehiscence, normal healing of the overlying soft tissue, absence of infection and high survival rates over long follow-up4.

On contact with body fluids, the initial exchange of cations (Na+, Ca2+) from the glass surface for cations (H+, H3O+) in solution causes alkalization of local pH. Hydroxyl (OH-) ions attack Si-O-Si bonds with subsequent loss of soluble silicagroups that re-polymerize onto the alkali deficient glass surface, as a silica-rich layer. Surface migration of Ca2+ and PO43- groups and incorporation of free Ca2+ and PO43- from the solution allows deposition of an amorphous CaPlayer over the silica-rich layer5. A mixed carbonated hydroxyapatite (HCA) layer is formed by the inclusion of OH and CO32-, which is believed to be biologically active and essential in bonding to living tissues. An interface thus established allows interaction with biological moieties such as collagen, fibronectin, plasma proteins, blood cells, fibroblasts and osteoblasts—and in due course of time a strong bonding between the biomaterial and the physiological environment is established6.

This paper explores in vitro bioactivity of two such materials as micro-particles, in order to characterize various parameters that influence their behaviour in physiological conditions.
Materials and Methods

1. Glass synthesis
The first glass, DB, was prepared previously. The composition was 49.47 mol% SiO2, 23.08 mol% CaO, 26.38 mol% Na2O and 1.07 mol% P2O5 [7]. This ratio of constituents has a theoretical network connectivity (NC) of 2.13 [8]. The second composition, Bioglass 45S5 [9] is an FDA approved commercial material with a composition of 46.2 mol% SiO2, 26.9 mol% CaO, 24.3 mol% Na2O and 2.6 mol% P2O5, and has NC of 1.90. Both glasses were ball-milled and sieved to a particle size < 38 μm.

2. Preparation of Medium
9.57 g L-1 of MEM powder (PAA) and 2.2 g L-1 NaHCO3 (Sigma-Aldrich) were dissolved in 1000 mL deionized water. 100 mL foetal bovine serum (Sigma-Aldrich) [10], 20 mL 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer solution (PAA Laboratories GmbH) and 1 g sodium azide (AnalaR BDH) were added and the pH was adjusted to 7.30.

3. Dissolution Experiments
75 mg glass powder was added to a sterile container. The pH of the dissolution medium was measured and 50 mL was pipetted into the container, based on previous studies [11, 12]. The container was placed in a temperature controlled incubator shaker (IKA® KS 4000i control) at 60 RPM and 37°C. All experiments were conducted in triplicates. At day 7, the solutions were filtered (VWR International, medium flow filter paper, 5-13 μm particle retention), the filtrate pH was measured and was stored at 2°C until required. The retentate was rinsed with isopropyl alcohol (IPA) and dried at 37°C for 24 h. The glass powder from each set of triplicates was combined. The filtered solutions, however, were kept separate.

4. Solution-Phase Characterization
pH measurements were carried out with a Thermo Scientific Orion 3 Star Plus pH meter, calibrated with standard solutions of pH 4.01, 7.00 and 10.

Inductively Coupled Plasma Emission Spectroscopy (ICP-ES) was performed using a Perkin-Elmer Optima 5300 DV ICP-OES spectrometer for quantitative analysis of Ca, P, Si, Na, K and Mg levels in the as-prepared medium (base-line) and the filtered solution at the end of each experiment.

5. Solid-Phase Characterization
Fourier Transform Infrared Spectroscopy (FT-IR) was performed on a Perkin-Elmer, Spectrum GX (wavenumber 500-1600 cm-1, 20 scans per spectrum). The spectra were normalized and the various absorption bands were assigned to different molecular structures [13, 14].

X-Ray Diffraction (XRD) was performed using a Siemens D5000 X-Ray Diffractometer (room temperature, 0.03° step-size, scan-range of 5-70°2θ, Cu-Kα radiation). The XRD patterns were compared with reference patterns from the Joint Committee on Powder Diffraction Standards (JCPDS) database: nos. 09-432 (hydroxyapatite); 19-272 (carbonated hydroxyapatite); 5-86 and 86-0174 (calcite).

6. Statistical Analysis
Student’s t-test was used for calculating the difference of means, and p-values <0.05 were considered statistically significant. Mean values ± standard deviations have been presented. All statistical analysis was performed using IBM® SPSS Statistics (v. 20) software for Mac.

Results

1. pH measurements
The initial pH of the dissolution medium was pH 7.3 ± 0.01. At day 7, the pH for Bioglass 45S5 was significantly higher (p=0.03) at 7.96 ± 0.32, whereas no difference was observed for DB glass, at 7.33 ± 0.40 (p=0.45). The final pH however, was not significantly different between the two glasses (p>0.05).

2. Ion Release
Calcium concentrations increased significantly in solution from 76.7 ± 0.1 mg L-1, to 197 ± 30 mg L-1 for Bioglass 45S5 (p=0.001) and 166 ± 4 mg L-1 for DB glass (p=0.0001). Phosphorus concentrations in solution decreased significantly, from 33.1 ± 0.1 mg L-1, to 8.1 ± 0.2 mg L-1 for DB glass (p<0.0001) and 27.7 ± 2.6 mg L-1 for Bioglass 45S5 (p=0.01). The as-prepared dissolution medium was nominally silicon-free (>0.15 mg L-1). After glass dissolution, Si concentrations increased significantly (p<0.0001 for both glasses), reaching 64.3 ± 0.7 mg L-1 for Bioglass 45S5 and 58.7 ± 1.3 mg L-1 for DB glass. These values correspond approximately to the solubility limit for Si, indicating further reaction highly unlikely. The concentration for Bioglass 45S5 was however significantly higher (p<0.02) than DB glass (Figure 1).

3. Apatite Formation
The different peaks on FT-IR spectra (Figure 2) represent the absorption bands for apatitic phosphate (PO43-): v4 (560-610 cm-1), v1 (960 cm-1) and v3 (1010-1040 cm-1); and carbonate (CO32-): v2 (870 cm-1, A/B-type substitution) and v3 (1400-1440 cm-1, A-type substitution). The split band (v4) at 560-610 cm-1 as seen for glass DB is
characteristic of crystalline apatite. The single broad band at 560-580 cm$^{-1}$ for Bioglass 45S5 represents amorphous CaP. On XRD (Figure 3), crystalline apatite and calcite phases were identified in relation to the DB glass. The reflections at 26, 28, 32-34 and 47 $^\circ$20 are characteristic of apatite. A high intensity peak at 29.5$^\circ$20 and several other reflections at around 23, 36 and 43$^\circ$20 were assigned to calcite. Bioglass 45S5 only showed amorphous halos at 20 and 30 $^\circ$20 and absence of any appreciable crystalline phase.

**Discussion**

The rate of glass degradation and consequently the tissue bonding ability is a function of glass structure and composition. Compositions such as 30-60 mol% SiO$_2$, 10-50 mol%CaO and 5-40 mol% Na$_2$O are maximally bioactive (15). The ratio of bridging oxygen (BO) to non-bridging oxygen (NBO) atoms, i.e. NC of the glass, determines dissolution kinetics. Na$_2$O and CaO are network modifier oxides that disrupt the three-dimensional network structure to linear chains, end members and isolated groups (6), making such silica glasses susceptible to dissolution.

Bioglass 45S5 is considered a highly bioactive glass, particularly when studies are performed using simpler media such as SBF (16), or tris-hydroxymethyl-aminomethane (Tris) buffer solution (17). However, in more complex, near-physiological solutions containing ionic concentrations similar to human plasma, amino acids, vitamins and serum proteins, the same glass composition may demonstrate failure to precipitate crystalline apatite, as in the current study. On the contrary, DB glass was able to form apatite even in such a challenging environment.

Traditionally, high CaO/P$_2$O$_5$ ratio within the glass structure has been considered essential for apatite formation (15). More recently, phosphate has been reported to act as a surface nucleation site only and not a critical component for bioactivity (18), and presumably it may be adsorbed onto the surface from the surrounding body fluids. The low-phosphate (1.07 mol%) DB glass demonstrated phosphorus uptake from a high-phosphate solution. Although it is suggested that high-phosphate content enhances apatite formation (19), the inability of Bioglass 45S5 to form apatite may be attributable to phosphate mediated repolymerization of the silica network (20), indicated by the presence of a broad Si-O-Si band on FT-IR. Additionally, a significantly higher Si concentration was observed in solution. The network connectivity of Bioglass 45S5 implies a linear structure, suggesting the ability of silicate chains to dissolve into solution without hydroxyl (OH$^-$) ion mediated Si-O-Si breakdown. However, glasses with NC just over 2 would still be able to react without Si-O-Si bond cleavage, as P-O bonds can also be readily broken (21).

For DB glass, high intensity carbonate (v$_2$, v$_3$) bands on FT-IR suggest the formation of carbonated hydroxyapatite (HCA) instead of carbonate-free hydroxyapatite (HAp). However, a secondary crystalline phase identified as calcite (CaCO$_3$) was also seen on XRD, indicating that once the solution is depleted of phosphorus ions, and an unfavourable Ca/P ratio exists, the Ca$^{2+}$ ions are then able to react with CO$_3^{2-}$. This suggests phosphate being a “limiting factor”, and that it may be more significant to have high phosphate concentrations in the local physiological environment instead of within the glass itself.

**Conclusion**

Apatite formation in physiological solutions as early as 7 days encourages the use of bioactive glasses for applications similar to hydroxyapatite scaffolds and granules. Bioactive glasses undergo time-dependent degradation and conversion to physiologically derived apatite, resulting in de novo bone formation and total replacement of the synthetic material. The possibility to tailor the composition to achieve rapid or slower dissolution rates or for the delivery of therapeutic ionic species (7, 22-24) makes such materials particularly attractive for applications in dentistry, orthopaedics, and tissue engineering and regenerative medicine.

**ACKNOWLEDGMENTS**

I would like to thank my supervisors Dr. Karin A. Hing, Prof. Dr. Delia S. Brauer, and Prof. Robert G. Hill. Financial support from QMUL International Science and Engineering Excellence Award is also gratefully acknowledged.

**References**


Figure 1: Concentrations of Ca, P and Si in dissolution media before immersion and after immersion for 7 days (Mean values ± SD)

Figure 2: FT-IR spectra of Bioglass 45S5 and DB showing the appearance of different absorption bands for PO4−, CO3− and Si-O-Si after immersion for 7 days

Figure 3: XRD patterns of Bioglass 45S5 and DB after immersion for 7 days (a = apatite, c = calcite)