The Antimicrobial Activity and Characterization of the Cast Titanium Copper Alloys with Variations of Copper Content

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Abstract. The effect of Cu addition on antimicrobial activity of Cast Ti-Cu Alloys against Staphylococcus aureus were studied. The Ti-Cu alloys were prepared with a variation of 0.5, 1, 2, 3, 4, 8 and 10 wt% of copper. Pure Ti metal was also prepared and tested as a comparison. The antibacterial was performed by killing activity test of colony forming unit (CFU) method with variation of contact time. Optical microscope observation, XRD, and the Vickers hardness test were carried out to characterize the investigated alloys. The results showed that Ti-Cu alloys were able to decrease the amount of bacteria by time. However, the activity of killing of bacteria in the varied range of Cu content did not show significantly different result. The highest bacterial kill ability in the alloy was observed in addition of 3 wt% Cu.

Keywords: Antimicrobial alloy, Ti-Cu alloys, Copper content, Staphylococcus aureus

1. Introduction

Titanium has been used as a biomaterial applied to dental, implant and cardiac valve retaining structures [1]. Titanium which is very light and has good mechano-chemical is more tolerant to the human body than stainless steel and cobalt alloys. It can also form a layer of titanium oxide (TiO2) quickly and spontaneously in surface called as a passive layer, so it is not dissolved in interstitial fluid and prevents the release metal ions which can react with tissue [2].

However, titanium has no antimicrobial properties so serious infections often occur during implant surgery. One of the bacteria which causes infection in the implant is the bacteria of Staphylococcus aureus (S. aureus) and Staphylococcus epidermis (S. epidermis). The infection which occurs in the implant has reached 2–30% of cases found in various literature. Mahan [3] reported that 75% of infections occurred in the pin tract region. S. aureus and S. epidermis bacteria form biofilm layers which are difficult to clean clinically because they are protected by phagocytosis and antibiotics [3]. Direct microbial contamination of opened wounds (such as
postoperative wounds) or post-traumatic infections (such as chronic osteomyelitis after opened fracture) is the cause of nosocomial infections [4,5].

Antimicrobial properties can be obtained with the addition of copper elements. Copper has an antimicrobial nature which is proven to rapidly kill bacteria and in the future copper alloys will be introduced as surfaces and objects that humans frequently touch [6]. The copper toxin is caused by Cu (I) ions in the bacterial cell. Cu (I) ions can damage cell membranes and kill bacteria. In a bacterial cell, there is an equilibrium of copper content or a homoeostatic condition, there is an excess copper exhaust mechanism by reducing copper to cuprous or Cu (I) ions. This Cu ion (I) is toxic for bacteria [7].

The amount of Cu released from the copper surface can increase significantly when contaminated with bacteria. This can occur due to the difference in the concentration of copper between the metal surface and the solution [8]. In addition, titanium combined with copper is reported [9] that it has good biocompatible properties, high corrosion resistance, low melting temperature, and it is easy when casting. Since 1950, many studies have focused on Ti-Cu alloys for industrial applications. The results of investigation show that Ti-Cu alloys have high mechanical strength and ability to be easily formed.

In the last 20 years, titanium dental foundry technology has made great progress. Kikuchi [10] showed that the Ti-Cu alloy as cast has a high yield strength reaching 650 MPa, a value greater than pure Ti and Co-Cr alloy dental casting. Yao et al. [11] showed that heat treatment could strengthen Ti-2.5% Cu alloy by forming Ti2Cu intermetallic particles after the β phase was decomposed into αTi and Ti2Cu.

Titanium applied as an implant combined copper become antimicrobial implant prevents the emersion of infection. Investigation of the antibacterial activity of Ti-Cu alloys has been carried out [12,13]. However, studies covering a larger percentage of Cu content are still very limited. Nevertheless, studies for low grade copper have not been performed on the casting process. The results of these studies will be compared with the other studies to determine the properties of antimicrobial in low grade Cu.

2. Materials and Method

The Ti-Cu samples used in this investigation included alloys with seven Cu variants 0.5, 1, 2, 3, 4, 8, and 10 wt%. Samples were produced by mini DC EAF furnace using a non-consumable tungsten electrode and water-cooled copper crucible in argon atmosphere with current flowed 70 A for 3 minutes. Five samples were cut in size of 100×50×20 mm, then homogenized at 800°C for 48 hours using muffle and furnace cooled to be prepared in the observation of microstructures, mechanical behavior, X-ray diffraction (XRD), and antimicrobial tests. Microstructural characterization was performed by optical microscopy and X-ray diffraction. Metallographic preparation included sandpapering with SiC paper in 60, 150, 240, 320, 400, 600, 800, 1000, 1500, and fineness of 2000 grit. Polishing used diamond and ethyl alcohol as a lubricant, and etching used “Kroll Etchant” with a solution of 5 vol.%HF, 10 vol.%HNO3, and 85 vol.% H2O, for 10-minutes dipping time.
Antimicrobial test began with the preparation of medium, physiological solution, bacterial culture, and bacterial colony-killing test. Nutrient broth (NB) is a liquid medium used to grow the inoculant of *S. aureus* bacteria in solution form. It was made by adding 13 g of nutrient broth powder to one litre of distilled water. After that, mixed, heated and sterilized. Nutrient agar (NA) is medium to observe the growth of bacterial colonies, made of 28 g of nutrient agar powder in one litre of distilled water and stir using magnetic stirred. Furthermore, nutrient agar was sterilized and poured in every sterile petri dish each 10−15 ml. Physiological solutions were used to inhibit bacterial growth, created by 0.85%(w/v) NaCl and dissolved in aquades. These solutions were inserted in every falcon tube for 9 ml, then sterilized. The entire mediums, solutions, and equipment for antimicrobial test should be sterilized in the autoclave for 20 minutes to prevent contamination. Fig. 1 shows schematic dilution of physiological solution to incubation in petri dishes at 24 hours.

The first step for liquid bacterial cultures was the bacterial culture stock of *S. aureus* in the tube taken with oce wire (sterilized by burning on fire bunsen) swiped at agar surface and transferred in sterile nutrient broth up to 2–3 oce. Medium nutrient broth which had been inoculated *S. aureus* bacteria then incubated in orbital shaker at room temperature for 18−24 hours before used. The bacterial colony-killing test was begun by cleaning laminar using 70% alcohol and exposed by UV light for 5−10 minutes. *S. aureus* culture was taken 1 ml with a micropipette and inserted in a falcon containing 9 ml physiological solution, then shaken by vortex for 15−20 seconds.

For the second step, the alloy samples taken with tweezers in an alcohol bath was dried near fire, and incorporated in physiological solution content 1 ml of bacterial culture (dilution 0), shaken by vortex for 15−20 seconds. The physiological solution in the falcon was the sample
stock. The solution was taken at dilution 0 and fed into 9 ml of physiological solution up to the 7th dilution. Each physiological solution at 5th, 6th, 7th dilution were taken using 0.1 ml micropipette and included in a petri dish containing sterile nutrient agar, thereupon flattened it in petri dish used L stick until dry. The second step were repeated with the duration of sample immersed in 1, 2, 3 hours in sequence. Petri dishes which been dried in the seal were incubated for 18–24 hours, then counted the number of the bacterial colonies formed on each petri dish.

3. Result and Discussion

3.1. Microstrutural characterization

Microstructure of Ti-Cu alloy with contents of 0.5, 1, 2, 3, 4, 8, 10 wt% Cu was examined. Fig. 2 shows the Ti-Cu alloy binary phase diagram [14]. In this phase diagram, the maximum solubility of Cu in Ti is 2.1 wt% Cu at 790°C, and the eutectoid point is in the composition of 7.1 wt% Cu. Above eutectoid temperature (790°C) Ti-Cu alloy containing 0.5–2 wt% Cu is in the alpha-Ti region. Alloy Ti-Cu with content of 3 and 4 wt% Cu is in the region between α-Ti and β-Ti. While alloys with 8 and 10 wt% content are in the beta-Ti and Ti2Cu regions.

![Fig. 2. Ti-Cu binary phase diagram [14]](image)

Homogenization at 800°C for 48 hours followed by furnace cooling will provide a phase and microstructure that correspond to the Ti-Cu phase diagram. Below eutectoid temperature, the
following reactions occurred. In the Ti-Cu alloys with composition in 0.5–2 wt% Cu intervals, Ti\(_2\)Cu particles undergo precipitation from the saturated \(\alpha\) phase due to the decreasing solubility of Cu at alpha phase with decreasing temperature from homogenization temperature (800°C) to room temperature:

\[
\alpha \text{ (supersaturated)} \rightarrow \alpha + \text{Ti}_2\text{Cu} \quad (1)
\]

For Ti-3 wt% Cu and Ti-4 wt% Cu alloys, below eutectoid temperature, \(\alpha\) (eutectoid) phase is formed from \(\beta\) phase with Ti\(_2\)Cu particles precipitated. The \(\alpha\) (proeutectoid) phase is formed from saturated \(\alpha\) phases, as follows:

\[
\alpha \text{ (proeutec.)} + \beta \rightarrow \alpha \text{ (proeutec.)} + \alpha \text{ (eutec.)} + \text{Ti}_2\text{Cu} \text{ (eutec.)} \quad (2)
\]

For Ti-8 wt% Cu and Ti-10 wt% Cu alloys, below eutectoid temperature, \(\alpha\) (eutectoid) and Ti\(_2\)Cu phases are formed from \(\beta\) phase, as follows:

\[
\beta + \text{Ti}_2\text{Cu} \text{ (proeutec.)} \rightarrow \alpha \text{ (eutec.)} + \text{Ti}_2\text{Cu} \text{ (eutec.)} + \text{Ti}_2\text{Cu} \text{ (proeutec.)} \quad (3)
\]

Fig. 3 shows the microstructure of Ti-Cu alloys with variations of 0.5, 1, 2, 3, 4, 8 and 10 wt% Cu. In Figs. 3a-c which shows the Ti alloy with a content of 0.5–2 wt% Cu, the visible microstructure is \(\alpha\)-Ti and Ti\(_2\)Cu intermetallic. \(\alpha\)-Ti is indicated by light phase while Ti\(_2\)Cu is shown by a dark phase in the form of fine elongated particles. The Ti\(_2\)Cu intermetallic particles became more pronounced by increasing wt% Cu. For Ti-3% Cu, the elongated precipitates begin to appear denser. At 4 and 8 wt% Cu (Figs. 3e & 3f), the eutectoid lamellar (dark colour) structure appears to be clearly observed. Meanwhile in Fig. 3g, hypereutectoid structure appear for 10 wt% Cu where lamellar structures are present with Ti\(_2\)Cu intermetallic phases. In this figure the lamellar structure is shown by a dark color while the Ti\(_2\)Cu (proeutectoid) precipitate is indicated by the bright phase. This lamellar structure has also been previously observed by Hayama [15] who studied Ti-Cu alloys for hypoeutectoid, eutectoid and hypereutectoid compositions.

Fig. 4 shows the relationship between Cu content in Ti-Cu alloys and Cu content in \(\alpha\) or Ti\(_2\)Cu phases. The Cu content of the phases is calculated simply by using Ti-Cu phase diagrams for variations of Cu content in Ti-Cu alloys. In the figure, it can be seen that the Cu content in Ti\(_2\)Cu continues to increase with increasing Cu content in the alloy, while the Cu content in the \(\alpha\) phase is relatively unchanged. This relationship needs to be made to consider the presence of Cu elements in solid solutions or intermetallic compounds in their role in bacterial killing activities.
Fig. 3. The optical micrographs of Ti-Cu alloys with variations in copper content of (a) 0.5 wt% (b) 1 wt% (c) 2 wt%, (d) 3 wt% (e) 4 wt% (f) 8 wt% and (g) 10 wt%. The microstructure is revealed by using Kroll's reagent.
The presence of phases in the Ti-Cu alloy was also examined using XRD. Fig. 5 presents the XRD pattern of the studied Ti-Cu alloy. The Ti-Cu alloy tested was Ti-Cu alloy containing Cu of 1 and 10 wt%. In the figure, diffraction peaks can be found for the alpha and Ti$_2$Cu phases. Furthermore, it was seen that in the addition of 10 wt% Cu, Ti$_2$Cu phase diffraction peaks were apparent and increased.

Fig. 5. XRD patterns for (a) Ti-1%Cu and (b) Ti-10%Cu alloys
3.2. Mechanical behavior

Fig. 6 shows the relationship between hardness and variation of Cu content in Ti-Cu alloys. Pure titanium is also shown as a comparison. The hardness of pure titanium was 181 HV, while the Ti-Cu alloys had hardness between 301–647 HV which was higher than pure titanium. In the figure, it can be seen that the addition of Cu to titanium increases the hardness of the alloy. In general, the higher the copper content, the higher the hardness of Ti-Cu alloys. At a content of 10 wt% Cu, the hardness reached the highest compared to other wt% Cu. The increase in hardness at the addition of 10 wt% Cu on Ti-Cu alloy is mainly due to the increase in the hard phase of Ti2Cu intermetallic compounds. Increased hardness occurs due to the strengthening mechanism of solid solution hardening of α phase and precipitation strengthening with the presence of Ti2Cu. This mechanism inhibits the movement of dislocations thereby increasing the hardness of the Ti-Cu alloy. High hardness will provide better wear resistance for implants. High hardness itself tends to provide high strength. Increased hardness with increasing Cu content is in accordance with the results obtained by Kikuchi [16]. However Hayama [15] reported different results in which an increase in Cu content did not increase hardness, and he even found a slight decrease in hardness with increasing Cu content in Ti-Cu alloys.

3.3. The effect of Cu composition Ti-Cu alloys to antimicrobial properties

Fig. 7 shows colonies of S. aureus bacteria incubated after 24 hours at 6th dilution. Figs. 7a and 7b are colony images for Ti-1% Cu alloys exposed for 0 and 3 hours. Figs. 7c and 7d are images of colonies for Ti-2% Cu alloys which are exposed for 0 and 3 hours. Figs. 7e and 7f are images of colonies for Ti-3% Cu alloys which are exposed for 0 and 3 hours. In the left figures (7a, 7c
and 7e), bacterial colonies were clearly observed, with nearly the same amount at the 6th dilution at 0-hour exposure. But after a 3-hours exposure, the bacterial colonies seemed to shrink and decrease. It is seen that Ti-Cu alloy is able to kill and reduce the bacterial colonies over time.

Fig. 7. Photographs of bacterial colonies after incubation at the 6th dilution for alloys: (a) Ti-1% Cu at 0 hour, (b) Ti-1% Cu at 3 hour, (c) Ti-2% Cu at 0 hour, (d) Ti-2% Cu at 3 hour, (e) Ti-3% Cu at 0 hour and (f) Ti-3% Cu at 3 hour
Fig. 8 shows a graph of the ability to kill bacteria by Ti-Cu alloys. In the figure, it can be seen the relationship between the time of exposure of Ti-Cu alloys to the number of bacterial colonies. The exposure period is carried out with a time variation of 0, 60, 120 and 180 minutes. The number of bacterial colonies is seen decrease with increasing the exposure time by Ti-Cu alloys. Pure titanium was also tested as a control variable in this experiment.

![Graph showing the killing power of bacterial colonies by Ti-Cu alloys](image)

**Fig. 8. The killing power of bacterial colonies in Ti-Cu alloys**

From Fig. 8 it can be seen that pure titanium appears to reduce bacterial colonies. However, pure titanium does not have the ability to kill bacteria except in the form of TiO$_2$ compounds. In this case, titanium is oxidized due to cutting of the sample during preparation which produces heat and forms a TiO$_2$ layer on the surface area. Sanding and sterilization using alcohol is not enough to remove this layer, so TiO$_2$ also kills bacteria and decreases the number of bacterial colonies. TiO$_2$ has photocatalyst properties that can absorb light and produce various chemical reactions. UV rays with H$_2$O and O$_2$ react with TiO$_2$ layers to form hydroxy (OH •) radicals on the surface and kill bacteria [17]. However, the killing power of TiO$_2$ is insignificant compared to Cu ions.

Increasing copper content in the Ti-Cu alloy is expected to increase the number of dead bacterial colonies. However, the results obtained from the test did not prove the statement. Fig. 9 shows a bar graph of the number of bacterial colonies that died during 3-hour exposure by Ti-Cu alloys. In the figure there is no tendency to decrease the number of bacterial colonies with variations in Cu content. Alloy with a content of 0.5 wt% Cu even has the ability to kill distant
bacteria compared to 10 wt% Cu. Together with Ti-1% Cu and Ti-4% Cu, Ti-10% Cu showed the ability to kill low bacteria in a 3-hour exposure interval. Meanwhile the Ti-Cu alloy with Cu content of 2, 0.5 and 3 wt% Cu showed the highest ability to kill bacteria, respectively.

![Graph showing number of dead bacterial colonies after exposure to Ti-Cu alloys]

**Fig. 9.** Number of dead bacterial colonies after being exposed to Ti-Cu alloys for 3 hours

Based on the theory, copper can kill bacteria by releasing Cu ions on the surface and dissolving into the surface of bacteria which then causes the cell membrane to break so that the components inside are lost. Stainless steel combined with copper has been designed and fabricated. In this study, it was explained that copper can release Cu ions from steel so that the corrosion formed in the steel is minimal in the biological environment [18].

Recommended daily intake of Cu for an adult per day by WHO is 2-3 mg [19]. Ma et al. [20] investigated the cytotoxicity of Cu on the human body stating that the daily average release Cu for a typical 50×15×2 mm bone fixation plate made of Ti-6Al-4V-5Cu Alloy is about 0.0001 µg, which is far below the recommended number. Therefore, the use of Ti-Cu alloy with 10 wt% Cu can be considered still in a safe range.

Antibacterial properties that did not show an increase in the addition of Cu to Ti-Cu alloys studied and even without a tendency were thought to be closely related to the state of the Cu element in the alloy, namely whether it is in a solute state in the α phase or as Ti2Cu precipitates. Ma et al. [20] stated that the Ti-6Al-4V-5Cu alloy was easier to release interstitial Cu atoms than in the Ti2Cu stable phase. Previously, in Fig. 2 we have shown a relationship between Cu content in Ti-Cu alloys and Cu content in α or Ti2Cu phases. In the figure it is shown that the number of Cu atoms in the α phase is relatively unchanged, while the number of atoms in Ti2Cu increases with increasing Cu content in the Ti-Cu alloys. An increase in the amount of Ti2Cu
does not give a role in antibacterial ability. Thus, it is the Cu atoms in the \( \alpha \) phase which are easier to release from the alloy and play a role in killing bacterial colonies.

4. Conclusions

Ti-Cu alloys with content of 0.5–3 wt% Cu shows \( \alpha \)-Ti and Ti\(_2\)Cu intermetallic elongated particles. On the addition of 4 wt% Cu, the alloy showed the presence of eutectoid lamellar structure. Meanwhile, the hypereutectoid structures (lamellar structures and Ti\(_2\)Cu phases) appear for the addition of 8 and 10 wt% Cu. The addition of Cu to titanium increases the hardness of the Ti-Cu alloy. The higher the copper content, the higher the hardness of Ti-Cu alloys. At a content of 10 wt% Cu, the hardness of the alloy reaches the highest value compared to hardness at the addition of other wt% Cu. There was no strong correlation between antimicrobial ability and variation of Cu content in Ti-Cu alloys. The highest bacterial kill rate is found in addition 3 wt% Cu. The Ti\(_2\)Cu phase increases with increasing Cu content in the alloy, but does not provide a significant role in antibacterial ability. The Cu atom in the alpha phase which is more easily released from the alloy, plays a role in killing bacterial colonies.

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6. References

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