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P. Hassanzadeh

A. W. Wren

Mark R. Towler Missouri University of Science and Technology, mtowler@mst.edu

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Silver Coated Bioactive Glass Particles for Wound Healing Applications

P. Hassanzadeh, A.W. Wren, M.R. Towler Alfred University 1 Saxon Drive Alfred, NY 14802

I. INTRODUCTION

Hench and colleagues discovered that bone could bond chemically to certain glass compositions in 1969 [1]. This group of glasses is known as bioactive glasses [1]. Some special compositions of bioactive glasses will bond to soft tissues as well as bone [1]. One of the important characteristics of bioactive glasses is their ability to release beneficial ions such as Ca^{2+} , Na^+ , Zn^{2+} , Sr^{2+} , and PO_4^{3-} in the body, which promote self-healing [2]. Bioactive glasses generally contain much less glass former (e.g., SiO_2 , B_2O_3 etc.) than conventional glasses [3]. Network modifiers encourage the formation of Non-Bridging Oxygens (NBO) groups. These NBO groups decrease aqueous durability and increase bioactivity [4].

Silver is a broad-spectrum antibiotic (against *E. coli* and *S. aureus* among other bacteria). Metallic silver is relatively inert. However, its interaction with moisture on the skin surface and with wound fluids results in release of silver ions [5]. Silver toxicity is low in humans [6]. The rate of healing of silver is proportional to the amount of silver release and the stage of the healing process [6]. Silica glass containing silver is expected to be a good candidate of antibacterial material for medical applications. However, preparing Ag_2O -SiO₂ glasses by the conventional melt-quench method with high concentration of silver is difficult due to the undesirable crystallization [7].

This work looks at determining the effect of coating the glass surface with Ag and also the antimicrobial properties of the glasses with respect to two species of prokaryotic organisms (*E. coli* and *S. epidermidis*). Both bacteria have been reported to cause septic complications relating to osteomyelitis and joint infection [8].

II. EXPERIMENTAL METHODS

A glass with composition of 0.42SiO_2 -0.15 CaO- $0.23 \text{Na}_2\text{O}$ -0.20 ZnO was prepared by weighing out appropriate amounts of analytical grade reagents and ball milling (1 h). The mix was then oven dried (100 °C, 1 h) and fired (1500 °C, 1 h) in a platinum crucible and shock quenched in water. The resulting frit was dried, ground and sieved to obtain glass particle sizes of < 90 μ m and 425-850 μ m, small and large respectively.

Silver-coated glass was prepared by adding two grams of each glass to the solution, which was prepared by dissolving silver nitrate in 100 ml ethyl alcohol for 1 h with addition of moderate heating while spinning on a magnetic stirrer. The glass was left to spin in this solution for 3 hours. After spinning, the glass particles were dried in an oven overnight and then heated to 300 $^{\circ}$ C to remove residual NO₃⁻.

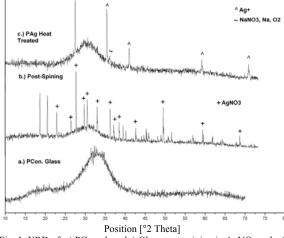
X-ray Diffraction was used to confirm the amorphous structure of uncoated glass (PCon) and existence of silver ions in the silver-coated glass (PAg). Scanning Electron Microscopy and Energy Dispersive X-ray Analysis were performed to evaluate morphology, composition and quantitative chemical identification of the surface of the silvercoated glass particles.

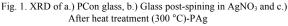
Antimicrobial testing against *S. epidermidis* and *E. coli* was performed to evaluate the antibacterial properties of silver ions and zinc ions in PAg and PCon. The agar diffusion method was used for this purpose. The inhibition zones were calculated using (1):

Inhibition Zone (mm) =
$$\frac{Halo \phi - Disc \phi}{2}$$
 (1)

III. RESULTS

X-ray Diffraction was initially performed on the glasses at each processing stage. Fig. 1a shows no crystallinity in the starting material PCon. Fig. 1b shows the presence of crystalline species after spinning in AgNO₃ for 3 hours, which was subsequently identified as AgNO₃. Fig. 1c shows the PAg after heating for 3 hours and presents crystalline peaks, which are predominantly associated with Ag⁺.





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287

Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Analysis (EDX) were performed in order to investigate the surface and bulk composition of the glass particles. Fig. 2 shows an image of large silver-coated glass particles (PAg._L) under the SEM and EDX. PAg._L was analyzed at the particle interior as shown in Fig. 2a and at the particle surface as shown in Fig. 2b. EDX of the interior of PAg shows the basic composition of the glass whereby Ca, Si, Na, Zn, and O are present as expected. When analyzing PAg surface with EDX, it is found that the Ag was surface-bound and did not diffuse through the bulk of the material.

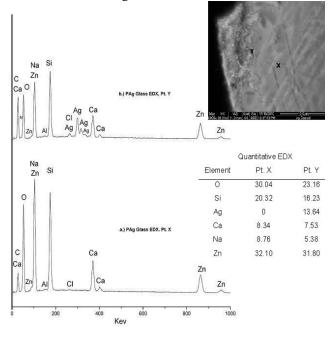


Fig.2. EDX analysis of a.) PAg glass interior, b.) PAg glass surface

The agar diffusion method was used to evaluate the antibacterial properties of glass particles against two strains of bacteria *E. coli* and *S. epidermidis*. Fig. 3 and 4 show the antibacterial testing results. Both particles sizes were used for this purpose. Large uncoated glass particles (PCon_{.L}) did not show any antibacterial properties. PAg_{.L} exhibited antibacterial properties in both bacteria. Inhibition zones observed were 4.19 \pm 0.66 mm when tested in *E. coli*, and 1.95 \pm 0.27 mm when tested in *S. epidermidis*. For small uncoated glass particles (PCon._s), the antibacterial effect was evident when tested in *S. epidermidis* where an inhibition zone of 3.95 \pm 0.91 mm was observed. The most prominent antibacterial effect was observed with small silver-coated glass particles (PAg._s) where an inhibition zones of 13.12 \pm 0.73 and 12.06 \pm 1.23 mm were observed using *E. coli* and *S. epidermidis*, respectively.

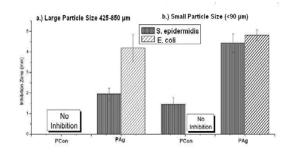


Fig. 3. Antibacterial testing of a.) Large particles and b.) Small particles in *E. coli* and *S. epidermidis*

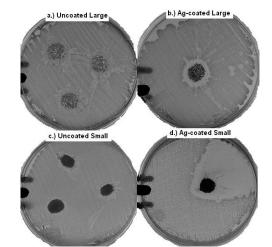


Fig. 4. Antibacterial testing of a.) Pcon._L, b.) PAg._L, c.) PCon._s and d.) PAg._s in *E. coli*

IV. CONCLUSION

Silver-coated bioglass expected to be a very good candidate as an antibacterial biomaterial. It seemed to show significant antibacterial properties against *E. coli* and *S. epidermidis*. Also, the smaller glass particles exhibited larger surface area, which led to the larger concentration of silver release. As a result, larger inhibitions zones were observed in small particle size of the glass versus the larger particle size.

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