Effects of Ce on the short-term biocompatibility of Ti–Fe–Mo–Mn–Nb–Zr alloy for dental materials

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Effects of Ce on the short-term biocompatibility of Ti–Fe–Mo–Mn–Nb–Zr alloy designed for implant materials were studied by acute toxicity test, hemolytic test, and MTT assay. The elements and their concentration in surface films and extraction media of Ti alloys were investigated with XPS and ICP, respectively. The primary compositions of the surface films of Ti alloys with 0.3% Ce and without Ce were TiO₂ and Nb₂O₅. There were 0.2 mg/l Fe and 0.16 mg/l Mn in the extraction medium of Ti alloy without Ce, while 0.27 mg/l Fe and 0.87 mg/l Mn in the extraction medium of Ti alloy with 0.3% Ce. The concentrations of Fe and Mn in the medium were too low to have any significant effects on human health. There was no sign of cytotoxicity in these tests. The cytotoxicity levels of Ti alloys without Ce and with 0.3% Ce were graded 0 and 1, respectively. The hemolytic degrees of Ti alloys without Ce and with 0.3% Ce were 0.558% and 0.67%, respectively. The cells being incubated in the extraction medium were normal. These phenomena indicated that Ce was innocuous within the concentration range of this study. In addition, the hemolytic ratio and toxicity level of Ti alloy with 0.3% Ce were a little higher than that of Ti alloy without Ce. This meant that Ce would slightly increase the toxicity of Ti alloy.

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1. Introduction

The necessity to substitute the hard tissues of the human body like artificial hip joints, bone, and teeth is growing because the population of those aged over 65 years of age is increasing. Because of high strength–density ratio, low elastic modulus, excellent corrosion resistance, and evident biocompatibility, the interest in applying Ti and Ti alloys to implant materials has been growing recently [1–5]. α + β Type Ti alloys such as Ti–6Al–4V ELI, Ti–6Al–7Nb, and Ti–5Al–2.5Fe have been used for orthopedic implant materials. However, the high elastic modulus of these alloys compared to teeth and the toxicity of the alloying elements such as Al and V have been pointed out [6,7]. The researches of Ti biomaterials are focused on β-type Ti alloys recently, which are of low elastic modulus and high strength [6,8].

The wear resistance of conventional dental Ti or Ti alloys in oral cavity condition is poor because the hardness of Ti or Ti alloys is only about half of that of the dentine, as shown in Table I. Based on cost accounting and d-electron alloy design methods, a series of new β-type Ti alloys, that is, Ti–Fe–Mo–Mn–Nb–Zr alloys were developed. The strength and hardness of these alloys were greater compared with conventional Ti alloys for dental materials, and the corrosion resistance was excellent [9].

Rare earth elements can refine the crystal structure and improve the mechanical properties of Ti alloys [10]; there have been a few reports about applying rare earth elements on artificial tooth materials. In our prior work, Ce was added into Ti–Fe–Mo–Mn–Nb–Zr alloy, the results showing that a suitable quantity of Ce can refine the crystal structure and improve the comprehensive mechanical properties of Ti alloys [9]. There were some debates about the cell toxicity of rare earth elements [14,15]. Therefore, the effects of Ce on the short-term biocompatibility of Ti–Fe–Mo–Mn–Nb–Zr alloy were examined in this work.

2. Materials and methods

2.1. Materials

Ti–2%Fe–17%Mn–10%Mn–14%Nb–6%Zr and Ti–2%Fe–17%Mn–10%Mn–14%Nb–6%Zr–0.3%Ce alloys,
whose compositions are shown in Table II, were melted in a cold-mold non-consumable-electrode electronic arc furnace.

The specimens (7 × 7 × 7 mm³) were polished with fine sand paper, cleaned with ultrasonic in physiological salt solution for 20 min, degreased with ethyl alcohol, rinsed with phosphate buffer saline thrice, and then kept at 37 °C for drying. Before testing, the specimens and physiological salt solution were disinfected with vapor at 121 °C for 1 h.

2.2. Examination methods

2.2.1. Preparation process of extraction media of Ti alloys

The extraction media of Ti alloys were obtained under standardized conditions (ISO 10993-5). For acute toxicity and hemolytic tests, the specimens were dipped in borosilicate glass tubes containing bacilli-free physiological salt solution for 120 h at 37 °C without shaking, and the medium was replaced with 1 mg/ml DME medium culture medium for MTT assay. The ratio between the specimen surface and the volume of the extraction vehicle was 3 cm²/ml.

2.2.2. Acute toxicity test

Thirty healthy white male mice were used as experimental animals. The weight of each mouse was about 20 g. All of these white mice were divided into three groups at random and there were 10 white mice in each group. The mice in the first group were injected the extraction medium of alloy 1 in the tail vein, and the mice in the second group were injected the extraction medium of alloy 2. As negative controls, the mice in the third group were injected the physiological salt solution. The ratio between the volume of the extraction medium and the mass of mouse was 50 ml/kg. The general state, toxicity expression, and mortality of white mice were observed for at least three weeks. The weight of mouse was measured at 0, 24, 48, and 72 h.

2.2.3. Hemolytic test

Twenty milliliters of blood of a healthy male rabbit was gathered and then heparin sodium was added to form incoagulable rabbit blood. Eight milliliters of incoagulable rabbit blood was diluted with 10 ml physiological salt solution. Ten milliliters of the extraction medium of every specimen was mixed with 0.2 ml incoagulable rabbit blood in a glass tube. As positive and negative contrasts, the extraction medium was replaced with distilled water and physiological salt solution, respectively. Every reagent was manufactured for three copies. Every tube was centrifuged for 5 min under 750 times acceleration of gravity after being incubated at 37 °C for 60 min in 5% CO₂ atmosphere, the absorbance of the supernatant of every reagent was read at 545 nm.

2.2.4. MTT assay

After an incubation period of 48 h, L929 cell lines were digested with 0.25% trypsinase, and then were executed with 10⁴ cells/ml suspension with 10% fetal bovine serum. Four pieces of 96-well plates were used. Thirty-two wells in every plate were divided into four groups, with eight wells in every group. After being added 100 μl cell suspension in every test well, the cells were seeded for 24 h to attach the cells to the surface. Then every well of the first group was added 100 μl of the extraction medium of alloy 1 and every well of the second group was added that the extraction medium of alloy 2. The third group was positive control, where every well was added 100 μl of the extraction medium of pure lead. The fourth group was negative control, where every well was added 100 μl DME. They were replaced into the culture box with 5% CO₂ atmosphere at 37 °C to culture continuously.

After being incubated for 24, 72, 140, and 188 h, one plate was taken out from the culture box. The shape of the cells was observed and pictures taken with an inverted microscope, and to every test well of the plate was added 50 μl 1 mg/ml MTT. The plate was replaced into the culture box for 3 h, the solutions were removed,