



Antibacterial Properties of TiAgN and ZrAgN Thin Film Coated by Physical Vapor Deposition for Medical Applications

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We deposited TiAgN and ZrAgN nanocomposite coatings on pure Titanium specimens, by using arc ion plating (AIP) with single alloy targets. TiAg ZrAg alloy targets of 5 wt.%, 10 wt.% silver content by vacuum arc remelting (VAR), followed by homogenization for 2 hours at 1,100°C in non-active Ar gas atmosphere and characterized these samples for morphology and chemical composition. We investigated the biocompatibility of TiAg and ZrAg alloys by examining the proliferation of L929 fibroblast cells by MTT test assay, after culturing the cells (4×10^4 cells/cm²) for 24 hours; and exploring the antibacterial properties of thin films by culturing *Streptococcus Mutans* (KCTC3065), using paper disk techniques. Our results showed no cytotoxic effects in any of the specimens, but the antibacterial effects against *Streptococcus Mutans* appeared only in the 10 wt.% silver content specimens.

Keywords: TiAgN, ZrAgN, Nanocomposites, Antibacterial, Biocompatibility

1. INTRODUCTION

Titanium(Ti) and zirconium(Zr) have been widely researched in the field of biomedical materials, due to their excellent mechanical properties and biocompatibility. They belong to group a in the periodic table, and are known to have similar chemical

properties, and no toxic effects. However, they do not possess antibacterial properties. Inflammation and infection, which are usually caused by adherence and colonization of bacteria on biomaterials, cause serious complications in patients [1,2]. Thus in order to enhance the antibacterial capability on the surface of biomedical devices, an effective approach to antibacterial agents was performed by means of surface coating techniques, to increase their antibacterial and mechanical properties [3-5].

Silver (Ag) and copper (Cu) are known to be effective antibacterial agents, due to their specific antibacterial activity [6]. It was reported that Ag-doped TaN and Cu-doped TaN with nanoparticles can decrease the multiplication of *Escherichia coli* bacteria,

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and showed improved antibacterial effect [7]. These experiments found that Ag is an interesting antibacterial biomaterial, due to the non-toxicity of the active Ag⁺ to human cells. Many authors have described multiple changes in cell physiology when treated with Ag, involving Ag ions binding to proteins, and enzymes in the cell wall, cell membrane, and peptidoglycan [8-10].

The materials used for biomedical applications must exhibit specific properties. The important properties of materials used for medical fields are biocompatibility, corrosion resistance, osseointegration, and excellent mechanical characteristics.

Vacuum arc re-melting (VAR) is widely used to improve the cleanliness, and refine the structure of standard air-melted or vacuum induction melted ingots, which are then called consumable electrodes [11]. Ti, Zr and its alloys are the materials most often processed with this method, and are used in a great number of high integrity applications, where cleanliness, homogeneity, and improved fatigue and fracture toughness of the final product are essential, such as medical and aerospace industries.

Plasma vapor deposition (PVD) coatings are well known for their good corrosion resistance, excellent adhesion force, and environmentally friendly vacuum coating process without hazard, compared with chemical vapor deposition (CVD) coatings. In particular, arc ion plating (AIP) has been widely used in industry, because of its advantages of dense metal vapor generation, high ionization efficiency, high deposition rate, and so forth [12].

So in view of the demands of rapid and mass product manufacturing, TiAgN and ZrAgN coatings were deposited using AIP. Each of the thin films was coated on the pure Ti specimen using a single target made from TiAg ZrAg alloy targets of 5 wt.%, 10 wt.% Ag content, and the surface properties were observed.

The objective of this work is to study the characteristics of the antibacterial performance on bacteria and their biocompatibility to improve their function in medical applications. *Streptococcus Mutans* was chosen as a model for this *in vitro* study. To verify the biocompatibility and cell proliferation activity of the TiAg and ZrAg alloys, the growth behavior of L929 fibroblast cells cultured on the specimens was also investigated.

2. EXPERIMENTS

2.1 Experimental Materials for single alloy targets

VAR was used to produce TiAg and ZrAg alloy targets, which were prepared at two different Ag content of 5 wt.% and 10 wt.%. VAR is the continuous re-melting of a consumable electrode by means of an arc under vacuum. 35-45 KW power is applied to strike an arc between the electrode and the base-plate of a copper mold contained in a water jacket. The intense heat generated by the electric arc melts the tip of the electrode, and a new ingot is progressively formed in the water-cooled mold. A high vacuum is maintained throughout the re-melting process. A homogenization treatment is performed for 2 hours at 1,100°C in non-active Ar gas atmosphere, in order to remove the microsegregation of alloying elements, thereby minimizing the concentration gradients developed during solidification, and ensuring a homogeneous distribution of particles.

2.2 Preparation of the TiAgN and ZrAgN coatings

Commercially pure (cp) titanium was chosen as the substrate materials. Prior to deposition, the samples were first ground with abrasive papers, and then polished with diamond paste. Finally, the specimens were successively ultrasonically cleaned in acetone, alcohol, and distilled water and dried. Specimens were prepared of 15 mm diameter and 3.5 mm height.

The TiAgN and ZrAgN coatings were prepared on cp titanium specimens by AIP. The distance between the target and substrate was 150 mm. The specimens were placed on a rotational substrate holder for deposition. The process pressure was less than 7.5 mTorr. The thickness of the coatings was controlled by a one-hour deposition time. The temperature of substrate during deposition was 350°C. The other process parameters were Ar and N₂ gas flow at 200 sccm, arc power of 60 A, and bias voltage of -100 V_{dc}.

2.3 Characteristics of alloys and thin films

Field emission scanning electron microscopy (FE-SEM, S-4800, Hitachi) at 15 kV was used to obtain images of the morphology and structure of the coating. Energy dispersive x-ray spectroscopy (EDS, QUANTAX, BRUKER) was performed on the alloys, to determine the chemical composition.

2.4 Biocompatibility testing of each alloy

Five alloy specimens of each group were subjected to biocompatibility tests. The L929 fibroblast cells were cultured in fetal bovine serum (FBS) containing 10% Dimethylsulphoxide (DMSO, SIGMA, USA). The proliferation of cells was examined by MTT test assay, after culturing the cells (4×10^4 cells/cm²) for 24 hours on the specimens. Then, 60 μ l of MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] per well was dispersed, and reacted to the cells at 1 hour.

The absorbance (optical density, OD) was quantified by microplate reader (ELx 800UV®, Bio-Tek Instrument, Inc, USA) measuring at 450 nm. The optical density of formazan reflected the level of cell viability, and higher OD values showed more living cells on the specimen, which presented better biocompatibility.

2.5 Antibacterial activity test

The antibacterial activity of each TiAgN and ZrAgN coating obtained against *Streptococcus Mutans* (KCTC3065) was studied by using the paper disk method for determination of the bacterial sensitivity to specimens: the relationship between the diameter of the zone of inhibition, and the content of Ag. The sizes of clear zone formed around specimen were measured, by observing the 4 points, and calculating the mean value.

0.5 McFarland turbidity (1.6×10^8 cells/ml) of the culture medium of *Streptococcus Mutans* were spread onto Brain heart infusion broth (BHI, Difco Lab., USA), supplemented with 10% (v/v) horse blood serum (Oxide, Italy). Each specimen on BHI was aerobic cultured in a 37°C incubator for 18 hours.

3. RESULTS AND DISCUSSION

The reductions in Ag content of the manufactured TiAg and ZrAg alloys were measured. During the VAR process, the Ag content decreased by about 20% (Table 1).

The morphological analysis gives an insight into the formation of structures. Fig. 1 shows SEM image of the TiAgN and ZrAgN nanocomposite coatings prepared by an AIP method using single alloy targets. At the Ag content of 10 wt.%, the thicknesses of TiAgN and ZrAgN thin film were observed to be 923 nm and 1,330 nm, respectively (Fig. 2). Independent of Ag content, the surfaces showed similar structures, and there were some residual clusters of the Ag nanoparticles throughout the overall coating surface. These tiny droplets appearing on the coating layer are known to be a characteristic of the AIP method. The emergence of Ag nanoparticles would influence the surface hydrophilicity and

Table 1. The decreased Ag content rates of TiAg and ZrAg alloy after VAR process (EDS, QUANTAX, BRUKER).

Alloy	Ag content (wt.%)	Measured content (wt.%)	Decreased Rate (%)
TiAg	5	3.86	22.8
	10	8.01	19.9
ZrAg	5	4.73	5.4
	10	7.78	22.2

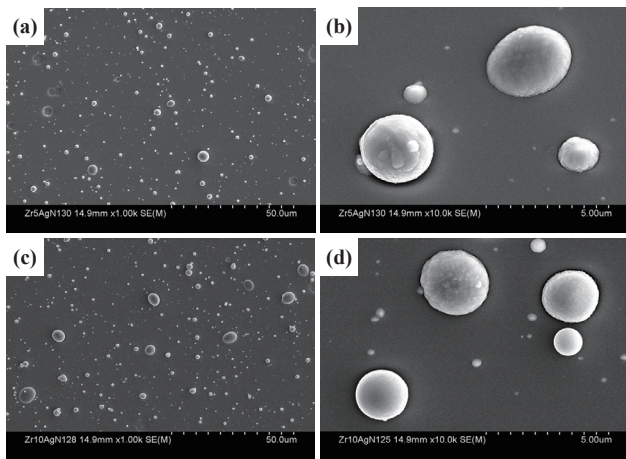


Fig. 1. FE-SEM images of the surfaces of the TiAgN and ZrAgN coatings: (a) TiAgN($\times 1$ K), (b) TiAgN($\times 10$ K), (c) ZrAgN($\times 1$ K), and (d) ZrAgN($\times 10$ K) (Ag : 10 wt.%, arc power : 60 A, bias voltage : $-100 V_{dc}$, deposition time : 1 h).

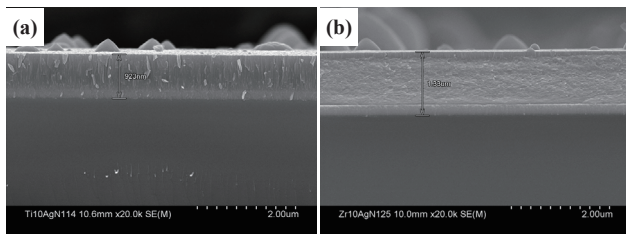


Fig. 2. FE-SEM images of the cross-section of the TiAgN and ZrAgN coatings: (a) TiAgN (thickness_923 nm) and (b) TiAgN (thickness_1,330 nm) (Ag : 10 wt.%, arc power: 60 A, bias voltage: $-100 V_{dc}$, deposition time: 1 h).

mechanical properties.

A cytotoxicity test is a screening method to determine whether a material has any toxic effect on living cells due to leachable components, before employing it in a medical device. In previous studies, the biocompatibility of Ti is attributed to surface oxide spontaneously forming in air, or other surface treatments, such as thermal oxidation and anodic oxidation. It is believed that the cellular behavior including proliferation, adhesion, and spreading is greatly influenced by this oxide layer of Ti [13].

In this study, an MTT assay test was used for evaluation. The optical density (OD) of the formazan produced by the L929 fibroblast cells grown on TiAg and ZrAg alloys was measured after 24 hours, as shown in Fig. 3. The OD of formazan reflects the level of cell metabolic activity, with higher OD values indicating a larger number of living cells on the specimen, and hence, better biocompatibility. All of the specimens possessed higher optical density value and it was clearly observed that there was no dif-

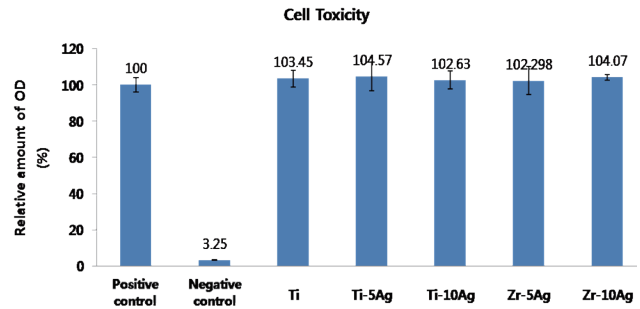


Fig. 3. Cell proliferation test of L929 fibroblast cells, after 24 hours of incubation.

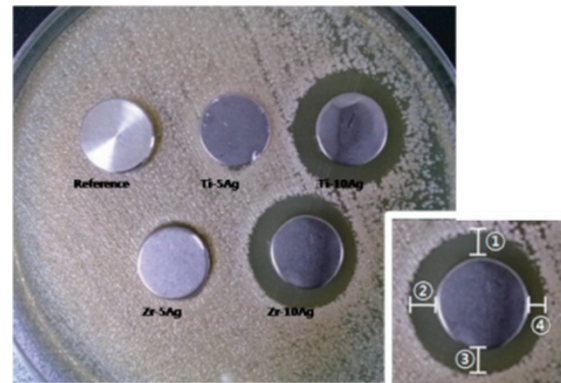


Fig. 4. The clear zone image of each aerobic cultured specimen against *Streptococcus Mutans* (after 18 hours).

ference in the viability and proliferation of L929 fibroblast cells among the specimens. The statistical correlation of the results of cytotoxicity tests was determined by student's t-test. Cytotoxicity of the alloys tested was not statistically different, compared to the positive control and cp titanium ($P > 0.05$).

In previous studies on the responses of soft tissue to the surfaces of oral implants, it has been shown that the surface treatment of the implant materials significantly influences the attachment of oral fibroblasts. By modifying the surface texture of the implant materials, the tissue-implant attachment can be enhanced, resulting in a material that should be at least as good as normal Ti [14].

Previous studies have confirmed the antibacterial activities of Ti-Ag and TiO_2 -Ag coatings against *S. aureus in vitro* [15,16]. Also, Huang et al. observed the effects of doping ZrO_2 coating with Ag and Cu on the antibacterial performance against *S. aureus* and *actinomycetemcomitans*. The antibacterial properties of surface coatings containing Ag and Cu can suppress microbial proliferation [17].

Fig. 4. shows the reaction of *Streptococcus Mutans* to TiAgN and ZrAgN coated specimens. The clear zone, which means the region of antibacterial activity, was identified for 10 wt.% Ag content of TiAgN and ZrAgN specimen. The sizes of clear zone were calculated as the average of four values, and were 3.55 mm and 3.35 mm, respectively (Table 2). Ag is known as one of the most interesting antibacterial materials. The use of a surface coating containing Ag can provide antibacterial action to suppress microbial proliferation, and thereby reduce bacterial counts. It may show a lower probability of implant-related infections [18]. As a result of the antibacterial test, *Streptococcus Mutans* showed inhibited growth, or was sterilized, in the case of over 10 wt.% Ag content specimens.

Table 2. The sizes of clear zone according to specimens.

Clear Zone(mm)	Ref.	Ti-5Ag	Ti-10Ag	Zr-5Ag	Zr-10Ag
①	-	-	3.7	-	3
②	-	-	4	-	3.6
③	-	-	3.5	-	4.1
④	-	-	3	-	2.7
Average	-	-	3.55	-	3.35

4. CONCLUSIONS

TiAg and ZrAg single alloy targets were prepared by VAR, and TiAgN and ZrAgN nanocomposite coatings were fabricated via AIP. At the Ag content of 10 wt.%, there were some residual clusters of Ag particles on the surface, and the thicknesses of TiAgN and ZrAgN thin films were observed to be 923 nm and 1,330 nm, respectively. We investigated the effects of Ag content of TiAgN and ZrAgN coatings on the antibacterial performance to *Streptococcus Mutans* and the L929 fibroblast cells proliferation activity of TiAg and ZrAg alloys. After 24 hours of cell culturing, there was no difference in the viability and proliferation of L929 fibroblast cells, compared to cp titanium control, and no cytotoxic effects were found. The bacterial test shows that the TiAgN and ZrAgN coatings have antibacterial activity, eliminating *Streptococcus Mutans* over 10 wt.% Ag content specimens.

In this study, the TiAgN and ZrAgN nanocomposite coatings on pure Ti materials not only showed remarkable antibacterial effect on *Streptococcus Mutans*, but also met the requirement of L929 fibroblast cells viability. The findings of this study suggest that TiAgN and ZrAgN coatings will have valuable applications in medical devices.

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