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Antibacterial effect of nickel-titanium alloy owing to nickel ion release

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ABSTRACT

This paper demonstrates that a NiTi shape memory alloy demonstrates antibacterial effect owing to the release of Ni ions from the alloy itself. Moreover, non-cytotoxic NiTi alloy with antibacterial properties can be prepared by simple post-heat treatment. We demonstrated that applying *Escherichia coli* to a NiTi surface resulted in 40% reduction in the number of cells following a 4 h incubation in an ambient atmosphere. When the alloy was heated at 450 °C in air, the antibacterial effect was slightly reduced but the cytotoxicity was drastically reduced. This result indicates that the production of an antibacterial NiTi alloy (without cytotoxic effects) is feasible with an appropriate surface modification. We further revealed that the appropriate Ni ion concentration for both antibacterial effect and biosafety was in the range from 0.05 mg L^{-1} to 3 mg L^{-1}. Our novel finding represents a new and unique strategy for improving the antibacterial performance of NiTi alloy.

1. Introduction

Nickel-titanium (NiTi) alloy has the unique properties of shape memory and superelasticity, and has been widely employed as a material for medical devices in cardiology and dentistry [1,2]. However, when Ni comprises around or above 50% of the total alloy constituents, serious issues can arise in medical settings. The interior of the human body is a severely corrosive environment, partly owing to the high concentrations of inorganic ions; accordingly, release of Ni ions from alloy is unavoidable [3]. High concentrations of Ni ions in the body can induce cytotoxicity, leading to tissue necrosis. Hence, material researchers have hitherto devoted a great deal of effort towards the fabrication of a novel shape memory alloy without nickel [4–6] and/or the formation of a surface layer with the ability to restrict Ni release from the surface [7–11].

While on one hand the release of Ni from medical device is problematic due to its toxicity, conversely Ni release can also be considered as a beneficial factor in medical settings because several researchers have reported that Ni ions have antibacterial properties. Miyano et al. investigated the antibacterial properties of the several metals, including Ni, against *Staphyloccus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). They demonstrated that the viability of the microorganisms was reduced following exposure to a Ni surface due to an antibacterial effect of Ni metal itself [12]. Hrenovic et al. also reported that Ni-loaded zeolite showed weak antibacterial effect against *E. coli* and *S. aureus* [13]. Ni-ion release from a NiTi alloy surface is likely to be lower than that from pure Ni metal, since the alloy surface is covered with the chemically stable thin Ti-oxide layer [15,16]. Nevertheless, it is expected that a NiTi alloy would have intrinsic antibacterial effect. A great deal of effort towards restricting the toxicity of NiTi has been made in the past decade. However, no report describing the antibacterial benefits of Ni ion release and the utilization thereof has been published. This is surprising since such an investigation could warrant the possibility of the usage of NiTi alloy as an antibacterial agent in medical settings. Therefore, in the present study, we evaluated the antibacterial efficacy and cytotoxicity of both untreated and heat-treated NiTi alloys, using animal cells and bacteria as model organisms. Furthermore, the optimal concentration range of Ni ions that had antibacterial effect without cytotoxicity was evaluated. Based on these results, we discuss the feasibility of a novel design for a NiTi alloy utilizing Ni-ion release in a therapeutic application.

2. Materials and methods

2.1. Material preparation and characterization

NiTi alloys (55% Ti) with sizes of ϕ15 mm × 3 mm, purchased from NEC TOKIN, were employed as specimen. The surface was ground with emery paper grade # 1200 or less and was then sonicated in ethanol for 10 min. After drying completely under ambient conditions, some of the polished alloys were heated at 450 °C in air for 5 h, in order to form an oxide layer on the surface [17]. The

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color of the treated surface was golden, indicating the successful formation of an oxide layer.

Morphological images of the surfaces were observed using secondary electron imaging of a scanning electron microscopy (SEM; JCM-5000 Neo Scope, JEOL, Japan). The layer structure of the surface was analyzed based on an elemental depth profile measured using X-ray photoelectron spectroscopy (XPS; PHI 5000 VersaProbe, ULVAC-PHI, Japan) with an Ar ion etching. The etching rate calculated from an SiO_2 layer was approximately 12.5 nm min^{-1}.

2.2. Ni ion release from the alloy

To determine the extent of Ni-ion release, the amount of Ni ions released into a physiological solution was measured. Phosphate buffered saline (PBS) solution was used as the solution. The specimens and a pure Ti were soaked in 6 mL of PBS in a polypropylene centrifuge tube. A pure Ti was used as a reference material. The tube was then incubated at 310 K in an incubator shaking at 65 rpm. Following incubation for 4 h, 600 μL of the solution was aliquoted from the centrifuge tube and the Ni concentration was determined using graphite furnace atomic absorption spectrometry according to the manufacturer's instructions (GF-AAS; Z-8000, HITACHI, Japan).

2.3. Antibacterial effect and cellular response on the alloy

The antibacterial effect of the alloy surfaces was examined using *Escherichia coli (E. coli)* strain ATCC 25922. Prior to the antibacterial test, *E. coli* was cultured on nutrient agar plates at 37 °C. A bacterial suspension of 2×10^6 CFU mL^{-1} was then prepared using a one five-hundredths diluted nutrient broth (NB). The untreated and the heat-treated alloy were sterilized by autoclaving for 30 min at 121 °C. A 25 μL aliquot of the bacterial suspension was dispensed onto the sterilized surface and covered with a cover glass (10 mm × 10 mm). The alloy was then placed into an incubator set at 37 °C with 95% humidity and incubated for 4 h. The bacterial suspension on the specimen was then washed into a broth of soybean-casein digest with lecithin and Polysorbate 80 (SCDLP). The SCDLP broth was then smeared on the surface of a nutrient agar plate, and the colonies formed on the plate were counted. The antibacterial effect of the alloys was represented as the survival rate of *E. coli*, defined as the ratio of viable bacterial colonies after the incubation to the initial number of bacterial colonies in the suspension.

Cellular response to the surface was evaluated using MC3T3-E1 cells (RIKEN BioResource Center, Japan), an osteoblast-like cell line. Before the tests, MC3T3-E1 cells were cultured in α-modified minimum essential medium (α-MEM; GIBCO BRL, USA) containing 10% fetal bovine serum (FBS; JR Scientific, USA) and 1% antibiotic-antimycotic (100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, and 0.25 μg mL^{-1} amphotericin B; GIBCO BRL, USA) at 37 °C under 5% CO_2 in a humidified atmosphere. Upon reaching confluence, the cells were detached by trypsin treatment, and a cell suspension was prepared by diluting the cells to the desired density. The untreated and the heat-treated alloy were sterilized by autoclaving and then placed into an individual well of a 24-well cell culture – plate. 500 μL of cell suspension at 1×10^3 cells mL^{-1} was pipetted onto the alloys in the wells. The plates were incubated for 168 h, after which the cells were retrieved from each well by trypsin treatment and counted using a hemocytometer after performing trypan blue staining.

2.4. Dependence of antibacterial effect and cytotoxicity on Ni ion concentration

The variation of antibacterial effect due to difference in Ni-ion concentration was also evaluated. 3 mL of bacterial suspension (2×10^6 CFU mL^{-1}) containing various concentrations of Ni ions was prepared by adding 30 μL of Ni solution of different molarities. The Ni solutions were prepared by diluting a Ni standard solution, including 1000 mg L^{-1} Ni in 0.1 M HNO_3 purchased from Wako Pure Chemical Industries, Ltd. in Japan. The suspension in the centrifuged tube was incubated for 4 h at 37 °C with 65 rpm shaking, and then smeared on the surface of a nutrient agar plate. The effect of Ni ion concentration upon antibacterial effect was assessed by counting the colonies formed on the plate. Here, the antibacterial effect was also represented as the survival rate of *E. coli*.

The cytotoxicity of Ni ions was also evaluated using the MC3T3-E1 cell. 30 μL of Ni solutions with various concentrations (0–10 mg L^{-1} Ni in 0.1 M HNO_3) was added into 3 mL of cell suspension (1×10^3 cells mL^{-1}) in a polypropylene centrifuged tube. Thereafter, 500 μL of the cell suspension was pipetted to an individual well of 24-well cell culture polystyrene plate. The plate was then incubated for 168 h at 37 °C under 5% CO_2 in a humidified atmosphere, after which the cells were retrieved from each well by trypsin treatment and counted using a hemocytometer.

2.5. Statistical analysis

The cell and antibacterial tests were performed with n = 3 and repeated at two different times at least. In the both tests, the statistical analyses were performed by ANOVA with SNK post-hoc test, to identify levels of significance ($p < 0.05$).

3. Results

3.1. Surface characteristics of the NiTi alloys

Surface morphologies of the untreated and the heat-treated NiTi alloys were confirmed using the SEM and no significant difference was found between the two alloys (Data not shown). The elemental depth profiles of the surface layer formed on untreated and treated surfaces are shown in Fig. 1. A naturally formed thin oxide layer comprising mainly titanium oxide was observed on the untreated surface. On the heat-treated surface, a comparatively thick oxide layer of about 150 nm was formed. In the region of 30–150 nm, the atomic ratio of O to Ti showed a constant value of approximately 2, which indicates the formation of TiO_2. A comparatively high concentration of Ni was detected in the depth range up to 30 nm. To clarify the chemical state, we analyzed the XPS narrow spectra (Fig. 2). The spectral shape of Ti 2p is symmetric, and the binding energy of Ti $2p_{3/2}$ was approximately 458.8 eV. These data indicate that the chemical state of Ti corresponds to TiO_2 [18]. On the other hand, the spectral shape of Ni $2p_{3/2}$ had a complex shape because it included two peaks corresponding to the different chemical states. The peak at 854.5 eV originates from NiO [19] and another peak at 856.0 eV may be $NiTiO_3$ [19]. The peak at 861 eV is the satellite peak accompanying both NiO and $NiTiO_3$.

3.2. Ni ion release from untreated and heat-treated NiTi alloy surfaces

The speed of Ni ion release from untreated and heat-treated alloy surfaces in PBS is shown in Fig. 3. Here, Ni-ion release from a pure Ti surface was also evaluated for comparison. Release of Ni ions was observed from NiTi surfaces, although no release from Ti was detected. The speed of release from the treated surface was about 1/15 of that from the untreated surface. Arndt et al. reported that the daily Ni release from commercial ready-to-use orthopedic NiTi wire was in the range of 0.2–7 mg cm^{-2} [3]. Thus, the result shown in Fig. 3 is consistent with previous data.