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Antibacterial effect of metallic glasses

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The antibacterial effect of Fe-based, Ni-based and Cu-based metallic glasses has been studied in this research. All test metallic glasses were found to inhibit growth of *Escherichia coli*. The optical density value of *E. coli* flocculation exposed to metallic glasses, from which the number of bacteria was determined, was 65% lower than that of the match group, and 57% lower than that of the ferrite group. Moreover, the antibacterial effect was not significantly different (σ=0.05) between Cu-based, Ni-based and Fe-based metallic glasses. These results extend our understanding of the antibacterial effect of metallic antibacterial materials, and suggest new applications for metallic glasses.

metallic glasses, antibacterial, *E. coli*

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Traditional antibacterial agents may be of natural biological, organic or inorganic form. Chitosan is one of the main natural biological antibacterial agents. It is the partial deacetylation product of chitin, which is the main structural component of invertebrate exoskeleton. Besides bacteriostatic action, this natural material has been utilized widely for a long time, in tasks such as soil improvement, hemostasis, enhancing healing. However, the commercialization of chitosan is hindered by limitations in current manufacturing techniques. Organic antibacterial agents are used extensively for sterilization, antisepsis and mildew proofing. Although efficient, broad-spectrum and durable, they are subject to poor heat-resistance, easy hydrolysis and biological safety doubts [1].

Among the inorganic antibacterial agents, metals and photocatalysts are most commonly used. Most metallic ions exhibit antimicrobial effect. For efficiency and safety reasons, silver, copper and zinc are the most widely available metallic antibacterial agents. Previous studies have referred to these metals as component or additive, since they can be combined with other materials to construct antibacterial materials [2,3].

Photocatalysts such as titanium oxide (TiO_2) reportedly inactivate bacteria, molds, viruses, and even cancer cells [4–8]. Since the post-separation of the powder catalyst in a slurry system is a major impediment in photocatalyst production [9,10], immobilized TiO_2 with high surface area (e.g., porous TiO_2 thin film) is favored for antimicrobial applications [2–4,11–14].

Nano materials are currently gaining favor as antibacterial materials. These are generally composites with nano antibacterial coating or containing nano antibacterial compounds. They have increased surface area and improved efficiency over conventional materials [15,16]. However, having entered the human body, the nano material indiscriminately destroys all cells, including human cells and symbiotic bacteria. Moreover, nano materials can easily penetrate the cell wall, and can pass the blood-brain barrier. The safety of nano antibacterial materials has yet to be demonstrated [17–19].

Metallic glasses such as ferrite core have already found use in industry [20–22]. However, apart from electronics, metallic glasses are rarely employed as functional materials, in particular, as antibacterial materials. In this paper, the

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antibacterial effect of Fe-based, Cu-based and Ni-based metallic glasses is presented and analyzed.

1 Materials and methods

1.1 Bacterial strain and flocculation

The bacterial strain (*E. coli* KNabc) was offered by Prof. Terry A. Krulwich (Department of Biochemistry, Mount Sinai School of Medicine of the City University, New York, USA). A single colony from an agar plate was inoculated into 5 mL potassium-modified Luria broth (LBK) and grown for 12 h in a shaker at 180 r/min at 37°C until the cultures reached mid-exponential phase. Mid-exponential phase cultures were stored at 4°C.

1.2 Metallic glasses

Excepting Fe-based metallic glasses $Fe_{78}Si_9B_{13}$, which were obtained from the Advanced Technology & Materials Co. (Beijing, China), all metallic glasses used in this work were produced by melting-spinning. A list of the materials tested is provided in Table 1. The metallic ribbons were cut into sections of length not exceeding the liquid level. The ribbon samples should not expose to the air. Each material was tested in triplicate, with mass of all 3 test samples maintained equal. Materials were sterilized under ultraviolet light for 30 min prior to experiment. An iron nail was used as a control sample.

1.3 Bacterial culture medium

The LBK nutrient broth (1% tryptone, 0.5% yeast powder, 87 mmol/L KCl) was prepared by dissolving 10 g tryptone, 5 g yeast powder and 6.48 g KCl fully in deionized water. The pH was adjusted to 7.0–7.2 with NaOH, water was added to a volume of 1 L and the solution sterilized by autoclaving at 121°C for 20 min. The medium was stored at 4°C for future use.

1.4 Antibacterial test

Prior to microbiological experimentation, all glass wares were sterilized by autoclaving at 127°C for 15 min.

(i) Shaking culture. The precursor flocculation of *E. coli* was diluted by a factor of 20 with LBK to yield the diluted *E. coli* flocculation. The 5 mL aliquots of the diluted flocculation were dispensed into test tubes, to which were then added the test metallic glasses. Test tubes were incubated in a shaker for 8 h at 180 r/min, 37°C. A tube without metallic glasses was used as the background in test. The number of bacteria in the sample was determined turbidimetrically by measuring optical density (A) at 600 nm.

(ii) Inhibition zone. A substrate was prepared by adding 20 g/L agar to LBK and was sterilized by autoclaving at 121°C for 20 min. After this mixture had been cooled to 50°C, 20±5 mL was poured into each petri dish and allowed to set. The 250 μL precursor flocculation of *E. coli* has smeared on the surface of the culture. For each metallic glass, 3 samples per dish were prepared. Zone of inhibition around the test sample (measured in mm) provides an assay of the antimicrobial activity of the specified metallic glass.

Table 1 Metallic glasses tested for antimicrobial activity

Code name	Contents
Cu1	$Cu_{64}Hf_{36}$
Cu2 [a)]	$Cu_{64}Hf_{36}$
Cu3	$Cu_{62}Hf_{38}$
Ni1	$Ni_{66}Hf_{34}$
Ni2	$Ni_{64}Hf_{36}$
Fe1	$Fe_{78}Si_9B_{13}$

a) Unannealed.

2 Results

The growth of shaking culture is shown in Figure 1. The A_{600} value of the control sample (iron nail) was 81.2% that of the blank control (CK). For test tubes containing metallic glasses, A_{600} was less than 60% that of CK. Apart from $Ni_{66}Hf_{34}$(Ni1), there was no significant difference ($\sigma = 0.05$) between the tested metallic glasses.

Before addition of metallic glasses, the 5% (v/v) diluted flocculation was cultured at 37°C by shaking at 180 r/min for 8 h. The optical densities of the resulting cultures are shown in Figure 2. The antibacterial effect of metallic glasses was greatly reduced or eliminated after prolonging the incubation period. Compared to Figure 1, the A_{600} value of tubes containing metallic glasses exhibited a dramatic rise, up to 107.15% relative to CK. Optical densities of cultures exposed to iron, however, did not alter appreciably under prolonged incubation ($\sigma = 0.01$).

The agar culture was used to measure diffusion of the metallic glasses in water. Figure 3 illustrates the radius of inhibition zone produced by different metallic glasses and

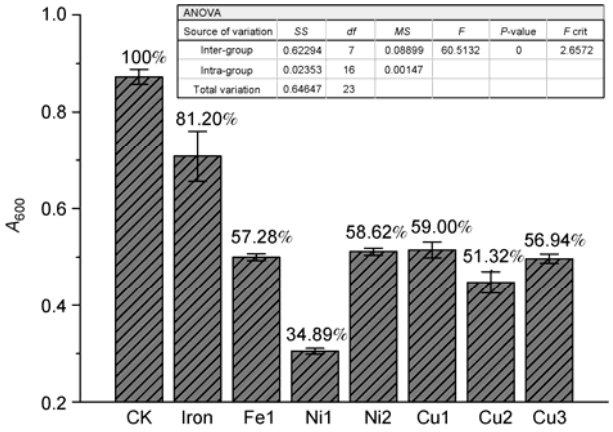

Figure 1 Optical densities of *E. coli* cultures exposed to different metallic glasses after shaking for 8 h.

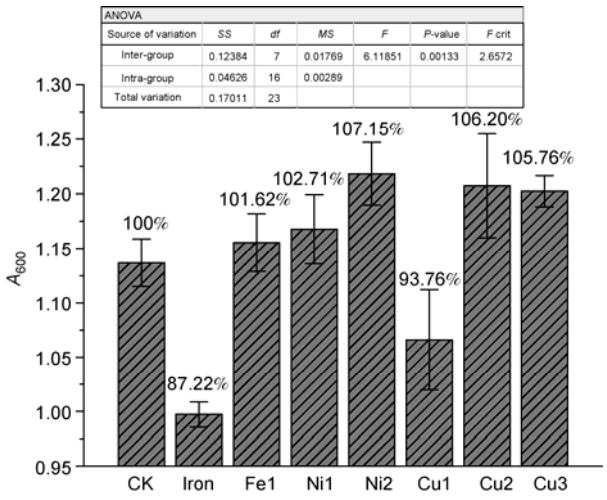

Figure 2 Optical densities of diluted *E. coli* colonies exposed to different metallic glasses (5% (v/v) dilution flocculation; cultivation prolonged by 8 h following addition of the glass).

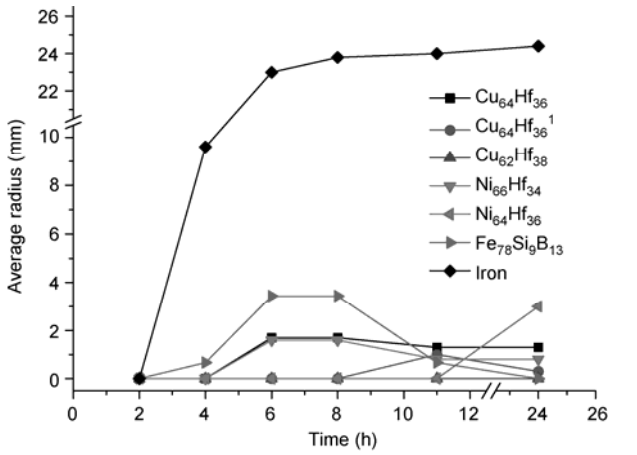

Figure 3 The radius of inhibition zone for different metallic glasses (with iron as the control) versus time.

iron as a function of time. For iron, the radius of inhibition expanded to 24 mm during the first 8 h and was thereafter stable, whereas the radius of metallic glasses reached negligible 4 mm at most. As time increased, the radius of metallic glasses fluctuated around some low value, frequently becoming lost.

The Fe-based metallic glasses $Fe_{78}Si_9B_{13}$ (Fe1) display the highest inhibition radius among the test metallic glasses. The inhibition zone of this material was enlarged at first and then narrowed. Ignoring the growing bacterial colony, two changing zones emerged; that appearing on the surface center of Fe1 enlarged constantly and almost covered the entire Fe1 surface after 24 h (see Figure 4).

Another zone (the inhibition zone) appeared around the Fe1. This zone exhibited the largest radius of the test metallic glasses and also faded the fastest. Interestingly, a clear white boundary was visible, created by the high density of *E. coli* within the colony. As time increased and the inhibition zone waned, the white boundary synchronously faded. After 24 h (see Figure 3), this boundary had disappeared.

3 Discussion

The effect of metallic glasses depends markedly on the concentration of flocculation in bacterial cultures incubated with shaking. The A_{600} value of tube containing $Ni_{66}Hf_{34}$ (Ni1) was 34.89% that of CK at normal culture, but up to 102.71% that of CK after prolonged incubation. The Ni1 appeared to lose its effect when the number of *E. coli* increased to high levels.

Tang et al. [17] have reported a similar phenomenon in bacterial cultures exposed to C_{60}. They reported a decline in *E. coli* growth after addition of C_{60} and a return to normal growth rate after several hours, at C_{60} concentrations of 20 and 40 mg/L. However, most of our cultures exposed to test metallic glasses have higher A_{600} values than that of CK (Figures 1 and 2). The possibility of bacterial growth as a cause of the phenomenon has been ruled out. Turbidimetric measurements are easily disrupted by suspended particles. Thus, the increased A_{600} value relative to CK might be at least partly caused by reaction products of metallic glasses.

In this experiment, the dosage of metallic glasses was maintained constant, but bacterial growth increased. By contrast, Tang et al. [17] increased metallic glass dosage in proportion to bacterial growth, and obtained the same results for metallic glasses as for C_{60}. Therefore, we suggest that metallic glasses are inhibitors rather than eliminators of bacterial growth.

Chitosan can prevent implantation of *E. coli*, cells by destroying the bacterial biofilm which protects them from being washed away [14]. The metallic glasses might be capable of the same action within their inhibition zones. The free bacteria could then implant only following departure from the inhibition zone. Within the region of the metallic glasses and their imposed inhibition zones, bacteria are free to

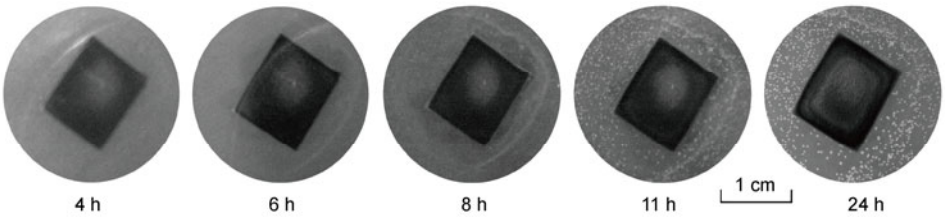

Figure 4 The $Fe_{78}Si_9B_{13}$ metallic glass and its inhibition zone at different time.

move but cannot attach. This hypothesis can account for the observed inhibition zone boundaries.

In former studies, inorganic antibacterial materials have been shown to destroy bacteria via atom and ion contact. The diffusion of metallic atoms or ions obeys Fick's law, given by

$$J = -D\nabla\phi, \quad (1)$$

where J is the amount of a material passing through a unit area perpendicular to the motion of the material, per unit second, D is the diffusion coefficient, and $\nabla\phi$ is the concentration gradient of the metallic atoms or ions.

Metallic antibacterial effect was found to increase in the order Hg>Ag>Cd>Zn>Ni. However, the Fe-based and Ni-based metallic glasses exhibit the same effect as the Cu-based metallic glasses. With the exception of $Cu_{64}Hf_{36}$ (Cu1) and $Ni_{66}Hf_{34}$ (Ni1), which demonstrate the abnormal behavior apparent in Figures 1 and 2, the antibacterial effect of metallic glasses is relatively consistent. In other words, the antibacterial effect of metallic glasses depends little on their metal constituents. The abnormal results of Cu1 and Ni1 can be attributed to human factors. The results for Cu2 do not exhibit the abnormal behavior of Cu1, despite Cu1, and Cu2 sharing a metal constituent.

Considering that diffusion of metal ions within metallic glasses is expected to differ between the test samples, it is interesting that the antibacterial effect of metallic glasses is independent of metallic atom or ion. This suggests that the antibacterial ability of metallic glasses arises from its non-crystalline structure.

4 Conclusion

Growth of *E. coli* was inhibited by metallic glasses, via prevention of bacterial adherence to the substrate surface. This kind of soft antibacterial action, which is similar to that of chitosan, is possibly invalidated by high bacterial density.

The antibacterial property of metallic glasses probably originates from their non-crystalline structure. In our study, the antibacterial effects of Fe-based, Ni-based and Cu-based metallic glasses were approximately the same. Metallic ions were found to play an insignificant role, and the difference in metallic constituents induced no effect on antibacterial properties, when metals were incorporated into metal/glass alloys.

These results extend our understanding of the antibacterial effect of metallic antibacterial materials, and offer potential for further application of metallic glasses.

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