PREPARATION OF Ag-DOPED AND UNDOPED BIOCERAMIC FROM BIO-WASTES

OLUWAFISAYOMI BABATOLA
The Federal Polytechnic Ilaro, Ogun State.
oluwafisayomi.babatola@federalpolyilaro.edu.ng

Abstract

The capacity of bioactive glasses to combine the dual functions of controlled drug release and bone regeneration has garnered them a lot of interest in recent years. But the high expense and toxicity of the conventional silica precursor, typically tetraethyl orthosilicate (TEOS), prevent the mass manufacture of bioactive glasses.. This has necessitated a paradigm shift from conventional alkoxysilanes to other silica substitutes. This research describes a novel sol-gel preparation of a silver-doped bioactive glass from biowastes as economic and eco-friendly routes to silica and calcium components of bioactive glasses. The silica was obtained from bamboo leaf while the calcium was extracted from waste chicken egg shells. The obtained bioactive glass samples were characterized for morphology and bioactivity using scanning electron microscopy (SEM). The samples' capacity to cause the creation of apatite to occur within three days of immersion in the simulated body fluid (SBF) was demonstrated by an in vitro bioactivity test, which was done using the doped sample as the control. This ability increased up until the fourteenth day at a faster rate. The results herein obtained indicates that silicate bioactive glass derived from waste bamboo leaf and eggshells can serve as candidate scaffolds in bone regeneration and a medium for release of antimicrobial agents.

Keywords: Bioactive glass; bioactivity, Bone regeneration, Bamboo leaf; chicken eggshells.

Introduction

Bone is an unpredictable tissue that consistently experiences dynamic natural renovating, i.e., the coupled procedure whereby osteoclasts resorb developed bone tissue followed by osteoblasts that create new unresolved issue solid homeostasis of bone (Boskey, 2007). Bone has the innate ability to regenerate as part of the healing process after an injury, as well as during skeletal development or ongoing remodeling as an adult. (Bates and Ramachandran 2007). Bone grafts are bone that is transplanted starting with one region of the skeleton then onto the next to help in recuperating, fortifying or improving capacity. Bone or bone-like materials utilized in bone grafts may originate from the person, from a contributor or from a man-made source. As a rule, they are utilized to occupy in a vacant space that may have been made in or between the bones of the spine by sickness, injury, deformation or during a surgery, for example, spinal combination. In most cases, bone grafting is necessary to promote bone healing. Blood is by far the most common transplantation tissue, with bone coming in at number two. (Boyce *et al.*, 1999; Van *et al.*, 1999).

This study is designed to explore a novel method of synthezing bioactive glasses using bioawastes gotten from bamboo leaves and chicken egg shells and doping it with silver. It would have an advantage for large scale production since the process can be expanded to meet commercial demand because the locally sourced material (bamboo leaf) is widely available.

Furthermore, one major complication for bone reconstruction is that, sometimes, it results in bacteria infection at the repair sites. Large bone defects remain after the contaminated bone tissues are removed; these defects must be repaired with an orthopedic implant. The ideal implant would be able to release an antibacterial agent in a controlled and continuous manner, treat the infection, and repair bone tissue. An infection might be specifically treated by the localized release of an anti-bacterial substance at the surgical site. The antibacterial properties of silver ions were well known. (Jones *et al.*, 2004; Crabtree *et al.*, 2003).

The traditional precursor for producing silicate bioactive glasses which is alkoxysilanes e.g trimethyl orthosilicate (TMOS) and tetraethyl orthosilicate (TEOS) are very expensive and toxic on inhalation (Essien, *et al.*, 2016). In orthopedics, neurosurgery, and dentistry, more than 500,000 bone grafting surgeries are carried out annually in the United States and 2.2 million globally to correct bone abnormalities. (Wenhao and Kelvin, 2017). Hence the needs to develop a material that is affordable and easily assessable and would also serve as a carrier system for antimicrobial agent.

Methodology

Sample collection

The dry bamboo leaf was collected from the Bells University of Technology, Ota and identified in an herbarium at the Botany Department of Lagos, Akoka. Waste chicken eggshells were collected from domestic waste in Ilaro, Ogun State.

Preparation of SiO_2 from bamboo leaves

The dried bamboo leaves were collected washed and sun dried to remove moisture. To create bamboo leaf ash(BLA), it was burned in a muffle furnace at 500ºC until all the organic chemicals were eliminated.

Extraction of Ca from Chicken eggshells

The chicken eggshells were cleaned with deionized water, dried in the oven for two hours at 120 degrees Celsius, and then ground into fine powders. In order to create a crystal-clear solution of $Ca(NO_3)_2$, the powder (4.4642 g) was carefully dissolved in 5 ml of pure HNO_3. (Essien *et al.*, 2016)

Synthesis of bioactive glass by the sol-gel method

Using a magnetic stirrer device, 99.9 g of citric acid was dissolved in 180 ml of deionized water to create the bioactive glass. Bamboo leaf ash (BLA), 14.286 g, was gradually added while stirring, and the mixture was heated at 120ºC for two hours to produce a clear solution. The eggshell stock solution was then dropped into the mixture while being stirred. Subsequently whilst the mixture was being mixed, the eggshell stock solution was added. For the whole reaction, the resultant mixture was agitated for an hour. The produced sol was then dried for 24 hours at 120°C after being placed in an oven at 100°C for 3 hours to create a gel. The material was then stabilized at 700°C for 2 hours in a muffle furnace to release nitrates, breakdown $Ca(NO_3)_2$, and create CaO. The material was then exposed to further radiation in an oven for 24 hours to further break the organic bonds in the substance. Desiccator samples that were taken from the furnace were kept there for future research. (Essien *et al.*, 2016)

Synthesis of Ag-doped bioactive glass by the sol-gel method

Using a magnetic stirrer device, 99.9 g of citric acid was dissolved in 180 ml of deionized water to create the bioactive Ag-doped glass. Bamboo leaf ash (BLA), 14.286 g, and 104 ml of 0.1 M $AgNO_3$ were slowly added while stirring, and the mixture was heated at 120ºC for two hours to produce a clear solution. The eggshell stock solution was then dropped into the mixture while being stirred. For the whole reaction, the resulting mixture was agitated for one hour. The produced sol was then dried for 24 hours at 120°C after being placed in an oven at 100°C for 3 hours to create a gel. It received the same treatment as the undoped. Samples obtained from the furnace were stored in the desiccator for further studies. (Essien *et al.*, 2016)

Preparation procedure of SBF

In order to evaluate the potential for apatite production, it is helpful to examine the in vitro bioactivity of bioceramics using this method. Using a stirring bar and 700 ml of deionized water in a 1000 ml plastic beaker, 1000 ml of SBF was created. It was placed on the magnetic stirrer in the water bath and covered with a watch glass. Under stirring, the water in the beaker was heated to 36.5ºC. NaCl (8.035g) , $NaHCO_3$ (0.355 g), $NaHCO_3$ (0.355 g), KCl (0.225 g), $K_2HPO_4.3H_2O$ (0.231 g), $MgCl_2.6H_2O$ (0.311 g), 1.0M-HCl (39 ml), $CaCl_2$ (0.292 g) and Na_2SO_4 (0.072 g) were dissolved one by one into the solution in this order at 36.5ºC. 900ml of deionized water was added to the solution to make it there. The pH of the mixture was measured, and the Tris buffer was added gradually while being dissolved into it bit by bit until the pH reached 7.40. The pH meter electrode was taken out of the solution, rinsed with deionized water, and then the washings were reintroduced back in. The pH-adjusted solution was transferred from the beaker into a 1000 ml volumetric flask, and the stirring rod, beaker surface, and washings were all cleaned with deionized water before being used again. The indicated line was filled with deionized water, a lid was placed on top, and the container was allowed to chill in the refrigerator between 5-10ºC (Kokubo and Takadama, 2006).

Bioactivity tests in SBF

The 70S30C doped and undoped bioceramics were immersed the simulated body fluid (SBF) to test for its bioactivity. 0.5 g of these glass samples were immersed in 100 ml of the SBF for 3, 7 and 14 days respectively at 36.5ºC in an incubator. After immersion for the different periods in the SBF, the specimens were taken out from the SBF and gently washed with deionized water thoroughly and dried in a desiccator for 5 days (Kokubo and Takadama, 2006).

Results

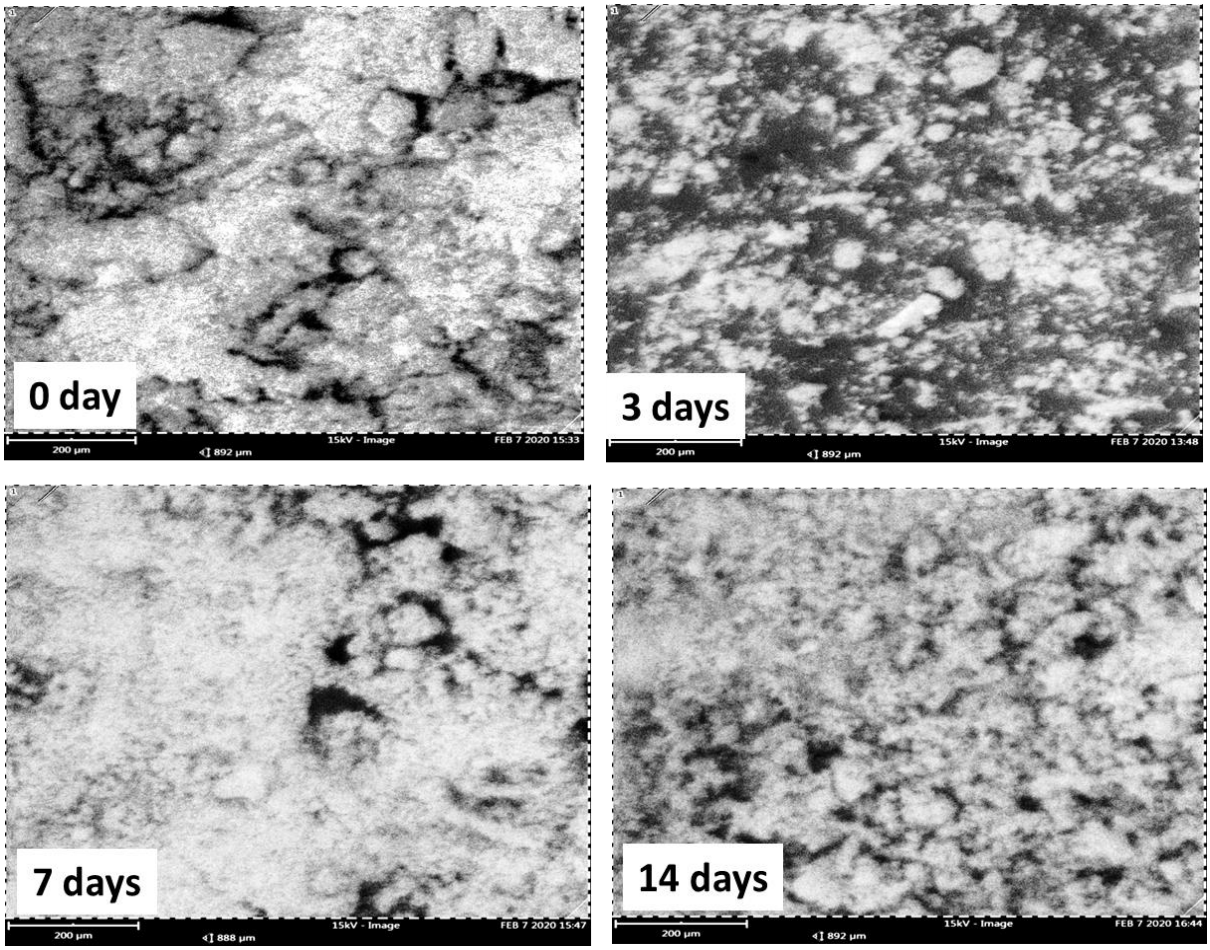

Figure 1: The SEM results for the undoped 70S30C bioactive glass at 0, 3, 7 and 14 days respectively after immersion in SBF.

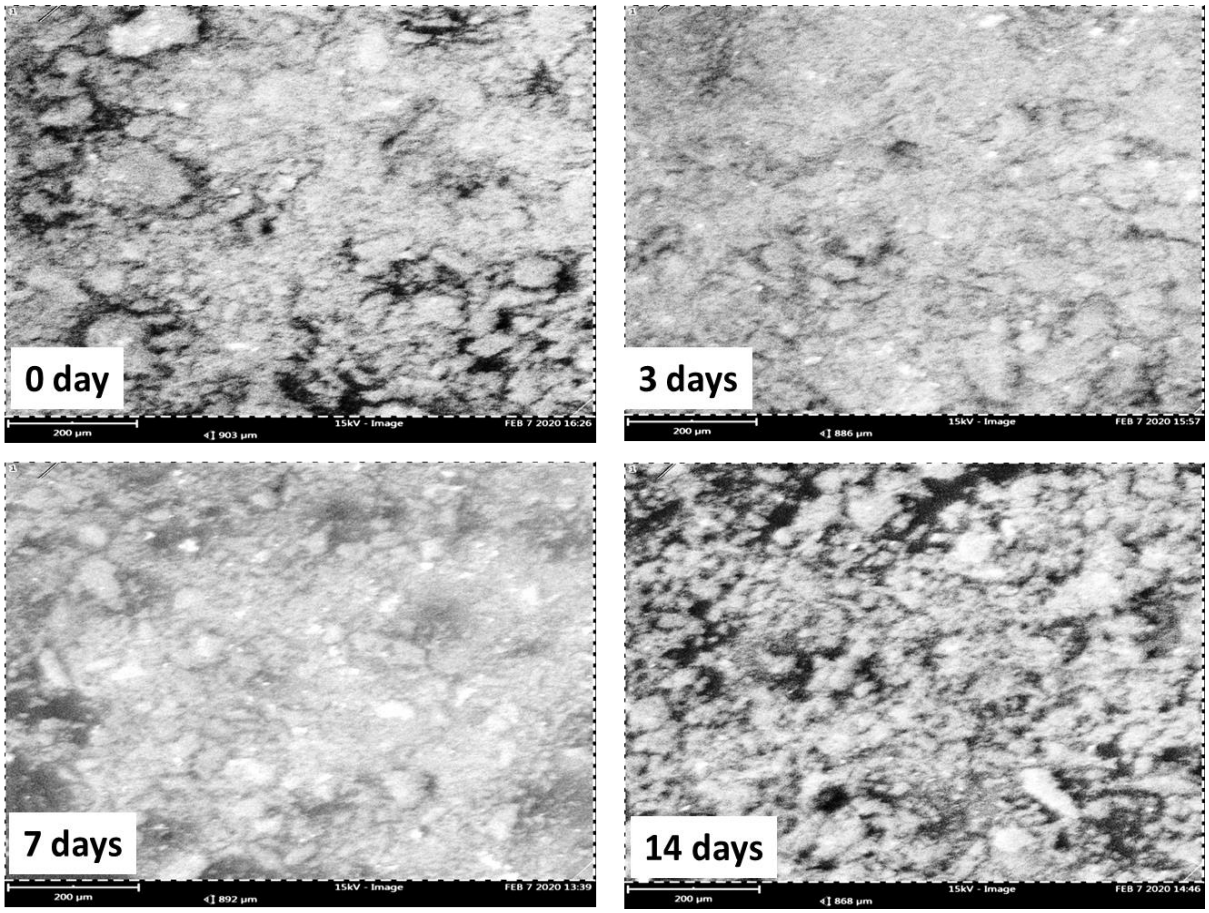

Figure 2: The SEM results for the 70S30C Ag-doped bioactive glass at 0, 3, 7 and 14 days respectively after immersion in SBF

Discussion

SCANNING ELECTRON MICROSCOPY (SEM)

Undoped Glass

The SEM micrographs of the doped and undoped bioceramic before and after immersion in the simulated body fluid are shown in Figure 8 and 9.

Figure 1 shows the microstructure of the bioactive glass at 0 day and after immersion the in simulated body fluid for 3, 7 and 14 days respectively. At 0 day, (Figure 2) the glass material shows surfaces dominated by glass particles, the spread of the glass particles over the surface is homogeneous that is they are well distributed giving it a wide surface area which indicates that if this glass sample is used for bone regeneration, it would stimulate adhesion of cells, proteins and growth factors at a very high rate on the surface towards formation of bone. Compared to the monodispersed glasses' smooth and regular texture, the sol-gel-derived glasses have a greater surface area and a texture that is extremely rough and porous. These surface features of the sol-gel-derived glasses speed up surface reactions and dissolve at a faster rate, releasing ions into solution more quickly. (Sepulveda *et al.*, 2002).

After immersion in SBF for 3 days, a few spherical balls were observed growing on the surface which is attributed to the formation of hydroxyapatite crystals on the surface. As further immersion period continues till 7 days, the apatite density increased on the surface of the material appearing to cover almost all the surface of the glass material. After 14 days of immersion in SBF, the surface is observed to be completely covered with apatite more than the previous days and also the appatite appears denser on the glass surface. This supports the fact that the material has the ability

to form apatite when immersed in simulated body fluids. As immersion period increased the rate of apatite formation on the glass surface increased which shows that the glass material has ability to grow apatite and is very bioactive.

Doped Glass

Figure 2 shows the microstructure of the Ag-doped bioactive glass at 0 day and after simulated body fluid for 3, 7 and 14 days respectively after immersion in the simulated body fluid. At 0 day, the surface appears to be more homogeneous with better distribution on the surface of the glass particles than the undoped material. After 3 days in SBF, the surface changed with the formation of tiny apatite balls growing on the surface of the material which appeared to cover the surface far better than the undoped material at 3 days. After 7 days the apatite changed to crystallites and colonized the surface of the material and became denser after 14 days of immersion in SBF. The increase in the apatite density could be attributed to the disruption of the silica network by the addition of the metal ion which led to the exchange of ions making the glass more soluble and thus led to formation of the apatite at an increased rate (Lin *et al.*, 2009)

Conclusion and recommendation

In this study, a bioactive glass was successfully synthesized and doped with silver in order to impart anti-microbial properties. The glasses were prepared using biowates obtained from bamboo leaves and waste chicken egg shells as cheap silica and calcium substitutes. The SEM results revealed a homogeneous surface dominated by glass particles before immersion in simulated body fluid. Further examination should be carried out to confirm the bioactivity of the bio ceramic produced.

References

Bates, P. and Ramachandran, M. (2007). Bone injury, healing and grafting. In Basic Orthopaedic Sciences. The Stanmore Guide. *Hodder Arnold*. 123-134.

Boskey, A. L. (2007). Mineralization of bones and teeth. *Elements.* 3; 385-391

Boyce, T., Edwards, J. and Scarborough, N. (1999). Allograft bone: the influence of processing on safety and performance. *Orthopedic Clinics of North America*. 30; 571—81.

Crabtree, J. H., Burchette, R. J., Siddiqi, R. A., Huen, I .T. and Handott, L. L. Fishman, A. (2003). The efficacy of silver-ion implanted catheters in reducing peritoneal dialysis-related infections. *Peritoneal Dialysis International.* 23; 368.

Essien, E. R. Atasie, V.N. and Udobang, E.U. (2016). Microwave energy-assisted formation of bioactive CaO-MgO-SiO_2 ternary glass from bio-wastes. *Bulletin Materials Science.* 39; 989-995.

Jones, S. A., Bowler, P. G., Walker, M. and Parsons, D. (2004). Controlling wound bioburden with a novel silver-containing Hydrofiber dressing, *Wound Repair and Regeneration*. 123-288.

Kokubo, T. and Takadama, H. (2006) How Useful Is SBF in Predicting in Vivo Bone Bioactivity? *Biomaterials.* 27; 2907-2915.

Lin, S., Ionescu, C., Pike, K. J., Smith, M. E. and Jones, J. R. (2009). Nanostructure evolution andcalcium distribution in sol–gel derived bioactive glass. *Journal of Material Chemistry*. 19; 1276–82.

Sepulveda, P., Jones, J. R., and Hench, L. L. (2002). In vitro dissolution of melt-derived 45S5 and sol-gel derived 58S bioactive glasses. *Journal of Biomedical Materials Research*. 61; 301–311,

Van H. A, Swiontkowski (1999). M. Bone-graft substitutes. *Lancet*. 353; 28—9.

Wenhao, W. and Kelvin, W. K. (2017). Bone grafts and biomaterials substitutes for bone defect repair: A review. *Bioactive Materials.* 2; 244-247.