Antibacterial Properties of Stainless Steel Coated on Ti6Al4V Alloy

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Abstract: Titanium alloys and stainless steel type 316L are used in biomedical applications due to their high corrosion resistance and good biocompatibility properties. In this study, a functionally graded material composed of titanium and stainless steel was fabricated using a powder metallurgical technique. Ti6Al4V alloy powder was placed as a substrate into a graphite crucible and stainless steel powder was added as a thin layer on the Ti6Al4V powder. The two layers were consolidated *in-situ* using a uniaxial hot press. The sintering process was carried out at 1050 ^{o}C for 30 minutes under 50 MPa. The pressure was maintained during the whole sintering process. A disc shape compact of 20 mm diameter and 5 mm thickness was obtained after sintering. The samples were metallographically prepared and their antibacterial properties were evaluated. A strong bonding was observed between the Ti6Al4V substrate and the 316L stainless steel layer, and no bacteria were observed on the stainless steel surface.

Index terms: Antibacterial activity, Stainless steel 316L, Ti6Al4V, pressure assisted sintering technique.

I. INTRODUCTION

Titanium and its alloys are used in many industrial applications due to their low density, high mechanical properties, excellent corrosion and good biocompatibility (1). In the biomedical field, titanium based alloys are commonly preferred for orthopedic implants and prostheses (2), in particular, type Ti6Al4V alloy is the most preferred among the titanium alloys (3). However, any implementation of biomaterials runs the risk of biomaterials associated infection (BAI), which is the most devastating postoperative complication because the infections can cause chronic suffering and high medical expenses. To overcome this problem, researchers developed different antibacterial surface solutions. Compared to the organic antibiotics, elements such as silver and copper have attracted great interest for bacteria resistant titanium alloys (4). This study attempts a different route to enhance the antibacterial activity of Ti6Al4V alloy by coating it with 316L stainless steel and developing its antibacterial performance.

Austenitic stainless steel type AISI 316L is the only stainless steel alloy used as biomedical material (3). Similar to titanium alloys, this type of stainless steel also shows good corrosion resistance and biocompatibility properties (5-6). The combination of stainless steel with Ti6Al4V alloy as a coating layer are considered in this study using powder metallurgy (PM) technique which has advantages over traditional production techniques, for example it provides near-net shapes of components with a fine and homogeneously distributed microstructure. Moreover, the powder particles are solid and the final products are obtained without melting. In addition to that, applying a pressure during the process provides enhanced densification which provide good mechanical properties (7).

II. MATERIALS AND EXPERIMENTAL WORKS

Pre-alloyed Ti6Al4V and 316L stainless steel powders were used for the biomaterial production. Fig.1 shows Scanning Electron Microscopy (SEM) images of the powders showing that Ti6Al4V particles have irregular shapes while stainless steel particles have a round shape. This is attributed to the production method of each powder, Ti6Al4V particles were produced by chemical methods, while 316L particles were produced by water atomization process. This means that the shape differences are the result of the production techniques. These shape characteristics have a negative effect during conventional sintering process resulting at low density ratios. However, in this study a pressure assisted sintering technique (hot pressing) was used.

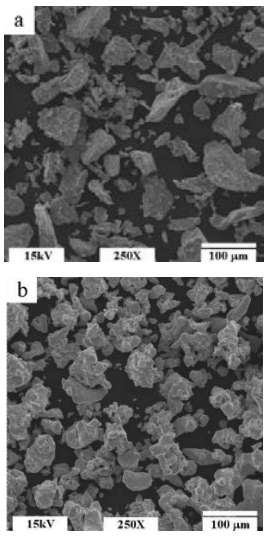

Fig.1 SEM images of (a) Ti6Al4V and (b) Stainless steel powders

Hot pressing is a pressure assisted sintering method which applies simultaneous pressure during the sintering process. This technique provides higher densification at lower temperatures and shorter production times compared to the conventional powder metallurgical production. Fig.2 shows a schematic diagram of the hot pressing process. In the hot pressing system, high pressure levels cannot be reached and the graphite die with punches are used because of their high

conductivity. However, the combined effect of temperature and pressure results in high density values. In this study, 50 MPa was applied during the sample production. Fig.2 shows the method of controlling the thickness of powder layers for each material. The Ti based powder was placed in the die as a substrate material and a thin layer of (250 μm) stainless steel was added on the titanium alloy particles. The powders were sintered at 1050 °C for 30 minutes under a 10^{-4} mbar vacuum atmosphere. Fig.3 shows the stainless steel coated titanium, 316L-Ti6Al4V, sample produced by the hot press method at 1050 °C.

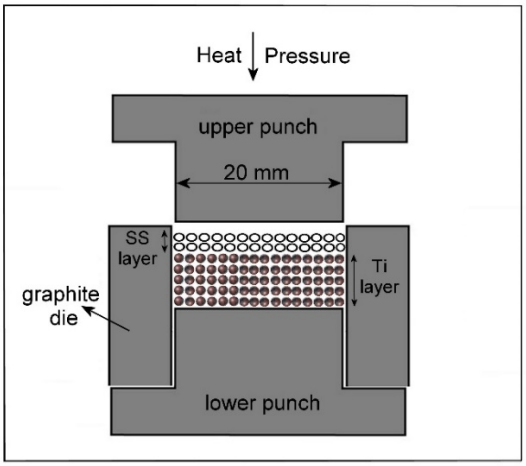

Fig.2 Schematic diagram of hot press die and punches

Samples were metallographically prepared following appropriate steps. Grinding papers with grades 320, 600, 1000 and 2000 were used for grinding, then 9, 6, 3 and 1 micrometer diamond suspensions were used for the polishing step. The samples were etched by using Kroll reagent. The density of the samples were determined by the Archimedes method. Optical and scanning electron microscopy were used to characterize the microstructure of the samples.

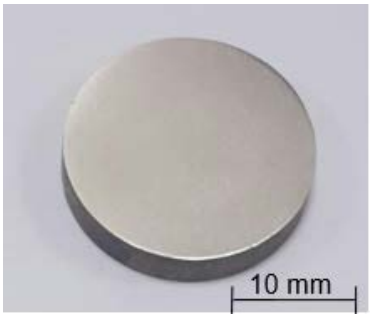

Fig.3 Stainless steel coated Ti6Al4V alloy produced by hot pres

To investigate the antibacterial activity of the produced samples, Escherichia coli (*E. coli*) ATCC 25922 and Staphylococcus aureus (*S. aureus*) ATCC 29213 bacteria cells were used in this study. The bacteria were prepared in Mueller-Hinton Agar (MHA) (Merck, Germany). The solution was prepared by dissolving 34 g of MHA in 1000 ml of distilled water and the nutrient broth was sterilized at 121 °C for 15 minutes. The bacteria were cultivated at 37 °C in the nutrient broth to a concentration of 10^8 cfu/ml and then diluted 10-fold by Phosphate Buffer Solution (PBS) to a concentration of 104 Colony Forming Unit (CFU/ml). 0.4 ml was taken from the suspension and dripped onto the samples. The samples with the cells were incubated at 37 °C for 18-24 h, then the solutions on the surface were harvested into sterilized tubes. The samples were vortexed, 0.02 ml of solution was taken with a glass baguette and cultivated in MHA. After incubation at 37 °C the active bacteria were counted.

III. RESULTS AND DISCUSSION

The density of the two-layer material was measured by Archimedes method and was found around 99.8%. The density ratio is relatively high for the powder metallurgical materials especially to be used in load bearing biomedical applications such as hip implants. The samples were cut and prepared metallographically. Fig.4 shows a cross section of a produced sample. The coated stainless steel layer on the Ti6Al4V alloy is shown. Kroll reagent affected the Titanium substrate, however stainless steel layer was not etched. There is also a diffusion layer between stainless steel and Ti6Al4V.

Table 1 shows the result of the antibacterial tests. Fig.5 shows bacterial colonies on the produced alloys. Coating of Ti6Al4V alloy by stainless steel has resulted to a bacteria free surface for both *E-coli* and *S. aureus* bacteria cells. Ti6Al4V alloy showed a good resistance to *S.aureus* bacteria. Fig.5 proves the results over the growth of bacteria on the samples. The number of colonies in the case of *E. coli* increased on the Ti6Al4V alloy surface but no *E. coli* colonies were observed on the stainless steel coating layer. *S. aureus* colonies were not observed in both stainless steel and Ti6Al4V surfaces.

Fig.4 Optical micrograph of hot pressed sample

Table 1. Antibacterial test results of stainless steel and Ti6Al4V alloy

Material	*E. coli* colonies number (CFU/ml)	*S. aureus* colonies number (CFU/ml)
Ti6Al4V	10800	0
316 L SS	0	0
Control	9300	12100

It is a well-known process that after the implant production process the titanium surface absorbs hydrocarbons from the atmosphere, water and cleaning solutions, as a result they easily become hydrophobic and the biological activity of titanium implants decreased continuously. Other researchers support these antibacterial activity results. ThItabashi *et al*. (8) studied the antibacterial properties of titanium alloys. They enhanced the antibacterial properties of pure titanium and Ti6Al4V alloy by treating the surfaces by low energy UV irradiation. Moreover, Walkowiak *et al*. (9) observed that bacteria colonization on titanium surface is more prone than 316L stainless steel. However, the interpretation of these results in more details need further investigation because the infection problems which are associated with implanting the metallic materials in patients body involve a serious of complicated steps.

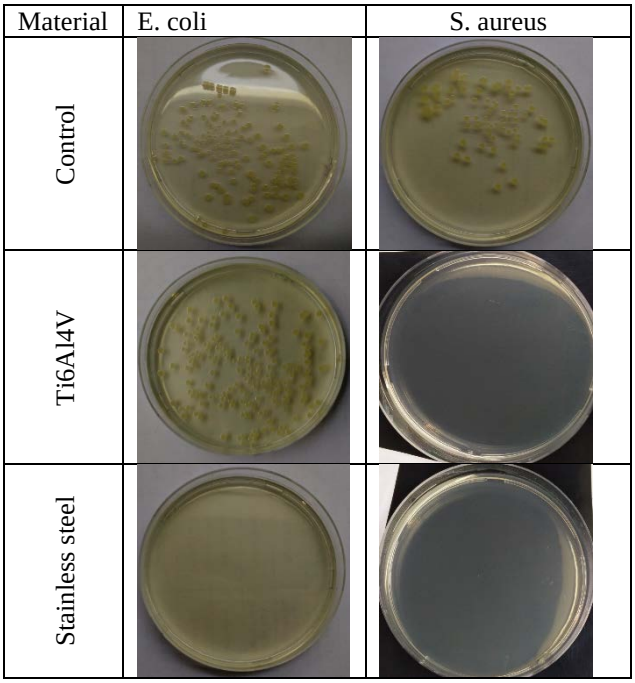

Fig. 5 Colony counts of E. coli (left) and S. aureus (right)

IV. CONCLUSION

In this study, antibacterial properties of titanium alloy by *in-situ* stainless steel coating was investiagted. A thin stainless steel powder layer was successfully produced on the Ti6Al4V powder using pressure assisted sintering technique (hot pressing) powder metallurgical method. Appropriate interlayer bonding between stainless steel coating layer and Ti6Al4V substrate material were obtained through a production process at 1050 ^{o}C for 30 minutes. *E. coli* and *S. aureus* bacteria were not observed on the stainless surface. As a result, the bacterial activity of Ti6Al4V is enhanced by a stainless steel coating layer.

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