

Research Article

Next Generation Antibacterial Hydroxyapatite Coating: Antibacterial Activity of Ag Ions in Serum

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Abstract We developed a novel thermal spraying technology for a silver-containing hydroxyapatite (Ag-HA) coating with antibacterial activity to reduce the incidence of implant-associated infections. In this study, we determined the concentration of Ag ions that show antibacterial activity in fetal bovine serum (FBS) and antibacterial activity of the Ag-HA coating in FBS. The minimum inhibitory concentration (MIC) of Ag ions for several bacteria in FBS was in the range of 4.0–7.9 ppm. When 10 ppm Ag were added (as AgNO₃ solution), 0.01 ppm of free Ag ions was detected. As the MIC of Ag ions approached the concentration that enabled formation of free Ag ions in FBS, the antibacterial activity of added Ag can be attributed to the free Ag ions. The Ag-HA coating showed strong antibacterial activity in FBS as well; the Ag concentration in FBS was 26 ppm for the antibacterial test of the Ag-HA coating. Because the Ag-HA coating can release sufficient free Ag ions in FBS, we observed that the Ag-HA coating shows a strong antibacterial effect in the biological medium studied.

Keywords hydroxyapatite; antibacterial; silver ions; serum; thermal spray

1 Introduction

Bacterial infection related to orthopaedic implants is currently a significant complication. We assumed that one of the ways to reduce the incidence of implant-associated infections is to introduce antibacterial activity into the surface of the implant. We have developed a novel thermal spraying technology for forming silver-containing hydroxyapatite (Ag-HA) coatings that have antibacterial activity, and we have evaluated the physical and chemical properties of the coating, the release rate of Ag ions from the coating, and other properties [8]. Although the Ag-HA coating releases

Ag ions slowly, these ions are known to combine with proteins [9]. It is also well known that the antibacterial activity of Ag is caused by free Ag ions [2]. However, Schierholz et al. have raised the concern that free Ag ions precipitate in albumin-containing environments, leading to concentrations too low to achieve bactericidal effects [9]. In this study, we selected fetal bovine serum (FBS) as the biological medium because FBS resembles body fluid to a great extent. We determined the concentration of Ag ions needed to introduce antibacterial activity into FBS; we also evaluated the antibacterial activity of the Ag-HA coating in FBS.

2 Materials and methods

2.1 Minimum inhibitory concentration (MIC) measurement

First, we measured the MIC of Ag ions for several bacteria in FBS to evaluate the antibacterial activity of Ag ions under biological conditions. A series of concentrations of AgNO₃ in FBS were used, and the MIC that inhibited bacterial growth was determined as the MIC for that bacteria type.

2.2 Free Ag ions determination

To confirm the existence of free Ag ions under biological conditions, the concentration of free Ag ions in FBS was determined. A given volume of AgNO₃ solution was added to FBS, and after removing serum proteins that had combined with Ag ions using ultrafiltration, the concentration of Ag ions was measured by Inductively Coupled Plasma-Mass Spectrometry (ICP-MASS).

2.3 Antibacterial test and concentration of Ag ions

The specimens of Ag-HA coating were prepared by flame spraying of HA containing 3 wt% Ag₂O onto the surface

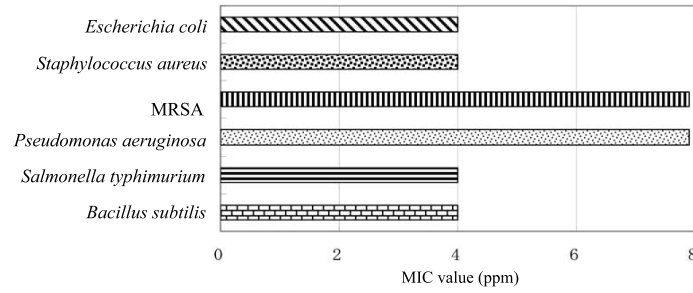


Figure 1: MIC values of Ag ions for several bacterial types.

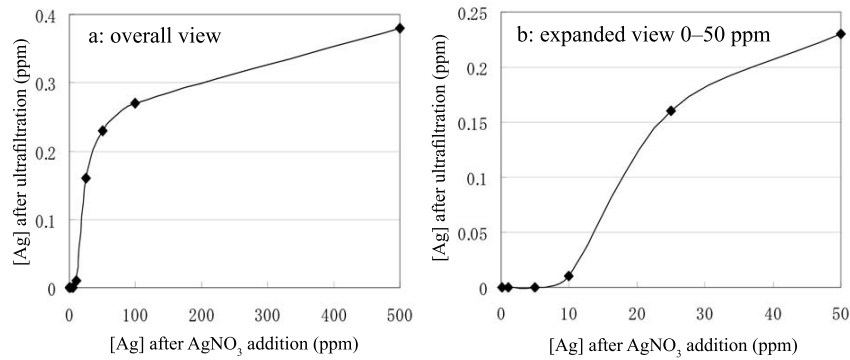


Figure 2: Release of free Ag ions in FBS. [Ag]: Ag concentration.

of CP-Ti discs [8]. The antibacterial tests of the Ag-HA coating referring to ISO 22196 [6] were performed with FBS as the medium. Specimens were incubated with bacteria for 24 hours at 37 °C, and then the number of viable bacteria was counted. Antibacterial activity was calculated using the following formula:

$$R = [\log(B/A) - \log(C/A)] = [\log(B/C)],$$

where R represents antibacterial activity, A is the average number of viable bacteria immediately after inoculation on the control specimen, B is the average number of viable bacteria on the control specimen after 24 hours, and C is the average number of viable bacteria on the antibacterial specimen after 24 hours. The concentration of Ag ions in FBS was measured using ICP-MASS.

3 Results

As shown in Figure 1, the MIC values of Ag ions were 4.0–7.9 ppm (calculated as Ag) for *Escherichia coli*, *Staphylococcus aureus*, MRSA, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Bacillus subtilis*. Figure 2 (a: overall view, b: expanded view around 0–50 ppm) shows the release of free Ag ions in FBS after AgNO₃ addition. When 10 ppm (as Ag) of AgNO₃ solution were added, 0.01 ppm of free Ag ions was detected. The more AgNO₃ solution added, the more free Ag ions were produced. The

results of the antibacterial test of Ag-HA coating in FBS are shown in Figure 3. The numbers of viable bacteria of each type on the Ag-HA coating were substantially decreased compared with that on the control (HA coating without Ag). Because R (the value of antibacterial activity) of the Ag-HA coating was much greater than 2.0, we showed that the Ag-HA coating had strong antibacterial activity in FBS. The Ag concentration in FBS in the antibacterial test was 26 ppm.

4 Discussion and conclusions

Antibacterial coatings on the surface of implants that provide antibacterial activity to the implants themselves have been studied as a possible way to prevent surgical site infections associated with implants [1,4,5,7]. In this study, we focused on Ag, which is widely used for antibacterial treatment in over-the-counter household medicines. Bacteria are considerably susceptible to Ag ions; bactericidal activity has been reported at Ag concentrations as low as 35 ppb [3]. However, the question remains whether Ag ions can show antibacterial activity in biological media: they may lose their antibacterial activity because they are known to combine with serum proteins through SH- and NH-residues [2]. The FBS used in this study contains many serum proteins; we found that free Ag ions could exist even in FBS. In addition, as the MIC values of Ag ions in FBS approached the concentration that enables release of free Ag ions, the antibacterial

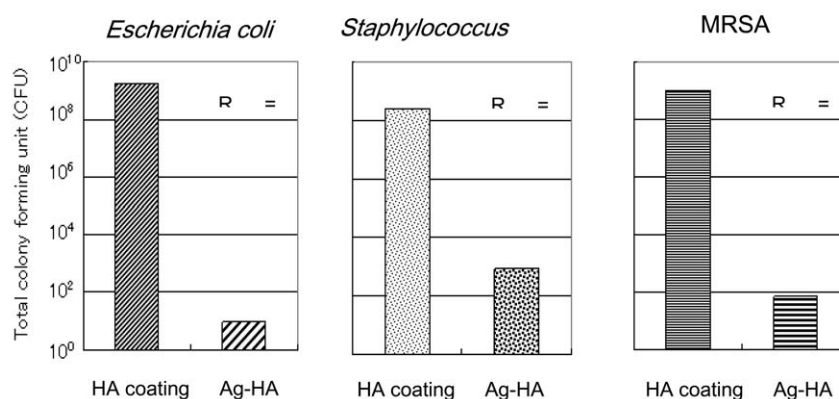


Figure 3: Results of the antibacterial test.

activity of Ag might be attributable to free Ag ions even in FBS. Therefore, we assumed that free Ag ions can exist and function as antibacterial agents under the biological conditions studied.

Similarly, the Ag-HA coatings showed strong antibacterial activity for several bacteria, including MRSA, in FBS. Therefore, we assumed that because the Ag-HA coating can release a sufficient amount of Ag ion to generate free Ag ions in FBS, the numbers of viable bacteria were greatly decreased on the Ag-HA coating. It has been shown that a high concentration of antibacterial agent at the bone-implant interface is essential to prevent bacterial infections [10]. Therefore, we expect the Ag-HA coating to show strong antibacterial behavior on the surface of cementless implants. It goes without saying that a comprehensive study of the toxic effect of a high concentration of Ag ions in a local area would be necessary in the future.

Our coating technology is based on the thermal spraying technique that has been widely used for medical and dental implants over the last two decades. This technology is promised as the next generation antibacterial HA coating for orthopaedic and dental implants.

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