Promising antimicrobial capability of thin film metallic glasses

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ABSTRACT

Thick film metallic glasses (TFMGs) are demonstrated to exhibit excellent surface flatness, high corrosion resistance and satisfactory hydrophobic properties. Moreover, the antimicrobial and biocompatibility properties of TFMGs are examined and the results are compared with the behavior of pure Ag and 316 L stainless steel. Three TFMGs, Al48Ag37Ti15, Zr54Ti35Si11, and Zr59Ti12Ag19, are prepared by sputtering to assess the antimicrobial performance against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa, which are the most common nosocomial infection pathogens. Experimental results show that the antimicrobial effect of the Al-, or Ag-containing AlAgTi and ZrTiAg TFMGs is similar to that of the pure Ag coating. The ZrTiSi TFMG with no Ag or Al shows poor antimicrobial capability. The physical properties of highly smooth surface and hydrophobic nature alone are not sufficient to result in promising antimicrobial ability. The chemical metal ion release still plays a major role, which should be born in mind in designing biomedical devices.

1. Introduction

The antimicrobial and biocompatibility properties of crystalline materials, such as 316 L stainless steel or Ag-based coating, have been widely investigated and applied in several fields [1]. However, the development and investigation of antimicrobial and biocompatibility properties for metallic glasses are still rare. Metallic glasses are typically hard and strong, free of grain boundaries and dislocations, exhibiting excellent surface flatness, high corrosion resistance [2–5] and satisfactory hydrophobic properties.

In comparison of the extensive research on bulk metallic glasses (BMGs), thin film metallic glasses (TFMGs) are relatively much less addressed. The antimicrobial properties of TFMGs are hardly reported in literature. TFMGs can be adopted as biological coatings for either biocompatibility [3–5], wear-resistant [6], or antimicrobial purposes [7]. By reducing the thickness dimensions to the micro-scale (about 1–3 μm) or nano-scale (about 100–300 nm), TFMG systems maintain the characteristic properties and thus become attractive in micro-electro-mechanical systems (MEMS), optical device, protective hard coating, or biomedical areas [8–13].

It is well known that Ag itself is an effective antimicrobial agent [14,15]. Silver is also found to be highly toxic to microorganisms and relatively low toxicity to human tissue [14,16,17]. However, the antimicrobial pathways of how Ag+ ions work are still not completely established. There have been some hypotheses [18]. Initially, Ag+ ions are bound to the bacterial cell wall and protein in the cell. Then, Ag+ ions would interfere with deoxyribonucleic acid (DNA) replication. Subsequently, DNA would change to a condensed form and would lose its replication abilities, leading to cell death when the Ag+ ions enter the cell. These effects have been observed in both Gram positive and Gram negative bacteria [15,18]. Besides, Ag+ ions promote formation of reactive oxygen species, which inhibit the respiration.

Silver nanoparticles have been demonstrated to be more efficient in antimicrobial effects than salts according to their extremely high surface areas, supplying better contact with microorganisms. In some previous researches, it shows that the nanoparticles preferably attack the respiratory chain, finally leading to cell division and cell death when silver nanoparticles enter the bacterial cell. These silver nanoparticles could release silver ions in the bacterial cells, enhancing the antimicrobial and bactericidal activity. Aymonier et al. [19] demonstrated that the hybrids of silver nanoparticles with amphiphilic hyperbranched macromolecules exhibited effective antimicrobial effects. Moreover, some studies reported that the antimicrobial effects of silver nanoparticles may be associated with characteristics of certain bacterial species [15,19]. Gram positive and Gram negative bacteria have differences in their membrane structure and sometimes exhibit different resistance against silver nanoparticles.

Recently, Chiang et al. [7] applied the Zr51Al5ZrNi Cu12Si4 TFMG to modify the 304 stainless steel surface, resulting in much better hydrophobic properties. Moreover, the surface antimicrobial properties for Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Acinetobacter baumannii and Candida albicans were also examined systematically. The experimental results showed that the Zr51Al5ZrNi Cu12Si4 TFMG coated on hospital equipments revealed antimicrobial effects and could reduce colonization...
and biofilm formation on the equipment surfaces. Although the detailed mechanism of antimicrobial effects for this Zr₅₄Al₇₅Ni₁₅Cu₂₇Si₄ TFMG has not been fully defined, this study suggested the potential of Zr-based TFMGs for antimicrobial applications. In this study, the antimicrobial efficiency of both Ag- and Zr-containing TFMGs is examined and discussed.

2. Experimental procedures

In order to explore the antimicrobial effect from only the coated films, biomedical-class glass, instead of 316 L stainless steel (SS), was applied as the substrate in this study. The glass is known to impose no impact on the antibacterial capability. AlAgTi, ZrTiSi and ZrTiAg thin films were selected to deposit on the glass by a multi-gun magnetron sputtering system. The heat of mixing for Ag–Al, Al–Ti, Ti–Ag, Zr–Ti, Ti–Si, and Si–Zr are −4, −30, −2, 0, −66, and −84 kJ/mol, respectively [20], favorable for forming amorphous structures. The resulting films have the compositions of Al₄Ag₈₅Ti₁₅, Zr₅₄Ti₃₅Si₁₁, and Zr₅₉Ti₂₂Ag₁₉. All in atomic percent. Pure Ag thin film was also prepared by sputtering. These uncoated glass substrates measure 40, 40, and 0.55 mm in length, width, and thickness, respectively. All the metallic targets for sputtering were 50.8 mm in diameter. The operating conditions of the sputtering system were set at a base pressure of 5 × 10⁻⁷ torr, a working pressure of 3 × 10⁻³ torr, and an Ar flow rate of 25 standard cubic centimeters per minute (sccm). The working distance from the holder to the sputtering guns was set to be 120 mm and the substrate adhered to the holder was rotated with an average speed of 15 rpm to obtain the uniform thickness of thin films during the deposition. The final film thickness was about 500 nm.

The structure of the as-deposited thin films was characterized by Bruker D8 Advance X-ray diffractometer (XRD). A JEOL ISM-6330 field-emission scanning electron microscope (SEM), equipped with energy dispersive spectrometer (EDS), was used to examine the surface morphology and film composition. The thicknesses of the as-sputtered films were measured by a 3D alpha-step profilometer. The surface roughness of coated and uncoated specimens was measured by a Digital Instrument NanoMan NS4 + D3100 atomic force microscope (AFM). Contact angles were measured by using the sessile drop method with a Dataphysics OCA-20 contact angle analyzer. The contact angle of each sample was measured three to five points using distilled water or ethylene glycol. The Nikon TS100 inverted research microscope was used to observe the cellular growth state in each well. The concentrations are determined at time zero which contain minor nanocrystalline phases. The fully crystalline Ag–Si, and Si–Zr are 30, 30, and 3.3 nm, as compared in Table 1. The fully amorphous ZrTiSi, ZrTiAg, and pure Ag films exhibit much rougher surfaces.

3. Results and discussion

The XRD scans of the AlAgTi, ZrTiSi, ZrTiAg, and pure Ag films are shown in Fig. 2. There is no obvious crystalline peak for the former three films; broad diffused peaks can be observed in these XRD patterns, characteristic of the amorphous atomic structure. But the diffused humps for the AlAgTi and ZrTiAg films are slightly sharper than that of ZrTiSi, suggesting that there might be minor nanocrystalline phases in the AlAgTi and ZrTiAg films. Furthermore, the compositions of sputtered the AlAgTi, ZrTiSi, and ZrTiAg thin films were identified as Al₄Ag₈₅Ti₁₅, Zr₅₄Ti₃₅Si₁₁, and Zr₅₉Ti₂₂Ag₁₉ (all in atomic percent), respectively.

The roughness and morphology of uncoated substrates and coated specimens were examined by AFM and SEM, as some samples compared in Fig. 3. The average roughness readouts Rₐ of the glass, AlAgTi, ZrTiSi, ZrTiAg, and pure Ag films were measured to be 0.3 nm, 0.7, 0.6, and 8.3 nm, as compared in Table 1. The fully amorphous ZrTiSi films appear to be slightly flatter than the AlAgTi and ZrTiAg films which contain minor nanocrystalline phases. The fully crystalline Ag films exhibit much rougher surfaces.

The contact angles of glass, AlAgTi, ZrTiSi, ZrTiAg, and pure Ag films in contact with water and ethylene glycol C₂H₄O₂ are demonstrated in Table 1. Obviously, the film coating can effectively result in hydrophobicity. The water contact angle increases from 26° for the glass up to
- 87°–105° with metallic thin film coatings, indicating that both Ag and TFMGs can effectively decrease the wettability of the surface. The Zr/TiAg TFMG shows the very promising contact angle. The hydrophobicity associated with TFMGs can be explained by the Cassie–Baxter theory [21]. In this study, the columnar structure of the thin films is similar to nanowires. The droplet is not only in contact with the solid surface but also in contact with the air which is trapped between droplet and cavities of columnar structure. The droplet is mainly supported by the air and a part of solid surface. Therefore, TFMGs are beneficial to improve the hydrophobic ability for biomedical devices.

In the beginning, the strains were examined by Baird Parker agar, CHROM agar, ECC agar, and Cetrimide agar. These selective agar plates may be used for identification of strains. The strains were cultured on the specimens for 24 h. All experiments were repeated in triplicate. Table 2 displays the colony-forming units (CFU) and antibacterial efficacy (AE) under various conditions. We first compare the performance of the glass, Ag, AlAgTi and ZrTiSi. For *P. aeruginosa*, the CFU number of the Ag-coated samples is ~3, while that of the AlAgTi TFMG is even lower to a level ~1, both AE values reaching above 99.999% kills against *P. aeruginosa*. But the ZrTiSi TFMG shows poor antimicrobial effect (only 9.302% kill), similar to the glass substrate. For *E. coli*, the antimicrobial effect of AlAgTi TFMGs is nearly the same as that of pure Ag, both showing greater than 99.999% kills against *E. coli*. Again, ZrTiSi TFMG does not provide any antimicrobial ability against *E. coli*. For *S. aureus*, CFU for pure Ag is ~1, but for AlAgTi TFMG is 1.3 × 10², meaning that Ag can reach greater than 99.999% kills and AlAgTi TFMG to 99.928% against *S. aureus*. The ZrTiSi TFMG result in only 15.230% kills, much lower than AlAgTi and pure Ag films, but better than the glass substrate. Overall, TFMGs exhibit better antimicrobial capability than glass, and the AlAgTi TFMG is highly effective, comparable to the pure Ag. But the ZrTiSi TFMG behaves much worse.

In this study, Ra for AlAgTi (0.7 nm) and ZrTiSi (0.6 nm) is not distinctly different. Thus the physical factor such as the surface roughness should not be the dominant and determining factor for the TFMG.
antimicrobial issue. Therefore, the chemical factor may be the most important aspect. Since it is well documented that Zr and Ti would not cause pronounced antimicrobial effects, we prepared the Zr59Ti22Ag19 TFMG, with the similar film thickness, amorphous nature, surface roughness and contact angles as the AlAgTi and ZrTiSi TFMGs, to explore the chemical effect solely from the content of Ag. In Table 2, it can be seen that the viable bacterial counts against P. aeruginosa, E. coli and S. aureus are all very low, AE readings are all greater than 99.999%, as promising as (or even better than) pure Ag and the AlAgTi TFMG, and much better than the ZrTiSi TFMG. It is thus demonstrated that even with the similar physical properties such as the amorphous atomic packing and surface roughness for these three TFMGs, the chemical content of, for example, Ag, would result in the difference in this study. From previous studies on the antimicrobial effect of Al [22], it is expected that the ZrTiAl TFMG containing Ag might also exhibit the similar performance as ZrTiAg.

The metal ion released by TFMG may be the deterministic factor for the chemical antimicrobial effect, therefore, we measure the ion released by TFMG. Ion released were measured by ICP-MS on the thin films exposed in nutrient broth for antimicrobial testing or the medium for biocompatibility MTS assay, both for 24 h. Table 3 displays that the concentrations of metal ions released from the thin films in the solutions. It can be found that the total amounts of released metal ions from the Al- and Ag-containing Al48Ag37Ti15 and Zr59Ti22Ag19 TFMGs are much higher than those from the Zr59Ti12Si11 TFMG. Al ions appear to be released to the highest amount in both the nutrient broth for antimicrobial testing (releasing 1.69 ppm) and the medium for MTS testing (releasing 8.27 ppm). The main reason of this result may be associated with the structure of Al48Ag37Ti15 TFMG, which shows the sharper XRD curve in Fig. 3 suggesting not fully amorphous with some minor nanocrystals. Consequently, the preferential corrosion would more readily occur at the phase interfaces. The other reason may be associated with the oxidized potential difference between Al, Ti, Zr and Ag. The oxidized potential of Al (+1.66), Ti (+1.37), and Zr (+1.55) is high than the oxidized potential of Ag (−0.8). The redox reaction was induced by potential difference. Therefore, Al, Ti, and Zr ions release would be inherently more significant from the TFMGs because the oxidation reaction of Al, Ti, and Zr occurred easily.

Note that in Table 3 that even with the very minor Ag ion releases from the ZrTiAg TFMG (of only 0.17 to 0.17 ppm), the antimicrobial CFU and AE readings are already very satisfactory, revealing the strong antimicrobial capability. The AE readings for the three bacterial are consistently greater than 99.99%; the overall antimicrobial performance of ZrTiAg TFMG, Ag and AlAgTi TFMG are nearly same in anti-Gram negative bacteria, for example, P. aeruginosa. Nevertheless, the very minor Ag ion releases from ZrTiAg TFMG (0.17 to 0.17 ppm Ag ions) result in stronger antibacterial capability than the much higher ion releases from the AlAgTi TFMG (1.69 to 8.27 ppm Al ions). This also implies that the Ag ions impose much more higher anti-bacteria ability than the Al ions, especially in anti-Gram positive bacteria, S. aureus. Besides, AlAgTi TFMG had less Ag ion released than ZrTiAg TFMG. The better anti-S. aureus character of ZrTiAg TFMG may come from Zr ion release or more Ag ion release.

The cytotoxicity testing by MTS array for stem cells is a rapid, standardized and sensitive method to determine whether a material contains significant quantities of biologically harmful extraction or not. Fig. 4 shows the cell viability results of coated glass compared with the reference 316 L SS (as a reference level of 100%). It can be observed that the cell viability of glass deposited with the ZrTiSi TFMG (~90%) is compatible to (but slightly lower than) that of 316 L SS. In previous research [23], it is well known that Ti has higher biocompatibility than 316 L SS. The possible reason of the current unusual result is that the surface roughness of the ZrTiSi TFMG (~0.6 nm) is too smooth for cells to adhere. Nevertheless, the current result still demonstrates that the ZrTiSi TFMG possesses high stem cell biocompatibility, similar to 316 L SS. Moreover, it can be observed that the cell viability of glass deposited with AlAgTi (near 70%) and ZrTiAg (near 75%) is compatible to the pure Ag thin film (also near 75%). The images of pluripotent mesenchymal stem cells on the culture dish with specimens show the morphology of cellular growth state, as shown in Fig. 5a and b for the 316 L SS and ZrTiSi TFMG reveal that the cells are wider, having expanded more and attaching better than the behavior shown in Fig. 5c and d for Ag and AlAgTi TFMG. Besides, it can also be observed that there are some white dots in Fig. 5c, d and e. The white dots are dead cells or detached cells. The results of microscope images correspond well with the results of cell viability.

### 4. Conclusion

The physical and chemical aspects of the Al48Ag37Ti15, Zr59Ti22Si11, and Zr59Ti12Ag19 TGMGs in terms of their antimicrobial capability against P. aeruginosa, E. coli and S. aureus and cytotoxicity with respect...
to stem cells are examined and compared with the behavior of pure Ag or 316 L SS. The following conclusions can be drawn.

1. The amorphous degree of these three TFMGs is found to best for ZrTiSi, followed by ZrTiAg, and last for AlAgTi. Minor nanocrystalline phases are present in the latter TFMG.

2. Due to no obvious grain boundaries and preferred orientation of the columnar growth of the sputtered films, the surfaces of the AlAgTi, ZrTiSi and ZrTiAg TFMGs are all extremely smooth. The average roughness Ra of the TFMGs is approximately 0.6–0.7 nm.

3. The water contact angles of all three TFMGs are around 70–100°, with similar hydrophobic nature as the pure Ag coating possesses.

4. Even with similar or compatible physical properties such as the amorphous atomic packing and surface roughness for these three TFMGs, the chemical content of, for example, Ag, would result in the difference in antimicrobial capability and biocompatibility behavior. The AlAgTi and ZrTiAg TFMGs containing Al or Ag exhibit promising antimicrobial effects, while ZrTiSi shows very poor antibacterial ability which may be due to low Zr ion released. It is suggested that metal ion release still plays a major role on antimicrobial activity. The best smooth surface alone for the ZrTiSi TFMG is not sufficient to result in promising antimicrobial ability.

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References


Fig. 5. The images of Inverted Research Microscope for pluripotent mesenchymal stem cells on the culture dish with (a) 316L SS, (b) Zr53Ti18Si11 TFMG, (c) pure Ag, (d) Al48Ag37Ti15 TFMG, and (e) Zr59Ti22Ag19 TFMG.