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# Antibacterial activity and cytotoxicity of chemically treating Ti alloy and bioglass doped with silver

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## Introduction

Bioactive Ti alloy and bioglass having high bone bonding capacity currently become essential materials for bone repair in orthopedic and dental fields. The former is used as load bearing implant such as hip joint, while the latter is used as bone substitute. Requirement of providing antibacterial activity to these implants is arising to avoid bacterial contamination which could lead to prosthetic failure and pain for the patients.

The aim of this study is to develop antibacterial and bioactive surfaces on Ti alloy and bioglass by chemical treatment using silver and defining characterization techniques suitable to compare a wide range of antibacterial and bioactive materials.

## Experimental Methods

Ti-6Al-4V alloy plates were soaked in 5M NaOH solution, and then in a mixed solution of 50 mM CaCl<sub>2</sub> and 50 mM SrCl<sub>2</sub>. They were heat treated at 600 °C for 1 h, followed by soaked in 1M Sr(NO<sub>3</sub>)<sub>2</sub> solution doped with 1 mM AgNO<sub>3</sub>. The resultant products were denoted by "Ti64(Sr+Ag)". Other alloy plates were pre-treated with a diluted HF aimed to remove the natural titanium oxide layer, and subsequently treated in H<sub>2</sub>O<sub>2</sub> solution added with 1 mM AgNO<sub>3</sub> (denoted by "Ti64(HF-H<sub>2</sub>O<sub>2</sub>+Ag)"). Bioglass in a system of SiO<sub>2</sub>-Na<sub>2</sub>O-CaO-P<sub>2</sub>O<sub>5</sub>-B<sub>2</sub>O<sub>3</sub>-Al<sub>2</sub>O<sub>3</sub> was also prepared and soaked in 30 mM AgNO<sub>3</sub> solution to incorporate silver ions on its surface (denoted by "BG+Ag"). The sample surfaces were analyzed by FE-SEM, EDX, TEM, XPS, XRD, Raman, zeta potential measurement. Apatite formation was examined by soaking the samples in SBF [1]. Cytocompatibility of the samples for Human fetal pre-osteoblasts (hFOB) were investigated by cultivating the cells on the samples at 37 °C, 5% CO<sub>2</sub>. Antibacterial activity of samples to multi-drug resistant *Staphylococcus aureus* was evaluated according to ISO 22196 standard.

## Results and Discussion

Ti64(Sr+Ag) and Ti64(HF-H<sub>2</sub>O<sub>2</sub>+Ag) showed nano-textured surfaces with an oxide layers about 1.5 μm and 200 nm in thickness, while BG+Ag showed smooth surface. These surfaces contains 0.2, 0.3 and 0.4 % Ag, respectively. XRD and Raman revealed that Sr+Ag containing calcium titanate, anatase and rutile were produced on Ti64(Sr+Ag), while hydrogen titanate was produced on Ti64(HF-H<sub>2</sub>O<sub>2</sub>+Ag). In contrast, only a broad halo attributed to glass phase was observed on BG+Ag. It should be noted that the surface of Ti64(HF-H<sub>2</sub>O<sub>2</sub>+Ag) could be analyzed by Raman, but not by XRD, probably due to its submicron thickness. XPS and TEM revealed that silver was doped as metal nanoparticles on Ti64(HF-H<sub>2</sub>O<sub>2</sub>+Ag), while they gave no evidences of the presence of silver particles on Ti64(Sr+Ag) and BG+Ag. It is considered that silver was doped as ions on these samples. All the alloy and bioglass samples were negatively charged and formed

apatite in SBF. Zeta potential measurement revealed that kinetics of reaction in SBF occurred faster on BG+Ag than alloy samples so as to induce apatite formation within 1 day. The mechanism of apatite formation was considered to be different for these samples: it is based on chemistry of micro environment for Ti64(Sr+Ag), surface charge for Ti64(HF-H<sub>2</sub>O<sub>2</sub>+Ag), or ion exchange for BG+Ag.

In antibacterial test, all the samples exhibited high antibacterial activity to multi-drug resistant *Staphylococcus aureus*: e.g. Ti64(Sr+Ag) and BG+Ag displayed 4.8 and 4.3-logs reduction compared with untreated alloy or bioglass without silver, respectively. Cytocompatibility test revealed no significant differences between Ti64(Sr+Ag) and untreated alloy, and also between BG+Ag and bioglass without silver.

### **Conclusion**

Three types of antibacterial and bioactive materials with similar amount of Ag were prepared. The surface properties were compared by various types of characterization techniques. Ag is present as metal nanoparticles on Ti64(HF-H<sub>2</sub>O<sub>2</sub>+Ag), while seems to be as ions on other materials. The materials formed apatite in SBF based on different mechanism and kinetics, indicating their high potential for bone bonding. Antibacterial activity was proved on all the materials without any cytotoxicity. These materials are promising for next generation implant because of their high antibacterial activity, cytocompatibility and apatite formation.

### **References**

T. Kokubo, H. Takadama. *Biomaterials*, 2006, 27, 2907-2915.

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