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A new antibacterial titanium–copper sintered alloy: Preparation and antibacterial property

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ABSTRACT

Copper element was added in pure titanium by a powder metallurgy to produce a new antibacterial titanium–copper alloy (Ti–Cu alloy). This paper reported the very early stage results, emphasizing on the preparation, mechanical property and antibacterial activity. The phase constitution was analyzed by XRD and the microstructure was observed under SEM equipped with EDS. The hardness, the compressive strength and the corrosion resistance of Ti–Cu alloy were tested in comparison with cp-Ti. The antibacterial property of the Ti–Cu alloy was assessed by two methods: agar diffusion assay and plate-count method, in which *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) were used. XRD and SEM results showed that Ti_2Cu phase and Cu-rich phase were synthesized in the Ti–Cu sintered alloy, which significantly increases the hardness and the compressive strength compared with cp-Ti and slightly improves the corrosion resistance. No antibacterial activity was detected by the agar diffusion assay on the Ti–Cu alloy, but the plate-count results indicated that the Ti–Cu alloy exhibited strong antibacterial property against both bacteria even after three polishing treatments, which demonstrates strongly that the whole alloy is of antibacterial activity. The antibacterial mechanism was thought to be in associated with the Cu ion released from the Ti–Cu alloy.

1. Introduction

Titanium alloys have been widely used as bone implant materials such as bone screws and plate and dental implants due to their good biocompatibility, good corrosion resistance and good mechanical properties. However, bacterial infection after implant placement is still a significant rising complication. It was reported that the infection rates ranged between 0.5% and 3.0% in total joint hip arthroplasties [1] and 2–30% in external fixation [2], despite strict antiseptic operative procedures, including systemic antibiotic prophylaxis and special enclosures using laminar flow. The bacterial infection might lead to implant loosening even implantation failure. Common causes of implant-associated infections are *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*) [3–5]. These indicate that the prevention of bacterial-related infections remains a major challenge for the delivery of quality medical care and the problem results in a high rate of mortality and morbidity thereby significantly increasing health care costs.

Besides the use of antibiotic during the surgical procedure, another measurement to reduce the bacterial infection is to use implants which have antibacterial properties. During the last decades, surface modification has been proven to be an effective way to fabricate implant with antibacterial surfaces. Many techniques, including ion implantation [6], plasma immersion ion implantation (PIII) [7–9], deposition [10–12], plasma spraying [13–15], magnetron sputtering [1,16], electroplating [17], dipping coating [18,19], UV irradiation [20], polymer modification [21,22] and oxidation [23,24], have been developed to enhance the antibacterial properties of implant devices. Both anti-adhesive and antibacterial surfaces with integrated antibiotics [11,25–27], antiseptics or ions (such as F^+ [8], Cu [6,16,18], Ag [10,13,17,19] and Zn [28]) have been studied extensively.

However, the antibacterial activity is significantly affected by the surface properties. For example, the poor adhesive strength always leads to the failing of the antibacterial coating of TiO_2 deposition coating, which results in the loss of antibacterial properties. For the ion implantation, the antibacterial surface is normally very thin. Once the antibacterial surface is destroyed by some reasons, the antibacterial property will disappear. Therefore, it is very necessary to develop a metal material, which has antibacterial activity in the whole alloy rather than on the surface. By the addition of Cu or Ag elements followed by proper heat treatment, antibacterial stainless steels were produced with excellent antibacterial properties [29–32]. Ag-containing titanium alloy [33] and NiTi alloy [34] also showed antibacterial properties. In this research, Cu element was added to titanium by a powder metallurgy in order to obtain a titanium alloy with antibacterial properties.

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Research results in this study are in a very early stage, focusing on the preparation and the antibacterial properties of the titanium alloy.

2. Experimental

2.1. Preparation of Ti–Cu alloy

High purity titanium powder (99.99 wt.%) and 10 wt.% high purity copper powder (99.99 wt.%) were ball milled for 3–6 h, then were hot pressure sintered into samples (named Ti–Cu sample) with 25 mm in diameter under vacuum condition under 15–30 MPa pressure at 850–1050 °C for 30–180 min and cooled in furnace down to room temperature. Samples with a diameter of 25 mm and a thickness of 1 mm were sliced from the sintered Ti–Cu sample for further experiments. Commercial pure titanium samples (named cp-Ti) with same dimension were used as control samples. All samples were ground with SiC paper up to 1000 grits and polished with 1 μm polishing liquid. The density of the sintered samples was measured according to Archimedes law and the relative density was calculated based on the measured value where the densities of pure titanium and copper were 4.51 g/cm^3 and 8.92 g/cm^3, respectively.

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. Micro-hardness and compressive strength

Micro-hardness was tested on a HVS-1000 microhardness meter (Huayin, China). The test load was 1000 g and the duration time was 20 s. Five different fields were selected randomly 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 for the compressive strength test 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.

2.4. Electrochemical test

Ti–Cu specimens and cp-Ti specimens for the electrochemical test were put into a sample holder as shown in Fig. 1 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 and a platinum electrode as the counter and the sample as the 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}) after immersion for 1 h. 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 [35]:

$$V = MI/nF \quad (1)$$

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. After testing, the Ti–Cu sample was ground by about 0.1 mm and polished, and then the electrochemical test was conducted again. This procedure was repeated three times in order to investigate the corrosion properties of the whole alloy, denoted as the first polished sample, the second polished sample and the third polished sample, respectively. Similar process was also applied to the antibacterial test.

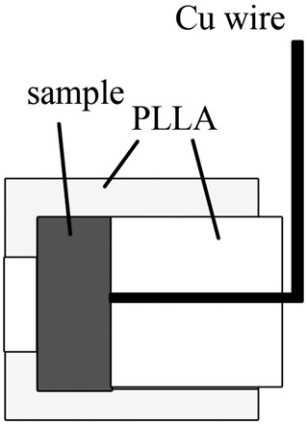

Fig. 1. Special equipment for the electrochemical test.

2.5. Cu ion release

To examine Cu ion release, Ti–Cu sample was immersed in 6 mL 0.9% NaCl solution at 37 °C for 72 h with a surface area-to-volume ratio of 1.76. The Cu ion concentration in the solution was analyzed by an inductively coupled plasma spectrometry (PerkinElmer, Optima 5300DV) with an accuracy of 0.005 mg/L.

2.6. Antibacterial properties

2.6.1. Preparation

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. PBS solution was prepared by dissolving 2.83 g Na_2HPO4, 1.36 g KH_2PO4 in 1000 mL distilled water and the pH value was adjusted to 7.2–7.4. NB and PBS solution were then sterilized by autoclaving at 121 °C for 20 min.

S. aureus, strain ATCC 6538 and *Escherichia coli* (*E. coli*), strain ATCC 25922 were used in this study. The bacteria 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 (bacterial suspension). All glassware and samples were sterilized with UV irradiation for 1 h before the experiments.

2.6.2. Agar diffusion assay

Agar diffusion assay was used to assess the antibacterial property with reference to the National Standard of China (GB/T 2738-2005) [36]. The hot agar solution was spread evenly with a Conrage stick on Petri dishes with a diameter of 90 cm and was allowed to cool down to room temperature under sterile conditions. A suspension of *S. aureus* or *E. coli* was sprayed over the total area of each Petri dish. A sterile Ti–Cu sample, a sterile cp-Ti sample (control sample) and antibiotic tablets (erythromycin, E/15, penicillin, p/101u, kanamycin, k/30 and gentamicin, Gm/10, as positive sample) were separately placed in the Petri dish. After this, the Petri dishes were incubated at 37 °C for 24 h under a humidity of 90%. The antibacterial property was accessed by the width of an inhibition zone around the sample. The inhibition zone was measured at three different points using calipers. The width of the inhibition zone was calculated by the following formula

$$W = \left(D_{zone} - D_{sample}\right)/2 \quad (2)$$