Exploring the Antimicrobial Potential of Heavy Metal Containing Zr-based Bulk Metallic Glasses

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Statement of Purpose: Bulk metallic glasses (BMGs) are a revolutionary group of alloys that have attracted tremendous research investment recently. Owing to the unique amorphous structure, BMGs exhibit a combination of excellent mechanical properties, high corrosion resistance, and facile thermal plastic formation, which are favorable for biomedical applications [1]. We have demonstrated that BMG are safe through in vitro cell culture assays [2, 3]. Additionally, many many biocompatible BMGs may possess potential antibacterial property, due to the presence of heavy metal elements (i.e., Cu, Ag, etc.). Prevention of bacterial infections in healthcare facilities and during surgical procedures is of great scientific and economic significance. However, reports on the capability of BMGs in this aspect are scarce. Our current research is directed to fill the knowledge gap regarding the antimicrobial potential of BMGs, to contribute to the further understanding of the fundamental properties of BMGs as well as induct new members to the antimicrobial biomaterial family.

Methods: The $(Zr_{55}Al_{10}Ni_5Cu_{30})_{1-x}Y_x$ (x = 0 or 0.01) and $Zr_{53}Al_{16}(Co_{0.75}Ag_{0.25})_{31}$ (at.%) BMGs were fabricated by arc melting and copper mold casting in a high purity argon atmosphere. Pure Cu (99.95 wt.%) or Ag (99.9 wt.%) plates were used as positive controls, while traditional biomedical alloys, Ti-6Al-4V and 316L stainless steels, were employed as negative controls. The antibacterial effect of the materials was investigated using the Gram positive bacterium *Staphylococcus aureus* (*S. aureus*, ATCC 6538) via moist contact assay. Briefly, the overnight culture of *S. aureus* was diluted in tryptic soy broth supplemented with 1 % of glucose to an optical density of 0.01, and allowed to grow for 4 h at 37 ^{o}C to a mid-log phase. Each alloy substrate was inoculated with bacterial suspension and statically incubated for 4 h at room temperature. The number of viable adherent cells was determined by serial dilution of the detached biofilm and the spread plate method. The amounts of heavy metal ions released from the testing specimens were monitored using inductively-coupled plasma optical emission spectrometry as a function of immersion time to produce an ion release profile.

Results: The antimicrobial effects against *S. aureus* of both Cu- and Ag-bearing Zr-based BMGs can be observed after the 4 h moist contact assays, which is evidenced by the significant reduction in cell viability on the BMG samples, as compared with that on the positive controls, as shown in Figure 1. The killing effect of the BMGs is related to the type and concentration of the constituent heavy metal elements. Minor additions of Ag (< 10 at.%) to the Zr-based BMG can lead to a more effective killing to *S. aureus* than the ones containing high Cu concentrations (~30 at.%). The biocidal effects of heavy metal elements are usually attributed to their ion forms, as illustrated in Figure 2. To better understand the underlying antimicrobial mechanisms of heavy metal containing BMGs, the release of heavy metal ions from the BMG samples was quantified and compared with that from the positive controls. It is found that the killing efficacy corresponded to the concentration of Cu or Ag ions dissociated from the BMG substrates.

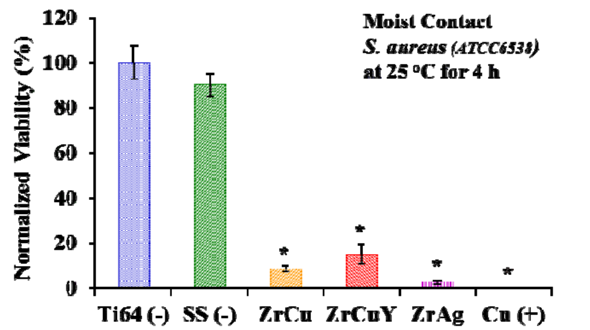

Figure 1. Viability of adherent bacterial cells after moist contact assay on Ti-6Al-4V (Ti64), 316L stainless steel (SS), Zr-Al-Ni-Cu BMG (ZrCu), Zr-Al-Ni-Cu-Y BMG (ZrCuY), Zr-Al-Co-Ag BMG (ZrAg), and pure Cu. Results were normalized to that obtained on Ti-6Al-4V (Mean ± SEM, * $p < 0.05$).

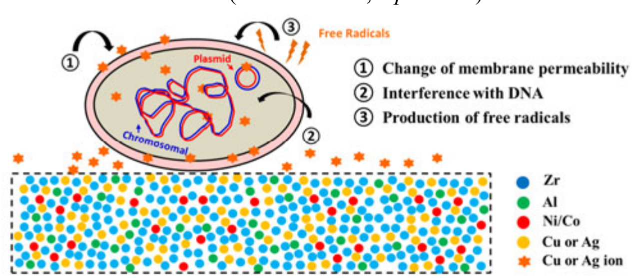

Figure 2. Schematic illustration of the possible antimicrobial mechanisms of heavy metal containing Zr-based BMGs.

Conclusions: Heavy metal containing Zr-based BMGs can kill *S. aureus*. The killing mechanism of the Cu- or Ag-bearing Zr-based BMGs is attributed to the release of metal ions. Other mechanisms involving the activities of free radical or dry metal surface may also contribute to the biocidal effect. This study presents a first effort to investigate antimicrobial biomedical BMGs, which can be useful for the future design of BMGs with enhanced killing efficacy.

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