Bioactive response of Ag-doped tape cast Bioglass® 45S5 following heat treatment

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The suitability of Bioglass® 45S5 to the tape casting process and the ability of the glass to retain *in vitro* bioactivity following heat treatment to increase strength has been established. In this research, tape cast Bioglass® was doped with silver prior to heat treatment in an effort to impart antimicrobial properties. The effect of initial dopant concentration and processing temperature was investigated. FTIR confirmed the presence of well established hydroxyapatite (HA) surface layers on Ag-doped tape cast Bioglass® following heat treatment and simulated body fluid (SBF) immersion. Solution analysis revealed the release of silver ion in concentrations similar to those reported in the literature to have antimicrobial effects. Additionally, mercury porosimetry was used to determine that silver enhanced the densification process relative to that of undoped tape cast Bioglass®. Ag-doped tape cast and sintered Bioglass® (TCSBG) appears promising for use in situations requiring rapid surface HA formation and antimicrobial properties.

1. Introduction

There is much interest in silver containing glasses and ceramics for use in bone replacement [1–3] as well as wastewater treatment [4, 5] owing to the demonstrated antimicrobial effects. In the orthopaedic realm, silver containing biomaterials with bone bonding properties would be especially desirable [6]. The ability of certain compositions of bioglasses and ceramics to bond to bone and soft tissue is well documented [7].

Tape casting offers the possibility of creating complex shaped forms given that the final material is built up thin layer by thin layer. The suitability of Bioglass® 45S5 to the tape casting process and the ability to retain *in vitro* bioactivity following heat treatment to increase strength (to values approximately equal to that of cortical bone) has been reported [8]. The purpose of this research was to characterize the *in vitro* bioactive response of silver doped tape cast Bioglass® (including silver release *in vitro*) as well as investigate the effect of silver ion on the phases and microstructure present following heat treatment.

2. Materials and methods

2.1. Processing

Bioglass® 45S5 particulate (average size 3 μm) was obtained from US Biomaterials Corp. (Alachua, Florida). Tape casting slurries were prepared by mixing Bioglass® with appropriate amounts of toluene, ethanol, polyvinyl butyral (10 000 MW), and phthalic acid as described previously [9]. The slurry was then tape cast onto a moving carrier film, forming a flexible tape of approximately 100 μm thickness following drying. Small disks were then cut from the sheets, stacked and laminated together under 30 MPa pressure at 150 °C. Differential thermal analysis (Stanton Redcroft Thermal Analyser STA-780) was used to verify the complete removal of organics from tape cast Bioglass® following heating under flowing air at 470 °C for 8 h.

Silver ion was obtained from nitrate ($AgNO_3$) solutions in ethanol. Tape cast Bioglass® pellets (6 mm dia. × 4 mm thick) were exposed to silver ion concentrations of 0.01 M or 0.1 M for 18 h. Following immersion, tape cast Bioglass® pellet samples were rinsed with ethanol, dried at 150 °C for 30 min, and then heated for 1 h under a flowing dry nitrogen atmosphere at 800 °C or 1000 °C.

2.2. Characterization

Fourier transform infrared spectroscopy (Genesis II FTIR, Spectronic Unicam) was used to characterize the hydroxyapatite (HA) surface layer formation on Ag-doped tape cast and sintered Bioglass® (TCSBG) surfaces during immersion (2 h, 24 h, and seven days) in simulated body fluid (SBF) at 37 °C. The SBF used herein, developed by Kokubo *et al.* [10], is a close approximation of the composition of human blood plasma.

The penetration depth of the FTIR beam is approximately constant and, therefore, the growth of the HA layer with time in solution can be quantified, as the intensity of the underlying silica layer decreases with increasing HA layer thickness. Quantification was achieved by taking the relative FTIR intensity ratio of the Si-O bending peak (457 cm^{-1}) to that of the larger

P-O peak (580 cm^{-1}). A lower ratio of Si:HA is therefore indicative of a HA layer of greater thickness.

The release of silver, silicon, calcium, and phosphorous ions into solution during immersion in SBF was quantified using inductively coupled plasma emission spectroscopy (ARL Fisons ICP Analyser 3580B).

Mercury porosimetry (Quantachrome Poremaster 33) was used to compare the pore size distribution of Ag-TCSBG and undoped TCSBG. X-ray diffraction of tape cast Bioglass® was performed using a Philips PW1729 X-ray generator and Cu_{α} radiation in effort to determine the predominant phases present following doping and heat treatment.

3. Results

3.1. Bioactivity

FTIR spectra of the Ag-doped TCSBG samples are shown in Fig. 1. The initial FTIR spectra of Ag-TCSBG do not appear to be affected by sintering temperature or concentration of silver ion dopant (Fig. 1a–d). The spectra for each of the four treatments (0.01 M/800 °C; 0.1 M/800 °C; 0.01 M/1000 °C; and 0.1 M/1000 °C) prior to immersion (initial) are similar: i.e. (a) each sample exhibits Si-O stretching modes in the range 1097–1110 and 1037–1050 cm^{-1} (b) non-bridging oxygen peaks are present for each of the four treatments in the range 920–940 cm^{-1} (c) dual P-O bending peaks are initially present in Ag-TCSBG near 620–625 and 576–582 cm^{-1} and (d) each of the four treatments initially has present Si-O bending peaks near 528–536 and 455–457 cm^{-1}. The initial calcium phosphate phase corresponding to the dual P-O peaks is not HA due to the absence of a P-O bending peak near 602 cm^{-1}.

HA layers were established on the surface of all four sample types following 2 h soaking in SBF (Figs 1a–d). P-O bending peaks indicative of HA are located between 600–604 and 576–580 cm^{-1}. Further evidence of HA layer development is demonstrated by the disappearance of the Si-O bending peak near 528–536 cm^{-1}.

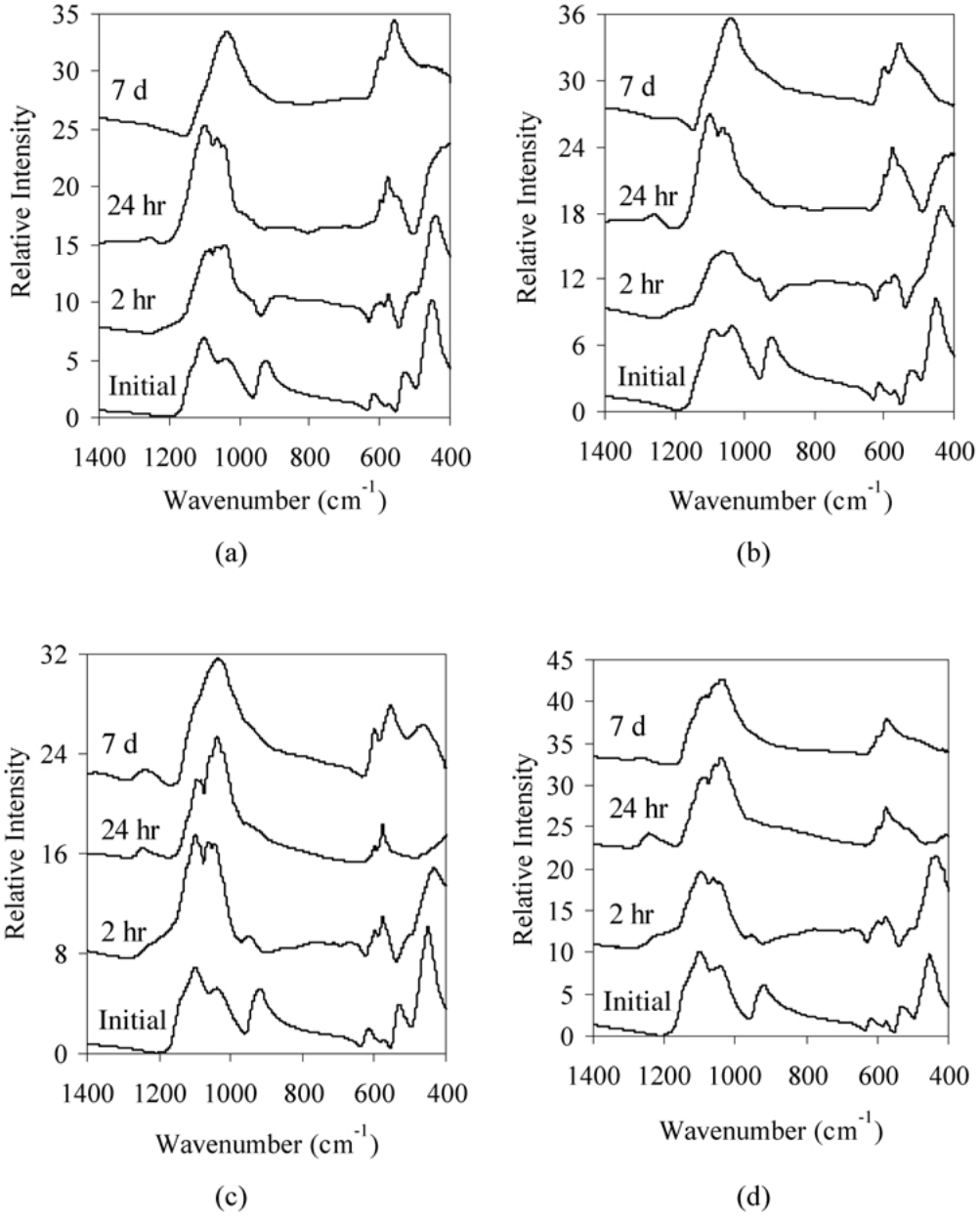

Figure 1 FTIR spectra of silver doped tape cast and sintered Bioglass®: (a) 0.01 M/800 °C, (b) 0.1 M/800 °C, (c) 0.01 M/1000 °C, and (d) 0.1 M/1000 °C.

After 24 h in SBF, the HA layer thickness increased (from 2 h values), as evident by the decrease in the Si-O bending peaks near 455–457 cm^{-1}. Dual P-O bending peaks are again present in the ranges 600–604 and 579–580 cm^{-1}. However, the P-O peaks near 579–580 cm^{-1} are more pronounced than the peaks near 600–604 cm^{-1}. The ratios of Si : HA for 800 °C Ag-TCSBG were 1.66 and 0.92 for the 0.01 and 0.1 M solution dopant concentrations, respectively. HA layers were slightly thicker on the 1000 °C Ag-TCSBG surfaces; Si:HA ratios for the 0.01 and 0.1 M samples were 0.62, and 0.21, respectively.

After seven days in SBF, HA layers were well established for each of the four sample types ($Si : HA \ll 1$); i.e. no Si-O bending peak was present after seven days in SBF indicating a thick HA layer had formed. P-O peaks were present in the ranges 600–602 cm^{-1} and 578–580 cm^{-1} for 0.01 M/800 °C, 0.1 M/800 °C and 0.1 M/1000 °C Ag-TCSBG. P-O bending peaks for 0.01 M/1000 °C Ag-TCSBG were present at 602 cm^{-1}, but there was a slight shift in the 560 cm^{-1} peak, in addition to a third P-O peak which appeared near 468 cm^{-1}. The presence of a third P-O bending peak associated with growth of HA has been observed previously [11].

3.2. ICP

The release profiles of Ag, Ca, Si, and P are shown in Fig. 2 for Ag-doped TCSBG soaked in SBF for 2 h, 24 h, and seven days. A gradual release of silver from TCSBG was observed over the seven days test period. Ag concentrations were similar at 2 h for the four treatments (0.01 M/800 °C; 0.1 M/800 °C; 0.01 M/1000 °C; 0.1 M/1000 °C). However, at 24 h the amount of silver released into solution was greater for the 0.1 M (800 and 1000 °C) samples than for those doped in 0.01 M Ag solutions. Furthermore, the 0.01 M/800 °C samples released a greater amount of silver (0.31 μg/ml) into solution after 24 h than did the 0.01 M/1000 °C samples (0.13 μg/ml). After seven days, the silver ion release from the 0.01/800 °C, 0.1 M/800 °C, and 0.1 M/1000 °C Ag-TCSBG samples were similar (1.18–1.24 μg/ml), whereas 0.01 M/1000 °C Ag-TCSBG released only 0.40 μg/ml into solution.

The decrease in calcium ion concentration (Fig. 2b) between 24 h and seven days for 0.01 M/1000 °C Ag-TCSBG suggests additional HA formation may have taken place. Gradual silicon ion release was also observed (Fig. 2c) over the seven days test period. The 0.01 M/1000 °C Ag-TCSBG released slightly more silicon into solution after seven days than did the other treatments. Phosphorous concentrations (Fig. 2d) decreased with time, indicating further growth of the HA layer with time for each of the four Ag-TCSBG treatments.

3.3. X-ray diffraction

For both undoped TCSBG and Ag-TCSBG, X-ray diffraction (XRD) analysis revealed the presence of

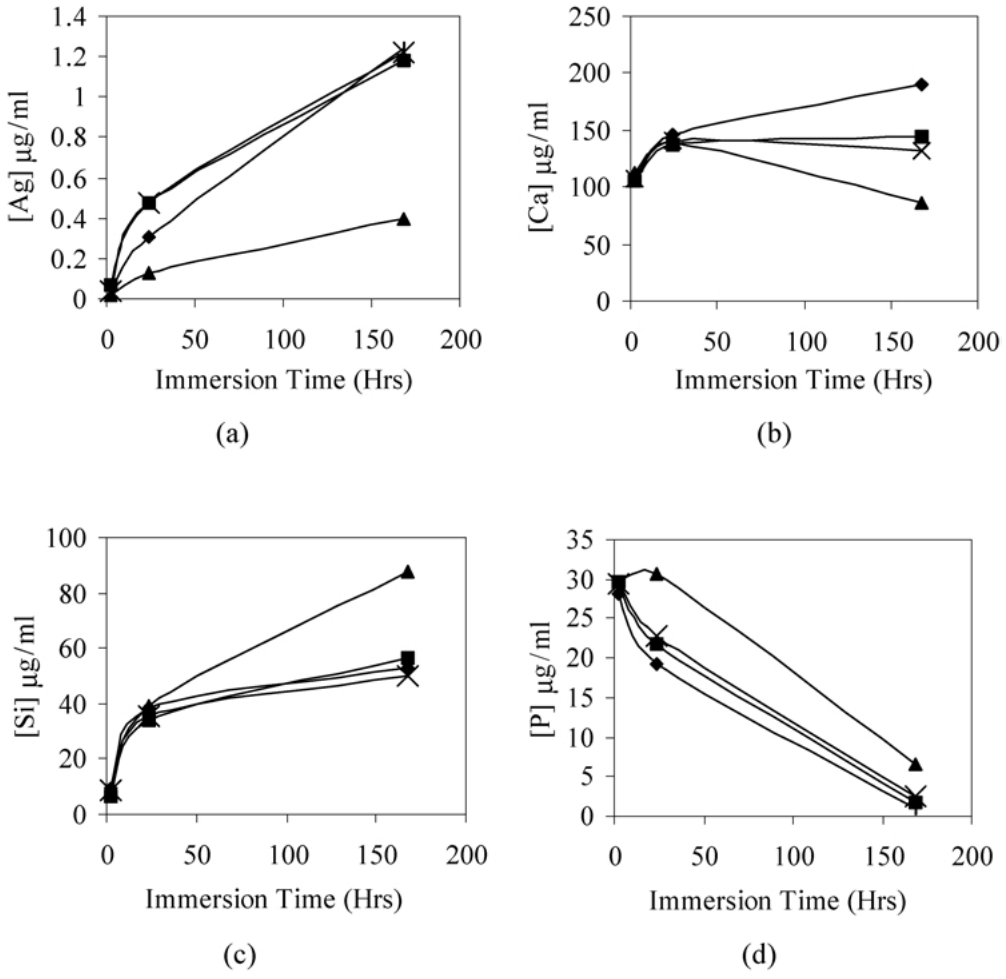

Figure 2 ICP data for (a) Ag, (b) Ca, (c) Si, and (d) P ions leached from Ag-doped TCSBG during SBF immersion. (◆) 0.01 M/800 °C; (■) 0.1 M/800 °C; (▲) 0.01 M/1000 °C; and (- × -) 0.1 M/1000 °C.

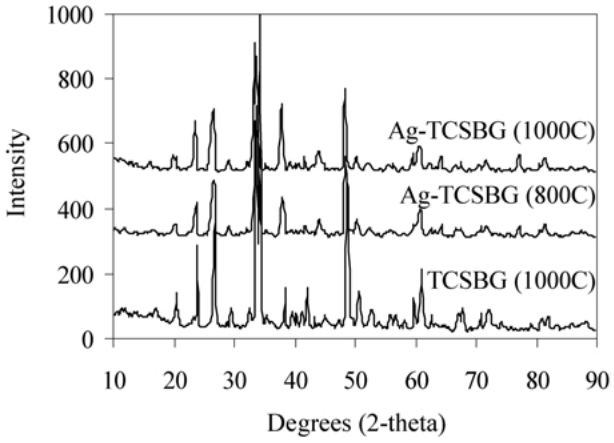

Figure 3 XRD spectra of tape cast sintered Bioglass® (1000 °C, 3 h), and Ag-doped tape cast and sintered Bioglass (800 and 1000 °C for 1 h).

$Na_2Ca_2(SiO_3)_3$ (*d* spacings 2.64, 1.88, and 3.35) as the predominant phase (Fig. 3). Silver was likely present in colloidal form within the pore surface of Ag-TCSBG, as XRD revealed the *d*-spacings of 1.24 and 1.45 which likely correspond to colloidal silver. These two peaks were not present in the undoped TCSBG.

3.4. Mercury porosimetry

In Fig. 4 is shown a pore size histogram for undoped TCSBG and 0.1 M/800 °C Ag-TCSBG. There was a significant amount of porosity in the range 0.5 to 0.8 μm diameter in undoped TCSBG sintered at 800 °C for 3 h. For 0.1 M/800 °C Ag-TCSBG (sintered for only 1 h) the pore size distribution was shifted to a lower range of (0.03–0.5 μm). The intrusion volume of mercury into undoped TCSBG ($5\,cm^3/g$) was greater than that of the Ag-doped TCSBG ($0.05\,cm^3/g$), which suggests that the presence of silver ion enhanced the densification of tape cast Bioglass®.

4. Discussion

When immersed in SBF, HA layers formed on the surface of heat treated (800 °C or 1000 °C) Ag-doped tape cast Bioglass®. The hydroxyapatite surface layers formed on 1000 °C Ag-TCSBG surfaces were of greater thickness than those formed on 800 °C Ag-TCSBG surfaces after 24 h immersion in SBF. Furthermore, the 800 °C Ag-TCSBG bioactivity was improved over that of undoped TCSBG processed at 800 °C [8]. This variation in the bioactive response due to processing temperature and presence of silver is thought to be related to the sample porosity. Silver doping had the effect of decreasing sample porosity relative to that of undoped samples, as confirmed by mercury porosimetry. TCSBG samples with high porosity were observed to undergo excessive ionic release during *in vitro* testing which had the effect of inhibiting HA formation [8].

ICP analysis revealed that silicon release in each of the four treatments of Ag-TCSBG was similar to those concentrations described previously which have been shown to stimulate bone formation *in vitro* [12]. Therefore, Ag-TCSBG may possess similar bone cell stimulating effects.

Silver was released from Ag-TCSBG gradually during immersion in SBF. Release ranges for the four treatments of Ag-TCSBG were 0.02–0.07 μg/ml at 2 h, 0.13–0.48 μg/ml at 24 h, and 0.40–1.24 μg/ml at seven days. The 0.01 M/1000 °C Ag-TCSBG released silver at a lesser rate than that of the 0.1 M samples. These silver ion release concentration ranges are promising in terms of potential Ag-TCSBG antimicrobial properties, as 0.1 μg/ml has been reported to be the minimum concentration for attaining bactericidal effects [13, 14].

5. Conclusions

Given (a) the ability of Ag-TCSBG to rapidly form HA surface layers *in vitro*, (b) silicon ion release similar to that reported to stimulate bone cells *in vitro* and (c) silver ion release above the minimum concentrations required to inhibit bacterial growth, Ag-doped TCSBG appears promising for use in clinical situations requiring a rapid bioactive response and additional antimicrobial properties.

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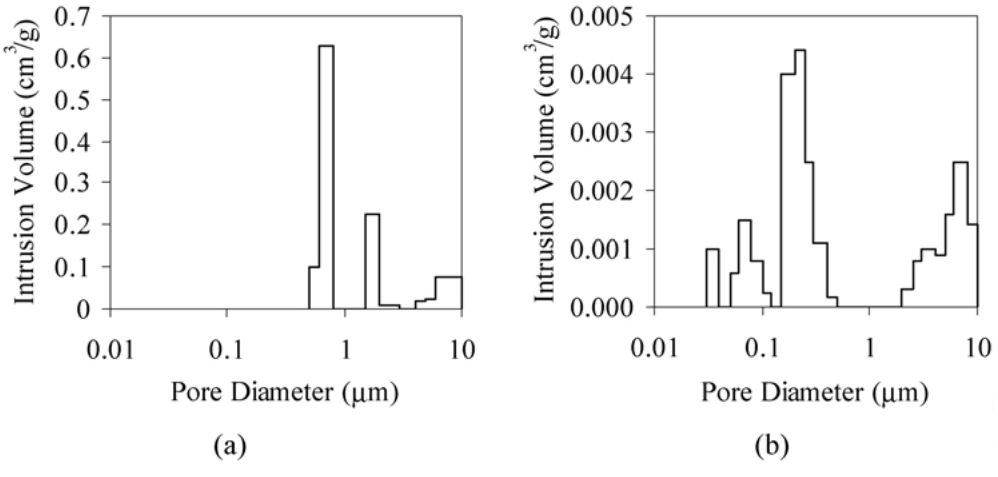

Figure 4 Histograms of intrusion volume vs. pore diameter for (a) non-doped TCSBG sintered at 800 °C for 3 h and (b) 0.1 M Ag-doped TCSBG sintered at 800 °C for 1 h.

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