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Effect of Cu content on the antibacterial activity of titanium–copper sintered alloys

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ABSTRACT

The phase constitution and the microstructure Ti–x Cu (x = 2, 5, 10 and 25 wt.%) sintered alloys were investigated by XRD and SEM and the antibacterial activity was assessed in order to investigate the effect of the Cu content on the antibacterial activity. The results have shown that Ti_2Cu was synthesized as a main secondary phase in all Ti–Cu alloys while Cu-rich phase was formed in the alloys with 5 wt.% or more copper. Antibacterial tests have showed that the Cu content influences the antibacterial rate seriously and only the alloys with 5 wt.% or high Cu have a strong and stable antibacterial rate, which indicates that the Cu content in Ti–Cu alloys must be at least 5 wt.% to obtain strong and stable antibacterial property. The Cu content also influenced the Cu ion release behavior. High Cu ion release concentration and high Cu ion release rate were observed for Ti–Cu alloys with high Cu content. It was concluded that the Cu content affects the Cu existence and the Cu ion release behavior, which in turn influences the antibacterial property. It was thought that the Cu-rich phase should play an important role in the strong antibacterial activity.

1. Introduction

Titanium and its alloys are widely used in the orthopedic and prosthodontic field because of their excellent biocompatibilities, mechanical characteristics, corrosion resistances and processibility. However, as a kind of bioinert materials, commercial available pure titanium and titanium alloys do not have bactericidal capability shortly after the implantation, the dental plaques can be identified around the implanted dentures [1,2]. The bacterial infection might lead to implant loosening even implantation failure [3]. Common causes of implant-associated infections are *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*) [4,5]. Therefore, strict antiseptic operative procedures are adopted to prevent from the clinical infection.

From the point view of implant materials, implants with antibacterial activity have been investigated in order to reduce the infection rate, such as antibacterial surface coated titanium implants and antibacterial stainless steel implants. Surface modification has been proven to be an effective way to provide implants with antibacterial activity in the past decades. During these studies, Ag [6–9], Cu [10–12] and Zn [13] were widely used as antibacterial agents. By the addition of Cu element followed by proper heat treatment, antibacterial stainless steel was produced with excellent antibacterial property [14–17]. In our previous study [18], it has been proven that Ti–10 wt.% Cu sintered alloy exhibited strong antibacterial property against *Escherichia coli* and *S. aureus* with an antibacterial rate of 99.9%. Shirai [19] also reported that Ti–(1 wt.% and 5 wt.%) Cu alloys showed antibacterial property with an antibacterial rate of 30%. In the studies of the antibacterial activity, it has been confirmed that the antibacterial activity of a Cu-surface modified cp-Ti [10] and Cu-containing steel [20] is strongly dependent on the Cu content.

Recently, copper element was selected as an alloying element to produce a low melting point cast alloy for dental application [21–24]. It was reported that the copper content in the titanium alloys influenced significantly the mechanical properties, such as elongation, modulus and hardness. In addition, titanium alloy with small amount of copper is reported to have adequate biocompatibility [25] and corrosion resistance for dental use [26,27]. However, excess copper intake causes stomach upset, nausea, and diarrhea and can lead to tissue injury and disease [28].

From above analysis, Ti–Cu alloy is considered as a candidate dental material and shows antibacterial activity. The Cu content in Ti–Cu not only affects the mechanical properties of the alloy, but also determines the final antibacterial activity and the biocompatibility. It is always desired to prepare Ti–Cu alloy with good antibacterial property and good mechanical property. Therefore, in this paper, the effect of the Cu content on the antibacterial property and mechanical property as well as the Cu ion release behavior has been investigated to understand the relationship between the chemical composition, the microstructure and

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phase constitute, the mechanical property and the antibacterial activity and to reveal the controlling antibacterial mechanism.

2. Materials and methods

2.1. Preparation of Ti–Cu alloy

High purity titanium powder (99.99%) and 2 wt.%, 5 wt.%, 10 wt.% and 25 wt.% high purity copper powder (99.99%) were ball milled for 3–6 h, then hot pressure sintered under vacuum condition under 5–35 MPa pressure at 850–1080 °C for 30–60 min. The sample (named Ti–Cu sample) with 25 mm diameter and 1 mm thickness were directly sliced from the sintered Ti–Cu sample for further experiments. Commercial pure titanium sample with similar dimension was used as control sample. Before testing, all samples were ground with SiC paper up to 1000 grits and polished with 1 μm polishing liquid.

2.2. Phase identification and microstructure

Phase identification was carried out on D/MAX-RB Rigaku X-ray diffraction (XRD) with a scan step of 0.04. Microstructure was observed on a JSM-6360LV scanning electronic microscope (SEM) with energy dispersive X-ray spectroscopy (EDS).

2.3. Hardness and compressive test

Hardness was conducted on a HR-150A Rockwell hardness meter (Huayin, China). The test load was 1470 N and the duration time was 30 s. Five different fields were selected randomly for hardness measurement and the result was a mean value with standard deviation. Compressive strength was conducted with reference to ASTM E9-891(2000). Samples with a diameter of 10 mm and a height of 15 mm were cut from the sintered sample. The testing was carried out on a *Suns* Testing System with a crosshead speed of 0.5 mm/min. At least five samples were tested for each condition. cp-Ti samples with the same dimension were used for comparison.

2.4. Electrochemical test

Ti–Cu and cp-Ti specimens for the electrochemical test were put into a sample holder with only one side of 10 mm in diameter exposed. Electrochemical test was carried out at 37 ± 1 °C in a beaker containing 500 ml 0.9% NaCl solution on a Versa STAT V3-400 automatic laboratory corrosion measurement system (Princeton Applied Research, USA) using a standard three-electrode configuration with a saturated calomel as a reference, a platinum electrode as a counter and the sample as a working electrode. According to ISO 10271:2001 Standard, the open-circuit potential vs. time curve was recorded for up to 1 h to determine the open-circuit potential (E_{OCP}). The potentiodynamic scan was started 5 min after finishing the open-circuit potential measurement at a scanning rate of 0.5 mV s^{-1}. The corrosion rate (V) was calculated by [29]:

$$V = MI/nF \tag{1}$$

where M is the molar mass of titanium (g mol^{-1}), I is the average corrosion current density measured in the electrochemical tests (A cm^{-2}), F is Faraday constant (96,485 C mol^{-1}) and n is the valence of titanium.

2.5. Cu ion release

To examine Cu ion release, Ti–Cu sample was immersed in 3.7 mL 0.9% NaCl solution at 37 °C for 24 h to 120 h with a surface area-to-volume ratio of 2.86 with reference of ISO Standard 10993-12:2002 and ISO Standard 10993-15:2000. The Cu ion concentration in the solution was analyzed by an inductively coupled plasma spectrometry (Perkin Elmer, Optima 5300DV) with an accuracy of 0.005 mg/L.

2.6. Antibacterial properties

Nutrient broth (NB) was prepared by dissolving 10.0 g peptone, 5.0 g beef extract, 5.0 g NaCl and 15.0 g agar in 1000 mL distilled water and the ph value was adjusted to 7.2 to 7.4. NB were then sterilized by autoclaving at 121 °C for 20 min. *S. aureus*, strain ATCC 6538 and gram negative *E. coli*, strain ATCC 25922 were used in this study. *S. aureus* and *E. coli* were cultivated at 37 °C in the nutrient broth to a concentration of 10^6 cfu/mL, and then were diluted 10-fold by PBS solution to a concentration of 10^4 cfu/mL.

Plate counting method was conducted with reference to Nation Standard of China GB/T 2591(JIS Z 2801-2000, ASTM G21-96, NEQ) [30]. The Ti–Cu sample and the control sample were placed in Petri dishes separately while nutrient agar was spread onto a Petri dish as a negative blank sample. Then, 0.4 mL of the bacterial suspension was dripped onto the samples. After this, the dishes were covered with a relatively large Petri dish and incubated at 37 °C for 24 h under a humidity of 90%. After the incubation, the inoculated strain was harvested into a sterilized Petri dish by 3.6 mL sterilized physiological saline solution washing. The samples were carefully washed in order to make sure that no bacterium was left on the sample. 0.02 mL solution was selected from the above washing solution and then inoculated onto nutrient agar plates and incubated at 37 °C for 4 h under a humidity of 90%. The active bacteria were counted in accordance with National Standard of China (GB/T 4789.2-2010) [31]. Three samples were assessed for each type of samples. The antibacterial rate R was calculated by the following formula:

$$R = \left(N_{control} - N_{sample}\right)/N_{control} \times 100\% \tag{2}$$

where, $N_{control}$ and N_{sample} are the average numbers of the bacterial colony on the control sample and the Ti–Cu sample, respectively.

3. Results

3.1. Microstructure

Fig. 1 shows the XRD patterns of Ti–Cu alloys with different Cu contents. At 2 wt.% Cu, as shown in Fig. 1a, the diffraction peaks of Ti_2Cu phase were observed beside the diffraction peaks of titanium matrix. With the increasing Cu content, as shown in Fig. 1b to d, the diffraction intensity of Ti_2Cu phase increases, indicating that more Ti_2Cu phases were synthesized.

Fig. 2 shows the microstructure of Ti–Cu alloys with different Cu contents. At 2 wt.% Cu, as shown in Fig. 2a, gray phases with flake

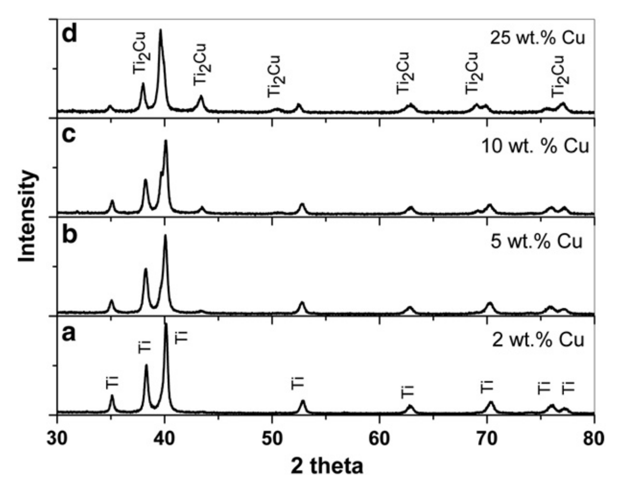

Fig. 1. XRD patterns of Ti–Cu alloys with different Cu contents. a) 2 wt.% Cu, b) 5 wt.% Cu c) 10 wt.% Cu, and d) 25 wt.% Cu.