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Antibacterial Ability of Alloy Materials Used in Surface Coating Techniques with Ag Films

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In response to increased attention to oral hygiene, materials of artificial dental implants and denture frameworks having antibacterial effects are increasingly valued. According to our literature review, most of the bacteria in the oral cavity are *Streptococcus mutans*, which causes tooth cavities. Therefore, in this paper, we discuss suitable materials for preventing the growth of *Streptococcus* on denture frameworks. We performed experiments to determine the antibacterial effects of different materials. In this experiment, the international standard method was used to detect the *S. mutans* culture, and the antibacterial method used solid and liquid media. The problem of the failure of the *S. mutans* culture was discussed. We used a 3D printing machine to manufacture Ti6Al4V alloys and a coating machine to deposit Ag films on Ti6Al4V alloy substrates (Ag/Ti6Al4V alloys), and we compared the antibacterial effects of Ti6Al4V, Co–Cr–Mo, and Ag/Ti6Al4V alloys with different Ag film thicknesses. Additionally, we observed films of Ag/Ti6Al4V alloys with different thicknesses by scanning electron microscopy (SEM). X-ray diffraction measurements of theAg/Ti6Al4V alloys revealed diffraction peaks of Ag (200), Ag (111), and Ag (200) for a 2.552-μm-thick Ag film for 4 min coating time. Results also showed that the growth rate was 0.0106 μm/s for 4 min coating time. Subsequently, we observed the sterilized test pieces after 18, 24, and 48 h to determine the antibacterial effect on *S. mutans*. We found that the antibacterial effects of Ti6Al4V and Ag/Ti6Al4V alloys were significantly greater than that of Co–Cr–Mo alloy. As a result, we propose that 3D printing is a suitable method for manufacturing Ti6Al4V alloy, which we found to be the most suitable material for making denture frameworks.

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1. Introduction

Ti6Al4V alloy removable partial dentures have gradually been replacing traditional dentures of stainless steel or Co–Cr–Mo alloy,(1) because Ti6Al4V alloys have a low risk of complications owing to their low density and ductility. However, since Ti6Al4V is difficult to process and expensive, Co–Cr–Mo alloy has continued to be used. Unfortunately, the nickel in Co–Cr–Mo alloy causes medical allergies. People having their teeth straightened traditionally used thick removable partial dentures, which felt heavy in the mouth. Therefore, they reverted back to Ti6Al4V alloy removable partial dentures. See Table 1 for a comparison of the characteristics of the two materials.(2,3) Until now, 3D-printed Ti6Al4V alloys have been typically fabricated by selective laser melting (SLM), which has also been used to fabricate bone plates/screws, hip joints, and braces. Products fabricated by SLM have gradually replaced traditionally forged titanium alloys and products and are increasingly used in implants and artificial tooth roots. However, the thickness of the Ag film used to coat these products also affects its antibacterial ability against *Streptococcus mutans*. This results from Ag ions being unable to kill *S. mutans* and strengthen oral hygiene and protection.(4) *S. mutans* is a strong acid-producing (acidogenic) bacterium that produces observable dental plaque, thereby triggering demineralization and decay.(5) Additionally, metallic materials are damaged by microbiologically induced corrosion, which is a major concern in many fields.(6) On the basis of the above concerns, in this study, we evaluate the effects of titanium and Co–Cr–Mo on *S. mutans* in the oral cavity by bacteriostatic tests.

Additionally, the characteristics of Ti6Al4V alloys make them suitable for fabricating various sensor devices, such as optical fiber sensors, biosensors, piezoelectric force sensors, and acoustic emission sensors. Regardless of whether Ti6Al4V alloys are employed for biomedical or sensor applications, it is important to improve their mechanical properties to increase their service lifetime. Thus, in this study, we have focused on the antibacterial properties of SLM-prepared Ti6Al4V alloys.

Table 1
Comparison of characteristics of forged and 3D-printed titanium alloys.

Material	Use	Advantages	Disadvantages
Forged	1. Bone plate/bone screw 2. Artificial hip joint 3. Spinal fusion cage 4. Braces	1. Can eliminate loose metal defects 2. Low price 3. Greater strength than 3D-printed alloys	1. Easy to waste materials 2. Mold required 3. Mechanical properties markedly different from those of bone
3D-printed	1. Bone plate/bone screw 2. Artificial hip joint 3. Intravascular stent 4. Prosthetic heart valve 5. Braces	1. Consistent quality 2. Can be formed directly 3. Easy X-ray penetration, good for observation after surgery 4. Similar mechanical properties to human bones	1. Subsequent surface treatment required 2. Printing process prone to internal stress residue

2. Materials and Methods

2.1 Alloy types

In this study, three materials used in removable partial dentures were selected: Co–Cr–Mo alloy, Ti6Al4V alloy, and Ti6Al4V alloy with a Ag coating film (Ag/Ti6Al4V alloy). We subsequently performed two bacterial tests (solid medium and liquid culture tests) to evaluate the bacterial growth rate of *S. mutans* on these alloys (Fig. 1).

2.2 Test strip production

We used a 3D printing machine to manufacture Ti6Al4V alloys by SLM in the vertical direction (Fig. 2). Ag/Ti6Al4V alloys were formed by using a coating machine (Fig. 3) to deposit Ag films with different thicknesses on Ti6Al4V alloy substrates. Following this technique, all alloys were prepared with dimensions of 10 × 10 × 3 mm^3, after which Co–Cr–Mo, Ti6Al4V, and Ag/Ti6Al4V alloys were forged (Fig. 3).

2.3 Bacterial strains and culture preparation

We used a bacterial test method to assess different materials in a removable partial denture (Fig. 4). First, we prepared a solid medium and Lysogeny broth (LB). To prepare the solid medium, 14 g of LB was added to 11.2 g of agar. The mixture was subsequently stirred with 700 ml of double distilled water (ddH$_2$O). We then prepared a liquid culture in 10 g of agar, into which 500 ml of ddH$_2$O was stirred. Next, the culture medium was autoclaved for 30 min, after

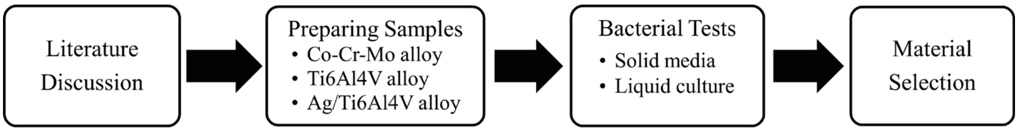


Fig. 1. Experimental bacteriostatic test method in this study.

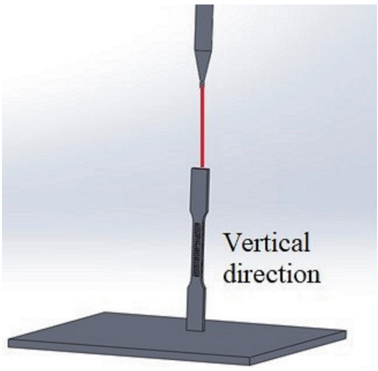


Fig. 2. (Color online) Ti6Al4V fabricated by SLM in vertical direction.

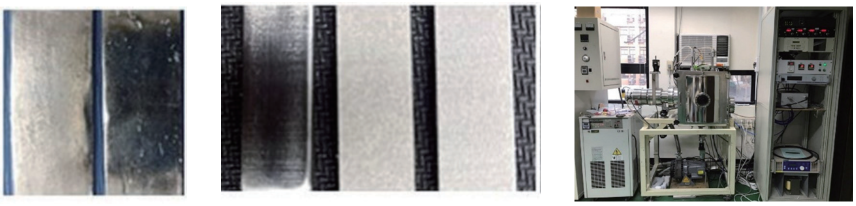

(a) (b) (c)

Fig. 3. (Color online) (a) Co-Cr-Mo alloy, (b) Ti6Al4V alloy, and (c) coating machine.

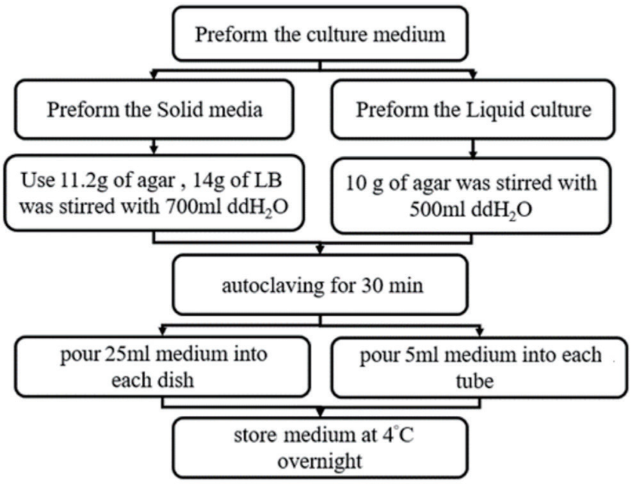


Fig. 4. Method of preparing solid medium and liquid culture.

which 25 ml of the solid medium was poured into each dish, whereas 5 ml of the liquid culture was poured into each tube. Finally, all cultures were stored overnight at 4 °C. After culture preparation, we activated bacterial strains by using an inoculating loop to infect the agar with one *S. mutans* culture, then using the streaking method to activate the *S. mutans* culture. Finally, one *S. mutans* culture was placed in 2 ml of sterilized LB broth, and the culture was incubated in an incubator at 37 °C for 24 h.

2.4 Fabrication process for Ag/Ti6Al4V alloys

Before coating the samples, Ti6Al4V alloys with dimensions of 10 × 10 × 3 mm^3 were prepared with a 3D printing machine, then sequentially abraded with 400, 600, 800, and 1000 grit silicon abrasive papers to examine the antibacterial mechanism of silver. Silver ions bind to the thiol group containing the amino acid cysteine, inhibiting vital enzyme and protein functions in bacteria, thereby causing their death.(7) We used a coating machine to deposit Ag films on Ti6Al4V alloys for 2 and 4 min to obtain Ag/Ti6Al4V alloys. We then evaluated the effect of the Ag film thickness on the bacteriostatic effect.

2.5 X-ray diffraction (XRD) and scanning electron microscopy (SEM) observations of Ag coating

In this study, the growth rates and thicknesses of Ag films were measured by SEM, after which the compositions of the Ag/Ti6Al4V alloys were determined by XRD. Additionally, the element compositions of the surfaces of the Ag/Ti6Al4V alloys were determined by X-ray photoelectron spectroscopy.

2.6 Solid medium bacteriostatic test

We performed a solid medium bacteriostatic test on the Co–Cr–Mo, Ti6Al4V, and Ag/Ti6Al4V alloys to determine their antibacterial effect on *S. mutans*. The three alloys were incubated at 37 ℃ for 18 and 24 h on an agar medium with *S. mutans*. After this test, the size of the zone of inhibition was used to judge whether each material had an antibacterial effect. The experimental steps are shown in Fig. 5.

2.7 Liquid culture bacteriostatic test

A convenient and widely applied method to determine the growth state of a bacterial cell culture is to determine the optical density (OD) spectrophotometrically at a wavelength of 600 nm, and the OD of the bacterial suspension was measured using a spectrophotometer (Fig. 6). This equipment detects the relative concentration of *S. mutans* under certain conditions. Before the test, the four different alloys (one control group and three test groups of forged Co–Cr–Mo,

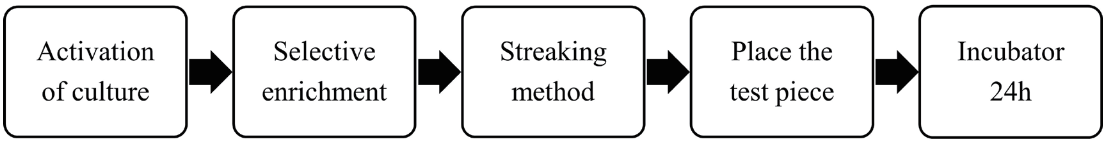


Fig. 5. Steps of solid medium bacteriostatic test.

Fig. 6. (Color online) Test process with spectrophotometer.

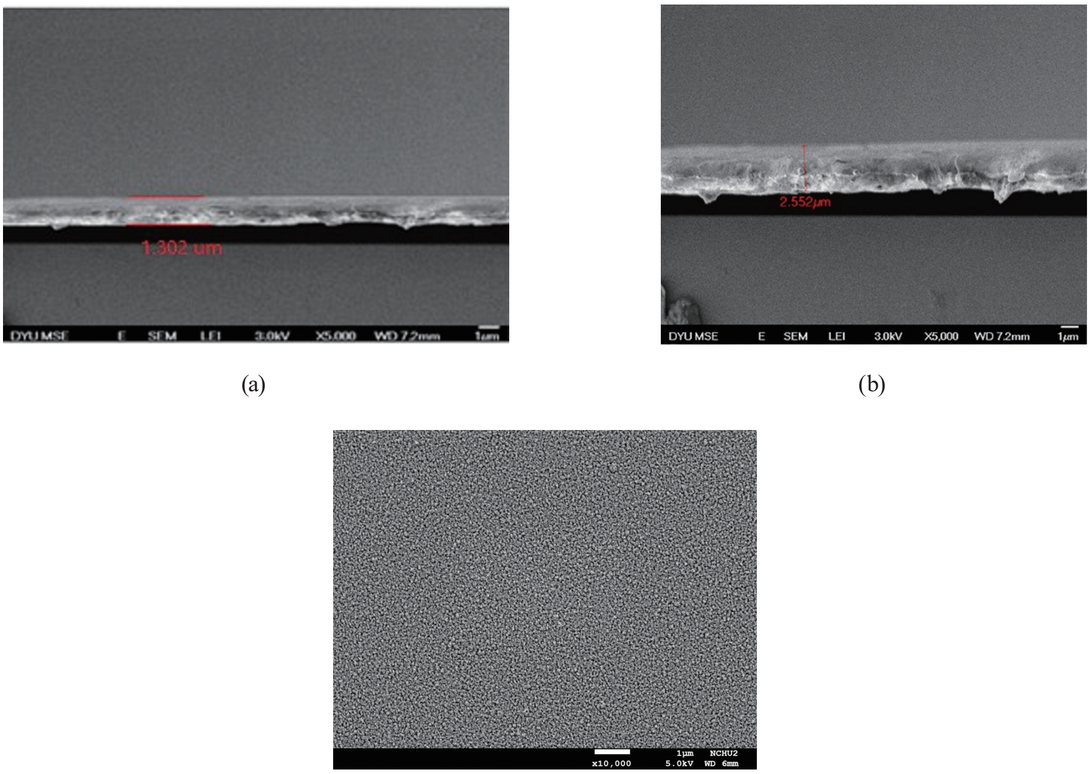
Ti6Al4V, and Ag/Ti6Al4V alloys) were placed in test tubes of bacterial suspension (including 5 ml of medium and 100 ml of *S. mutans*) for 24 or 48 h.

3. Results and Discussion

3.1 XRD and SEM observations of silver coating

The morphologies of Ag films deposited on Ti6Al4V substrates were observed by SEM. Figure 7 shows plan-view SEM images of 1.302- and 2.552-μm-thick Ag/Ti6Al4V alloys. As observed, the growth rates were 0.0108 and 0.0106 μm/s for 2 and 4 min coating times, respectively. The results also confirmed the presence of many grains of uniform size on the sample surface [Fig. 7(c)].

As shown in Fig. 8, we observed only three diffraction peaks, Ag (200), Ag (111), and Ag (200), in the XRD spectra of the Ag/Ti6Al4V alloys coated for 2 and 4 min. From the experimental results, it was confirmed that the films were crystalline phases of Ag. In addition, the longer the coating time, the stronger the diffraction peaks. Therefore, it is speculated that the formation of the Ag film is through the growth of the film in the thickness direction, which also indicates that the film has large grains.



(a) (b)

(c)

Fig. 7. (Color online) Plan-view SEM images of (a) 1.302-μm-thick and (b) 2.552-μm-thick Ag/Ti6Al4V alloys, and (c) sample surface with grains of uniform size.

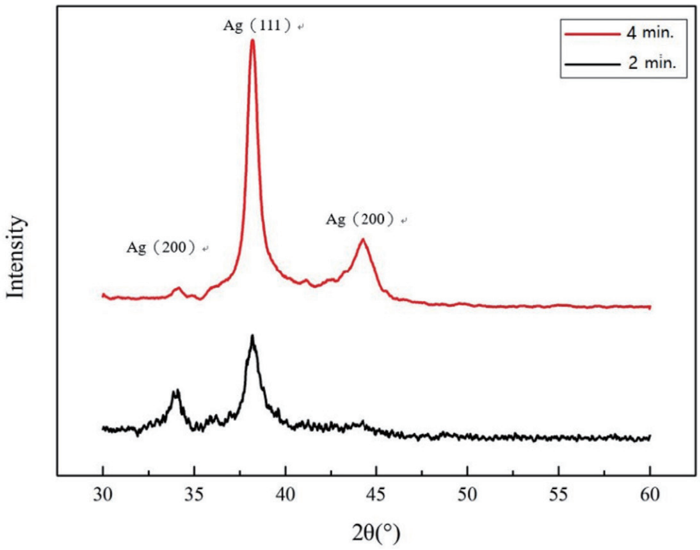


Fig. 8. (Color online) XRD patterns of Ag/Ti6Al4V alloys.

3.2 Results of solid medium bacteriostatic test

Alloys with dimensions of 10 × 10 × 3 mm^3 were prepared then subjected to the solid medium bacteriostatic test. Figures 9(a) and 9(b) show the Co–Cr–Mo and 3D Ti6Al4V alloys after the solid medium bacteriostatic test, respectively. The inhibition zone for the Ti6Al4V alloy was evident. Figures 10(a)–10(c) show the uncoated Ti6Al4V alloy, the Ag/Ti6Al4V alloy with the Ag layer coated for 2 min, and the Ag/Ti6Al4V alloy with the Ag layer coated for 4 min after the solid medium bacteriostatic test, respectively. Figures 9 and 10 show that the Ag/Ti6Al4V alloy with a Ag coating deposited for 4 min formed a clear inhibition zone after 24 h.

3.3 Results of liquid culture bacteriostatic test

OD measurement is the standard approach in microbiology for characterizing bacteria concentrations in culture media, and OD qualitatively reflects the concentration or quantity of microorganisms. Test alloys with dimensions of 10 × 10 × 3 mm^3 were prepared and sequentially wet-abraded in the liquid culture bacteriostatic test (Fig. 11). The Co–Cr–Mo, Ti6Al4V, and Ag/Ti6Al4V alloys were placed in test tubes containing a bacterial suspension (100 ml of *S. mutans*) for 24 or 48 h, then the OD values of the alloys were measured using a spectrophotometer. We used Eq. (1) to obtain the inhibition rate (%) for the alloys.

$$\begin{aligned}\text{Inhibition rate (\%)} &= (\text{OD value of control group} - \text{OD value of test group})\\ &\quad \times 100\% / (\text{OD value of control group})\end{aligned} \tag{1}$$

The OD value of each group after the liquid culture bacteriostatic test for 24 or 48 h, the OD value of each test group, and the OD value of the control group were entered into Eq. (1) to

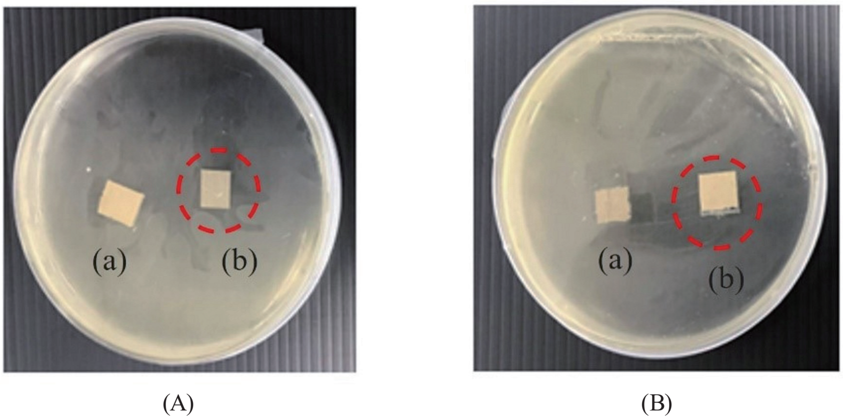


Fig. 9. (Color online) Bacteriostatic results obtained from (a) Co–Cr–Mo and (b) Ti6Al4V alloys after 18 h (A) and 24 h (B) on solid medium.

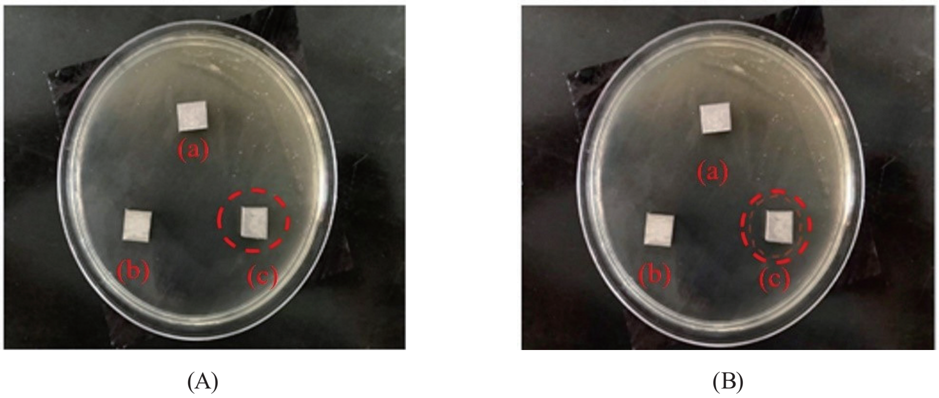


Fig. 10. (Color online) Bacteriostatic results obtained from (a) uncoated Ti6Al4V alloy, (b) Ag/Ti6Al4V alloy with 2 min Ag coating, and (c) Ag/Ti6Al4V alloy with 4 min Ag coating after 18 h (A) and 24 h (B) on solid medium.

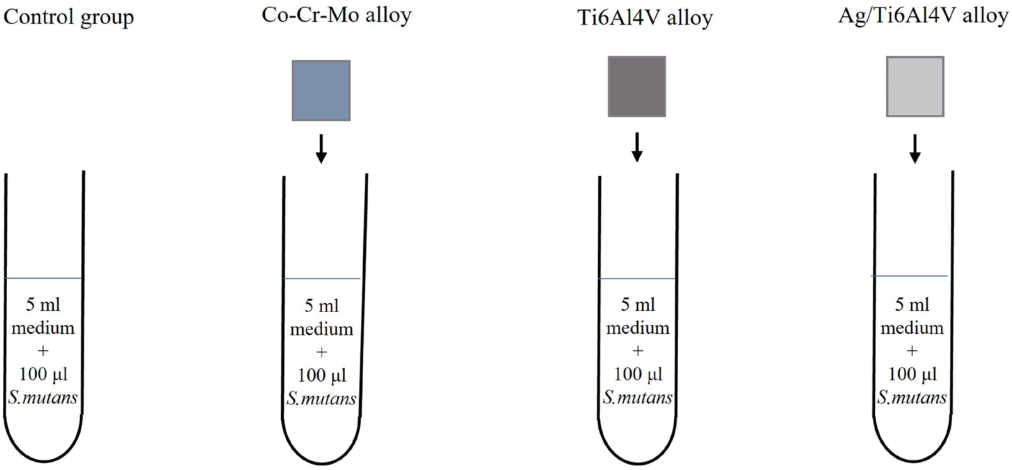


Fig. 11. (Color online) Test group tubes of three alloys and control group tube

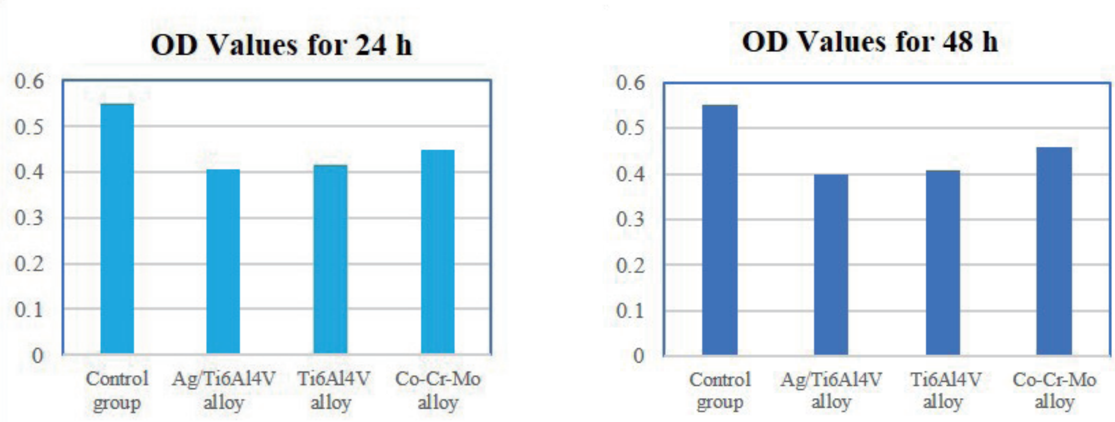
obtain the inhibition rate (%) of each alloy (Tables 2 and 3). The highest inhibition rates of 28% (24 h) and 27% (48 h) were obtained for the Ag/Ti6Al4V alloy, which was thus demonstrated to have the strongest antibacterial effect. Additionally, the inhibition rates of the Ti6Al4V alloy were 26% (24 h) and 25% (48 h) and those of the Co–Cr–Mo alloy were 17% (24 h) and 18% (48 h). After 48 h, the Ag/Ti6Al4V alloy had better antibacterial effects (Fig. 12).

Table 2
Results of antibacterial tests in liquid culture for 24 h.

OD value	Material			
	Control group (a)	Ag/Ti6Al4V alloy (b)	Ti6Al4V alloy (b)	Co-Cr-Mo alloy (b)
First group	0.566	0.433	0.449	0.498
Second group	0.548	0.392	0.398	0.441
Third group	0.542	0.376	0.380	0.438
Average	0.552	0.400	0.409	0.459
Inhibition rate (a − b) × 100% / a	—	28%	26%	17%

Table 3
Results of antibacterial test results in liquid culture for 48 h.

OD value	Material			
	Control group (a)	Ag/Ti6Al4V alloy (b)	Ti6Al4V alloy (b)	Co-Cr-Mo alloy (b)
First group	0.572	0.419	0.436	0.456
Second group	0.550	0.406	0.416	0.448
Third group	0.525	0.390	0.390	0.446
Average	0.549	0.405	0.414	0.450
Inhibition rate (a − b) × 100% / a	—	27%	25%	18%



(a) (b)

Fig. 12. (Color online) OD values for different materials after 24 h (a) and 48 h (b) liquid antibacterial tests.

4. Conclusion

In this study, Ti6Al4V was observed to have a strong antibacterial effect after surface modification. When Ag^+ came in contact with the *S. mutans* cell membrane, oxidative stress was induced with the assistance of reactive oxygen species, thereby inhibiting the expression of adhesion protein genes. However, when Ag^+ entered the cytoplasm, the mitochondria and DNA in the cytoplasm were destroyed, causing cell death. Moreover, *S. mutans* came in contact with polygonal nanoparticles on the modified Ti6Al4V surface, which caused the outer membrane of *S. mutans* to be stretched and destroyed. In summary,

1. the XRD spectra of the Ag/Ti6Al4V alloys showed diffraction peaks of Ag (200), Ag (111), and Ag (200);
2. the growth rate of the Ag layer was found to be 0.0106 μm/s from SEM images of the Ag/Ti6Al4V alloy with a 2.552-μm-thick Ag layer;
3. Ti6Al4V obtained by 3D printing had a strong antibacterial effect on *S. mutans*, indicating the suitability of the material for complete dentures;
4. after 18 and 24 h of solid-state cultivation, an inhibition zone was observed for the forged Co–Cr–Mo and 3D-printed Ti6Al4V alloys;
5. after 24 h of solid-state cultivation, the strongest antibacterial material was Ag/Ti6Al4V alloy with a Ag coating deposited for 4 min, which formed a clear inhibition zone;
6. after 24 h of liquid-state cultivation, the strongest antibacterial effects were observed for Ag/Ti6Al4V alloy (28%), and after 48 h of liquid-state cultivation, the Ag/Ti6Al4V alloy had better antibacterial effects.

Acknowledgments

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References

1. L. He, D. Dai, L. Xie, Y. Chen, and C. Zhang: Mater. Design **207** (2021) 109890. https://doi.org/10.1016/j.matdes.2021.109890
2 R. D. Carlo, S. Zara, A. Ventrella, G. Siani, T. D. Ros, G. Iezzi, A. Cataldi, and A. Fontana: Nanomaterials **9** (2019) 604. https://doi.org/10.3390/nano9040604
3 D. Merker, B. Popova, T. Bergfeldt, T. Weingärtner, G. H. Braus, J. P. Reithmaier, and C. Popov: Diamond Related Mater. **93** (2019) 168. https://doi.org/10.1016/j.diamond.2019.02.003
4 L. N. Xu, X. Y. Yu, W. Q. Chen, S. M. Zhang, and J. Qiu: RSC Adv. **10** (2020) 8198. https://doi.org/10.1039/D0RA00154F
5 J. C. Souza, P. Ponthiaux, M. Henriques, R. Oliveira, W. Teughels, J. P. Celis, and A. Rocha : J. Dent. **41** (2013) 528. https://doi.org/10.1016/j.jdent.2013.03.008
6 Y. Yang, M. Masoumeh, E. Zhou, D. Liu, Y. Song, D. Xu, F. Wang, and J. A. Smith: Corrosion Sci. **182** (2021) 109286. https://doi.org/10.1016/j.corsci.2021.109286
7 E. J. Fatani, H. H. Almutairi, A. O. Alharbi, Y. O. Alnakhli, D. D. Divakar, M. Abdulaziz A. Alkheraif, and A. A. Khan: Microb. Pathogen. **112** (2017) 190. https://doi.org/10.1016/j.micpath.2017.09.052

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