Characteristic Studies on the Zr-Based Metallic Glass Thin Film on Antibacterial Capability Fabricated by Magnetron Sputtering Process

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Abastract- Silver and copper are commonly used as antibacterial elements in most of biomedical apparatus. Because silver (Ag) is relatively expensive and has some tissue-toxicity as the concentration of ionized silver reaches a critical value, it is necessary to find an alternative element for treating some kinds of bacterial or fungal infection. Copper-based antibacterial material can be a good candidate because of its lower cost, better antifungal ability, and higher chemical stability than those of silver. A series of ZrCuNiAl thin films are coated on 304 stainless steel by a DC sputtering method with different sputtering power. The amorphous feature of these ZrCuNiAl thin films is confirmed by glancing incident X-ray diffraction (GIXRD). The porosity and surface roughness are observed by field emission scanning electron microscopy (FESEM) and atomic force microscopy (AFM), respectively. The adhesion capability and hardness of these amorphous thin films are conducted by scratch test and nano indentation, respectively. In addition, the organisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter baumannii* and *Candida albicans* are used in this study to evaluate the antimicrobial effects for ZrCuNiAl thin films which coating on 304 stainless steel substrate.

Keyword- Biomedical Antibacterial; Material Amorphous Material; Microstructure; Thin Films; Physical Vapor Deposition (PVD)

I. INTRODUCTION

In hospital, the different microbes such as fungus, bacterial or virus grew well besides the people everywhere [1-3]. Infectious disease related with hospital was so called nosocomial infection. Nosocomial infections might result from endogenous flora (i.e., microbes that were normal commensals of the skin, respiratory tract, gastrointestinal tract, or genitourinary tract), reactivation of latent infectious agents (e.g., Mycobacterium tuberculosis, Pneumocystis carinii, herpes viruses), or exogenous flora (i.e., microbes from the environment) [3-10].

Nosocomial infection was frequent transmission the causative pathogens from the hospital environment (such as air, water, instrument or equipment) especial direct contact the pathogens via the hands of health-care providers to the people in hospital [3]. The causative pathogens were found Staphylococcus aureus (52%), Escherichia coli (8 %), Pseudomonas (6%) and polymicrobial (48%, especially aerobes) in nosocomial infection. In Taiwan, the Acinetobacter baumannii was another important pathogen in recent years [11, 12].

The 304 stainless steel was a material widely used on the hardware for working surfaces and door fittings (such as handles and push plates) and in many medical apparatus (such as surgery tools) in hospital [9, 13, 14]. Therefore, to improve the environment of the hospital organism-growth on the area where touched frequently by most people, such as 304 stainless steel door-contactor, it might be an excellent method to control the nosocomial infection.

The first step of getting infection should be good adhesion between microbes and people. Using atomic order amorphous metal thin films to change the hospital environment might be an effective way to control the nosocomial infection [1, 4, 10, 13, 15, 17, 18].

Among Mg-, La-, Ti-, Zr-based systems metallic glasses, the Zr-based amorphous alloys were particularly interesting because they had a high glass-forming ability with critical cooling rates as low as 1 k/s and had an extremely wide supercooled liquid region exceeding 100K[16, 19-28]. In addition, Zr-based bulk amorphous alloys exhibited good engineering properties of high tensile strength, high elastic modulus, relatively high impact fracture energy, and high corrosion resistance [25, 29-33]. Moreover, Zr-based bulk amorphous alloys exhibited relatively good thermal stability, homogeneous distribution of multiple elements, and easy fabrication[16, 19-24, 29, 34, 35]. In this study, we focus on evaluating the effect of sputtering power on the microstructural, mechanical, and antimicrobial properties of ZrCuNiAl thin films on 304 stainless steel substrate.

II. EXPERIMENTAL

The as-prepared bulk metallic glass ZrCuNiAl was selected as the target material of the vacuum direct current (DC) MDX1000 sputtering system with Varian V550 turbo pump. Sputtering involves firing ions toward a ZrCuNiAl bulk amorphous alloy target to displace atoms, which were then deposited on a 304 stainless steel substrate to form a ZrCuNiAl thin

film. The operation condition of the DC sputtering system was set as base pressure 6 x 10^{-6} torr, working pressure 4 mtorr, Argon flow 5.4 sccm, sputtering time 30 minutes, and sputtering power 15 W, 20 W, 25 W, 30 W, 35 W and 40 W respectively.

The amorphous feature of these ZrCuNiAl thin films was confirmed by the PANalytical X'Pert Pro glancing incident X-ray diffraction (GIXRD). The composition distribution of these Zr-based alloy thin films was analyzed by HORIBA energy dispersive spectrum (EDS) analysis of Hitachi S4700 field emission scanning electron microscopy (FESEM). The porosity and surface roughness were examined by FESEM and NT-MDTP7LS atomic force microscopy (AFM), respectively. The alpha step was conducted to measure the thickness of ZrCuNiAl thin films which prepared by different sputtering power. The adhesion capability between thin film and 304 stainless steel substrate was evaluated by a TEER 2330 scratch tester under a maximum load of 3 kg. Nano-indentation was applied to measure the hardness of ZrCuNiAl thin films associated with sputtering power at 30 W.

For evaluating the bactericidal, bacteriostatic or mycostatic effect [2, 36] of these ZrCuNiAl thin films, the organisms such as Staphylococcus aureus(ATCC 25923), Pseudomonas aeruginosa (ATCC 27853) , Escherichia coli (ATCC 25922), Acinetobacter baumannii (isolated from patient) and Candida albicans (isolated from patient) were isolated. A drop of 0.1 ml of different microbes from incubated tubes respectively, which corresponding to the bacterial count 7.5 x 10^7 colony/ml (under Vitek colorimeter keeping 80-88% turbidity), was sampled and placed on each ZrCuNiAl thin film and 304 stainless steel plate. Then the ZrCuNiAl thin film and 304 stainless steel which with microbes on their surface were placed above the Mueller-Hinton (M-H) agar plate and were incubated in a Mueller-Hinton agar plate (consists of beef soup 300gm, peptone 17.5gm, starch 1.5gm and agar 17.0gm) at 37.5℃ for evaluating the antimicrobial effects of these ZrCuNiAl thin films after different incubation time, namely 24, 48, and 96 hours. The different microbial areas at 24, 48, and 96 hours cross different photography size of same sample were calculated by the software of Image-pro-Plus to adjust the bactericidal, bacteriostatic or mycostatic effect. Streaking some tissue from surface of the infected material (ZrCuNiAl or 304 stainless steel) and Mueller-Hinton agar respectively was transferring for inoculation of blood agar plate (BAP) and eosin-methylene blue agar (EMB) for semi-quantitative colony counts under aseptic procedure. These organisms were identified by two agar mediums (BAP and EMB) without unfavorable bias.

III. RESULTS AND DISCUSSION

A. Properties Characterization of ZrCuNiAl Thin Film

The thickness of ZrCuNiAl thin film as a function of sputtering power as shown in Fig.1 exhibits a reasonably linear relations.

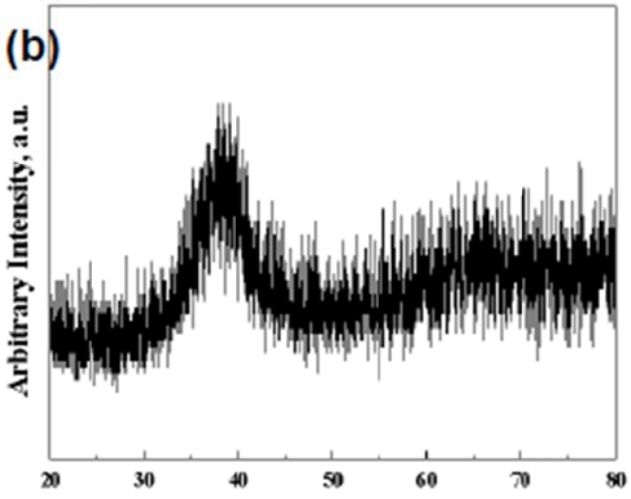

Fig. 1 X-ray pattern of as-deposited ZrCuNiAl Thin film

A 550 nm thickness of ZrCuNiAl thin film can be fabricated within 30 min under 40 W sputtering power. In parallel, the result of TEM diffraction reveals that a typical amorphous feature for all of these ZrCuNiAl thin films which produced by different sputtering power, as shown in Fig. 2.

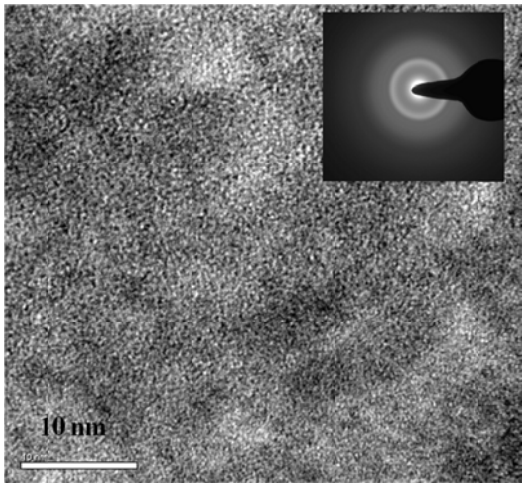

Fig. 2 Bright-Field TEM images with selected area diffraction pattern of ZrCuNiAl

Because these thin films are much thin, several sharp peaks that belong to the crystalline structure of 304 stainless steel occur on the TEM diffraction patterns as shown in Fig. 2. The compositions of ZrCuNiAl bulk metallic glass keep as same as sputtering ZrCuNiAl thin films under energy dispersive spectrum (EDS) examination. The sputtering procedure may be not even on 304 stainless steel substrate, the compositions of specimens shows somewhere had target material, somewhere had substrate material which produced by sputtering power of 15W. The un-homogenous distribution [29, 35] is found in the low sputtering power, such as 15 W. When Cr, Fe elements [37-38] are submitted from the plate, the percentage of Zr, Ni, Al, Si [39-43] will be changed, not like more than 20W.

The surface roughness is measured by AFM as a function of sputtering power as shown in Fig. 3 presents a decreasing trend with sputtering power. The roughness about 1 nm can be obtained for the thin film which is prepared by the sputtering power more than 25 W.

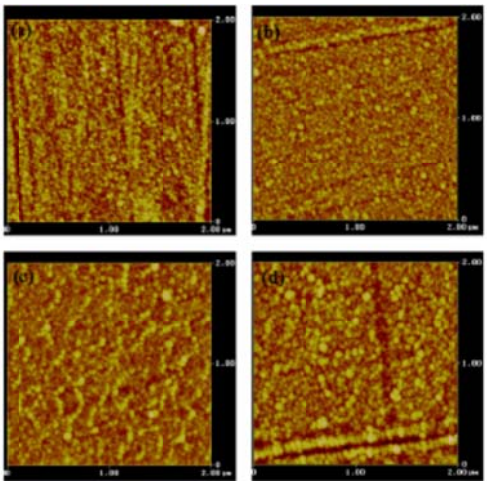

Fig. 3 The AFM images of ZrCuNiAlthin films deposited with different sputtering powers on SUS304 stainless steel: (a)15, (b)20, (c)25 and (d)30 watt

The result of scratch test reveals that the adhesion capability [25, 29, 33, 34] increases with sputtering power, a saturated value of 70 N occurs on the thin film which coated by the sputtering power more than 35 W. This value is compatible with the adhesion capability of commercial hard coating, such as CrN on tungsten carbide tool. In manufacturing, ZrCuNiAl thin film sputtering on 304 stainless steel substrate is an acceptable procedure.

The hardness of ZrCuNiAl thin film which produced by 30W sputtering power is measured to be an average of 5.83±0.41 GPa and 6.01±0.17 GPa under the loading of 2500iN and 3000iN, respectively.

B. Evaluation of the Antimicrobial (Bactericidal, Bacteriostatic, or Mycostatic) Effects

Cu-ions will kill bacteria by destroying cell walls, cell membranes and directly exerts. That the antibacterial has influence because of Cu-ions have strong reduction and can extract the electrons from the bacteria, causing their cytoplasm to run off and oxidizing their cell nucleus.

Therefore, in ZrCuNiAl amorphous thin film, the nano-structured surface (which has about 1 nm roughness) and homogenous distributed atomic-scaled Cu-rich cluster [10, 29, 35, 49-53] may play an important role on bactericidal effect.

Different microbes own different bio-adhesion. The adhesin is microbial molecules. A single adhesin may have more than one receptor, and a single receptor may be recognized by many different adhesins. Adherence to host (or environment) is the initial interaction of a pathogenic microorganism. Bacterial adhesion to solid surfaces is due to the relationship between the number of adhering bacteria and the wettability or surface free energy. In the double control study of bactericidal or bacteriostatic or mycostatic effect of ZrCuNiAl thin films, the different isolated organisms are incubated in Mueller-Hinton (M-H) agar plate for 24 hours, 48 hours and 96 hours under 37.5°C, as shown in Fig. 5. Different microbe [2] is dropped on ZrCuNiAl thin film and control (304 stainless steel) under aseptic procedure respectively.

For Escherichia coli [9, 55] (Gram positive cocci), the ZrCuNiAl thin film has the bacteriostatic effect for 24 hours as shown in Fig. 4.

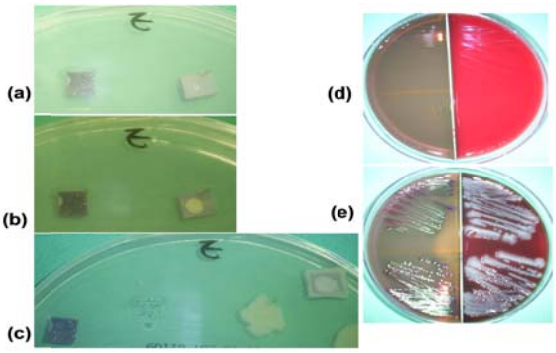

Fig. 4 (a, b, c) Escherichia coli (Gram negative bacilli) on M-H plate ZrCuNiAl thin film (Lt side) and 304 stainless steel (Rt side), (a) for 24 hrs, (b) for 48 hrs, (c) for 96hrs; (d, e) scratching tissue from agar surface & material surface to BAP(Rt) and EMB(Lt) for 24 hrs, (d) for ZrCuNiAl thin film, (e) for 304 stainless steel

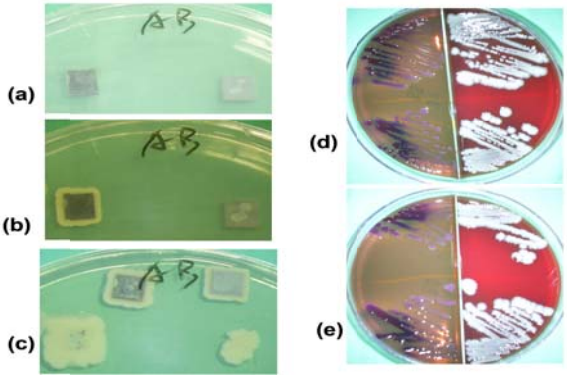

Fig. 5 (a, b, c) Acinetobacter baumannii(Gram negative cocci)on M-H plate ZrAlNiCuSi thin film(Lt side) and 304 stainless steel(Rt side), (a) for 24 hrs, (b) for 48 hrs, (c) for 96hrs; (d, e) scratching tissue from agar surface & material surface to BAP (Rt) and EMB (Lt) for 24 hrs, (d) for ZrAlNiCuSi thin film, (e) for 304 stainless steel

For Pseudomonas aeruginosa (Gram negative bacilli), thin film has bacteriostatic effect within 24 hours, but grown well following 48 hours as illustrated in Fig. 4. For Escherichia coli [55-57] (Gram negative bacilli), the ZrCuNiAl thin film has bactericidal effect for 96 hours as shown in Fig. 5. For Acinetobacter baumannii [11, 12] (Gram negative coccobacilli), thin film has bacteriostatic effect within 24 hours, but grown well following 48 hours as illustrated in Fig. 6. For Candida albicans [17, 18, 36, 56] (fungus), ZrCuNiAl thin film has mycostatic effect within 48 hours than 304stainless steel.

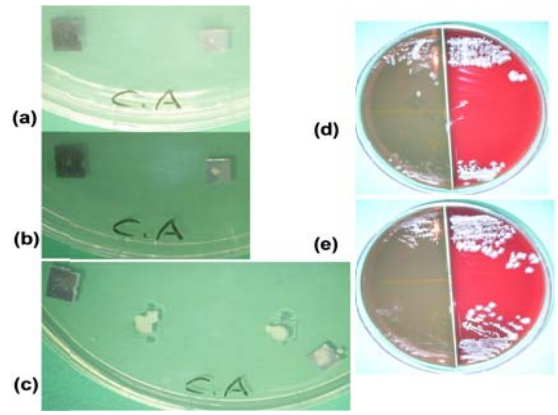

Fig. 6 (a, b, c) Candida albicans (Fungus) on M-H plate ZrAlNiCuSi thin film (Lt side) and 304 stainless steel (Rt side), (a) for 24 hrs, (b) for 48 hrs, (c) for 96hrs; (d, e) scratching tissue from agar surface & material surface to BAP (Rt) and EMB (Lt) for 24 hrs, (d) for ZrAlNiCuSi thin film, (e) for 304 stainless steel

After placing the specimens (ZrCuNiAl thin film and 304 stainless steel with microbes on surface) above the Mueller-Hinton agar plate, incubating for 24 hours, 48 hours and 96 hours, these ZrCuNiAl thin films was found to exhibit the bactericidal or bacteriostatic effect in comparison with 304 stainless steel plate. Using the software of Image-pro-Plus to calculate the areas of different microbes at 24, 48, and 96 hours respectively, then cross different photography size of same specimens (ZrCuNiAl thin film and 304stainless steel) at 24, 48, and 96 hours make adjustment to evaluate the bactericidal, bacteriostatic or mycostatic effect.

Streaking some tissues [2] from each surface of the infected material (ZrCuNiAl or 304 stainless steel) and Mueller-Hinton agar transfer for inoculation of blood agar plate (BAP) and eosin-methylene blue agar (EMB) for semi-quantitative colony counts under aseptic procedure. The Staphylococcus aureus (ATCC 25923), Pseudomonas aeruginosa (ATCC 27853), Escherichia coli (ATCC 25922), Acinetobacter baumannii (isolated from patient) and Candida albicans (isolated from patient) were identified by two agar mediums (BAP and EMB) without unfavorable bias as showed Fig. 6.

In a serial study, ZrCuNiAl thin films have better bactericidal effect for Escherichia coli than 304 stainless steel plate after 96 hours incubation time. For the microbes of Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumannii and Candida albicans, ZrCuNiAl thin films exhibit good microbiostatic effect in these four kinds of microbe within first 24 hours. For incubating Staphylococcus aureus and Candida albicans 24 hours to 48 hours, ZrCuNiAl thin films present better microbiostatic effect than 304 stainless steel.

In addition, there is no significant difference on antimicrobial (bactericidal, bacteriostatic or mycostatic) effects for the ZrCuNiAl thin films which prepared by different sputtering power (35W or 40W).

IV. CONCLUSION

According to the results of X-ray diffraction, AFM examination, scratch test, nano-indentation, bacterial incubation test, and bacterial identification test for the ZrCuNiAl thin film coating on 304 stainless steel, the evaluation of microstructures and antimicrobial capabilities of thin film can be summarized as:

Microstructure analysis of X-ray diffraction confirms that an amorphous state can be achieved for the ZrCuNiAl alloy thin film fabricated by DC sputtering method. A 550 nm thickness of ZrCuNiAl thin film can be fabricated within 30 min under 40W sputtering power. The surface profile of the ZrCuNiAl film exhibits the very smoothly in morphology with surface roughness about 1 nm can be obtained for the thin film which was prepared by the sputtering power more than 25 W. A saturated value of 70 N occurs on the thin film which coated by the sputtering power more than 35W. This value is compatible with the adhesion capability of commercial hard coating, such as CrN on tungsten carbide tool. In manufacturing, ZrCuNiAl thin film sputtering on 304 stainless steel substrate is an acceptable procedure. The hardness of ZrCuNiAl thin film which produces by 30W sputtering power is measured to be an average of 5.83±0.41 GPa and 6.01±0.17 GPa under the loading of 2500 ìN and 3000 ìN, respectively. This value is harder than the commercial tool steel. Using well mechanical properties of sputtered ZrCuNiAl thin films to different pathogens, ZrCuNiAl thin films have better bactericidal effect for Escherichia coli than 304 stainless steel plate after 96 hours incubation time. For the microbes of Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumannii and Candida albicans, ZrCuNiAl thin films exhibit good microbiostatic effect in these four kinds of microbe within first 24 hours. For incubating Staphylococcus aureus or Candida albicans 24 hours to 48 hours, ZrCuNiAl thin films present better microbiostatic effect than 304 stainless steel.

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