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Introduction of antibacterial function into biomedical TiNi shape memory alloy by the addition of element Ag

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ABSTRACT

A new kind of biomedical shape memory TiNiAg alloy with antibacterial function was successfully developed in the present study by the introduction of pure Ag precipitates into the TiNi matrix phase. The microstructure, mechanical property, corrosion resistance, ion release behavior in simulated body fluid, cytotoxicity and antibacterial properties were systematically investigated. The typical microstructural feature of TiNiAg alloy at room temperature was tiny pure Ag particles (at submicrometer or micrometer scales with irregular shape) randomly distributed in the TiNi matrix phase. The presence of Ag precipitates was found to result in a slightly higher tensile strength and larger elongation of TiNiAg alloy in comparison with that of TiNi binary alloy. Furthermore, a maximum shape recovery strain of ~6.4% was obtained with a total prestrain of 7% in the TiNiAg alloy. In electrochemical and immersion tests, TiNiAg alloy presented good corrosion resistance in simulated body fluid, comparable with that of CP Ti and TiNi alloy. The cytotoxicity evaluation revealed that TiNiAg alloy extract induced slight toxicity to cells, but the viability of experimental cells was similar to or higher than that of TiNi alloy extract. In vitro bacterial adhesion study indicated a significantly reduced number of bacteria (*S. aureus*, *S. epidermidis* and *P. gingivalis*) on the TiNiAg alloy plate surface when compared with that on TiNi alloy plate surface, and the corresponding antibacterial mechanism for the TiNiAg alloy is discussed.

1. Introduction

TiNi alloy has several unique features, ranging from the physical and chemical properties to biological performance, which make it appropriate for use in biomedical applications in general [1,2] and can be summarized as follows.

(1) Unique superelasticity: the self-expandable TiNi alloy biomedical devices can pass through very small openings and then elastically spring back into the desired shapes. The maximal recoverable strain as high as 8% [3] benefits the deployment of TiNi alloy devices with three-dimensional freedom, where the constancy of shape and stress are essential, including permanent implants, stents, blood filters, bone staples and needles.

(2) Excellent shape memory effect: TiNi alloy is termed a thermal shape memory alloy because it expands and flexes when cooled, but resumes its shape and size when it returns to its normal temperature. A large number of TiNi alloy devices take advantage of this property, including thermal-activated arch wires and endovascular devices [4,5].

(3) Low elastic modulus: the reported values of elastic modulus of TiNi alloy vary from 20 GPa to 50 GPa for martensite and 40 GPa to 90 GPa for austenite [6], which is much closer to that of natural bone (~30 GPa). In the orthopedic area, the low modulus is beneficial for transferring the stress needed to adjacent bone without resulting in bone resorption [2]. Thus TiNi alloy, with an excellent combination of high strength and low modulus [4] close to the bone, is very suitable for implantation to avoid loosening of implants and giving a longer service period to avoid revision surgery.

(4) Good corrosion resistance and biocompatibility: many in vitro and in vivo studies [7–10] indicate that TiNi alloy has extremely good corrosion resistance and biocompatibility, mainly due to the formation of a passive dense titanium-oxide layer (TiO_2).

(5) Non-ferromagnetic property: TiNi alloy implant provides a clear, crisp image in magnetic resonance imaging, which is used increasingly frequently [11] in modern medicine.

From the viewpoint of material science, there are many reports on further alloying TiNi alloy by adding various third elements to develop new functions, such as wide transformation temperature

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hysteresis by Nb [12], high transformation temperature by Hf [13], low transformation temperature by Fe [14], narrow transformation temperature hysteresis by Cu [15], and so on. Yet to the authors' knowledge, there are only three papers regarding the addition of Ag into TiNi alloy. The first report in 2006 focused on the corrosion behavior and cytotoxicity of TiNiAg alloys with Ag concentration (from 0.12 to 0.26 wt.%) [16], with the intention of using it as a dental alloy. In 2007, Zamponi et al. [17] used a multilayer-like co-sputtering technique to achieve high Ag concentration (~4, 7 and 9 at.% Ag) in TiNi film, wherein the silver was incorporated into the alloy as pure Ag nano-precipitates. Later, they fabricated TiNi-Ag films with Ag concentration up to 11 at.% [18], which revealed that Ag addition did not alter the shape memory transformation characteristics.

From the viewpoint of biomedical engineering, the element silver is well known for its broad-spectrum antibacterial effect at very low ppb concentrations [19], and it possesses many advantages, such as good antibacterial ability, excellent biocompatibiliy and satisfactory stability [20,21]. The purpose of the present work is to test the feasibility of introducing the antibacterial function to TiNi alloy by the effective addition of alloying element Ag (a much high concentration than that in Ref. [16]).

2. Materials and methods

2.1. Materials preparation

A ternary TiNiAg alloy ingot with an actual composition of $Ti_{49.3}Ni_{47.3}Ag_{1.4}$ (in atomic percentage as identified by energy dispersive spectrometry (EDS)) was prepared from 99.8% purity Ti (Grade 0), 99.9% purity Ni and 99.9% purity Ag by means of arc melting in vacuum with a water cooling Cu bath. The alloy button was re-melted four times for homogeneity, and the as-cast button with height 15 mm was hot-rolled at 800 °C into plate 1.5 mm thick. Specimens were cut using electro-discharge machining and were mechanically polished to remove the surface oxide layer. Then the specimens were solid solution treated at 800 °C for 2 h, followed by quenching in water. After the heat treatment, the specimens were polished by mechanical method again to a mirror finish and then ultrasonically washed in acetone and alcohol. CP Ti (Goodfellow, Cambridge, UK) and $Ti_{50}Ni_{50}$ alloy (at.%) samples were used as controls, with treatment identical to that for the experimental TiNiAg alloy.

2.2. Characterization of materials

2.2.1. Scanning electron microscopy observation

A Hitachi S4800-type microscope, equipped with an EDS-microanalysis unit for chemical microanalysis was used for the scanning electron microscopy (SEM) characterization of the microstructure.

2.2.2. Transmission electron microscopy

Transmission electron microscopy (TEM) observation was carried out on a JEOL-2010FX microscope operated at 200 kV using a top-entry-type double-tilt specimen stage. Specimens for TEM examinations were mechanically polished into slices with thickness ~50 μm, and then ion-milled in a Gatan 691 Precision Ion Polishing System.

2.3. Phase transformation

2.3.1. Differential scanning calorimetry measurement

The phase transformation behavior of the experimental specimens was characterized by differential scanning calorimetry (DSC), using a Perkin–Elmer Diamond calorimeter with a heating and cooling rate of 20 K min^{-1}.

2.3.2. X-ray diffraction

X-ray diffraction (XRD; Rigaku DMAX 2400 diffractometer) using Cu Kα radiation was employed for the identification of the constituent phases at ambient temperature.

2.4. Tensile test

The stress–strain curves of TiNi and TiNiAg alloys were studied in tension. The tensile testing was performed using an Instron 3365 universal testing machine at a strain rate of 0.0004 s^{-1}.

2.5. Shape memory effect testing

The tensile tests were used to evaluate the shape memory effect of TiNi and TiNiAg alloys. All specimens were dipped into liquid nitrogen before the tests. The tensile stress was applied until the tensile strain reached ~7%, and then the stress was removed. After unloading, the specimen was heated to ~230 °C, and the distance between two bars within the gauge range was measured before and after the above testing to calculate the shape recovery ratio.

2.6. Corrosion behavior and ion release in simulated body fluid

2.6.1. Electrochemical measurement

A three-electrode cell was used for electrochemical measurements. The counter electrode was made of platinum, and the reference electrode was a saturated calomel electrode (SCE). All the measurements were carried out on an electrochemical workstation (CHI660C, China). The test solutions were artificial saliva solution [22] and 1% lactic acid solution [23].

2.6.2. XPS

X-ray photoemission (XPS) analysis was performed with an Axis Ultra spectrometer using mono Al Kα (1486.6 eV) radiation at a vacuum pressure of 10^{-9} bar, 15 kV and 15 mA. The binding energy was calibrated using a *C1s* hydrocarbon peak at 284.8 eV.

2.6.3. Immersion test

A 1% lactic acid solution was prepared for the immersion tests. Specimens with size 10 × 10 × 1 mm were submerged into 7 ml test solution at 37 °C for 168 h. Inductively coupled plasma atomic emission spectrometry (Leeman, Profile ICP-AES) was employed to measure the concentrations of the alloying element ions which had dissolved from the experimental alloy plates. An average of three measurements were taken for each group.

2.7. In vitro cytotoxicity assay

Murine fibroblast cells (L-929) and osteoblast cells (MG-63) were selected to evaluate the cytotoxicity of the experimental materials. They were cultured in Dulbecco's modified Eagle's medium (DMEM), 10% fetal bovine serum, 100 μg ml^{-1} penicillin and 100 μg ml^{-1} streptomycin at 37 °C in a humidified atmosphere of 5% CO_2. The cytotoxicity tests were carried out by indirect contact experiments. Extracts were prepared using serum-free DMEM as the extraction medium, with surface area of extraction medium ratio 1 ml/3 cm^2 in a humidified atmosphere with 5% CO_2 at 37 °C for 72 h. The control groups involved the use of DMEM medium as a negative control and 10% DMSO in DMEM medium as a positive control. Cells were incubated in 96-well cell culture plates at 5×10^3 cells per 100 μl medium in each well and incubated for 24 h to allow attachment. The medium was then replaced with 100 μl of different experimental extracts. After incubation of the