Research Article

Biocompatibility of Nanoporous TiO₂ Coating on NiTi Alloy Prepared via Dealloying Method

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Received 2 March 2012; Accepted 30 March 2012

Academic Editor: Xiaoming Li

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This paper investigated the biocompatibility of nanoporous TiO₂ coating on NiTi shape-memory alloy (SMA) prepared via dealloying method. Our previous study shows that the dealloying treatment at low temperature leads to 130 nm Ni-free surface titania surface layer, which possesses good bioactivity because of the combination of hydroxyl (OH⁻) group in the process of dealloying treatment simultaneously. In this paper, the biological compatibility of NiTi alloy before and after dealloying treatment was evaluated and compared by direct contact method with dermal mesenchymal stem cells (DMSCs) by the isolated culture way. The interrelation between the biological compatibility and surface change of material after modification was systematically analyzed. As a consequence, the dealloying treatment method at low temperature could be of interest for biomedical application, as it can avoid sensitization and allergies and improve biocompatibility of NiTi shape-memory alloys. Thus it laid the foundation of the clinical trials for surface modification of NiTi memory alloy.

1. Introduction

Nowadays, nickel-titanium (NiTi) shape-memory alloy (SMA) becomes one of important biomedical metal materials because of its special shape-memory effect, the hyper-elasticity, and excellent biocompatibility [1, 2]. However, NiTi alloy containing high-concentration nickel (atomic ratio at 50%) can have a large number of nickel ions dissolved out after corrosion; especially in body fluid containing chlorine ion, point corrosion-resistant performance is not ideal, which will cause larger chronic host negative response, such as sensitization, teratogenicity, and even carcinogenic change [3]. These negative properties make NiTi alloy in the body of safety questioned, and patients are worried, which become one of the obstacles for NiTi alloy biomedical application. In view of the above questions, researchers have done a lot of work, the use of physical, chemical, and electrochemical, and so forth diversified method on NiTi shape-memory alloy surface modification [4, 5]. Recently

our research team have used dealloying technology at low temperature to realize the surface Ni ions removal and obtained the titanium oxide of porous structure on the surface [6–10]. Li et al. studies show that the formation of the porous structure is beneficial to cell compatibility [11, 12]. The nanoporous surface can obviously improve the cellular compatibility [13].

Stem cell with the capacity of quick proliferation and itself renewal can be differentiated into many different target cells, and its potential of multilineage differentiation brought new chance for biological tissue engineering [11, 14–16]. Dermal mesenchymal stem cells (DMSCs) as a part of the stem cells, compared with other mesenchymal stem cells, are characterized by drawing materials conveniently, unrestricted position, and the advantages of fast growth and renewal; yet they are able to keep those characteristics in vitro culture. Jean et al. have confirmed dermal sources of stem cells with differentiation potential to be the fat, cartilage,

and bone, which starts the important role of dermal stem cells in the connective tissue repair for recognizing [17–22]. In this paper, first of all, the dermal stem cells were separated and subcultured by the traditional program successfully, then used direct contact method to the evaluation and comparison of NiTi alloy biological compatibility with that before and after dealloying treatment and performed a systematic analysis of the relationship between the nature change of material surface after modification and the biological compatibility. Thus it is laid the foundation of the clinical trials for surface modification of NiTi SMA.

2. Experiments

2.1. Dealloying NiTi Alloy. NiTi shape-memory alloy performance for experiment reaches the country demanding on “using NiTi shape memory alloy to processing material standard in medical equipment and surgical implants”; the alloy components are Ni: 50.7% and Ti: 49.3%. Sample was processed into 20 mm × 10 mm × 2 mm, grinded and polished gradually with metallographic sand paper from 400× to 1200×, and then put, respectively, into acetone and anhydrous alcohol with ultrasonic cleaning. The 200 mL self-developed dealloying treatment liquid (a typical formula as follows: nitro dioctyl phthalate:H₂O₂:HCl:H₂SO₄ = 4:1:2:3, volume ratio) was hold in the beaker with sample and stirred at 50°C, low temperature for 15 h. The sample was taken out to clean with deionized water and dry. The dealloyed sample was heated treatment at 400°C for an hour and prepared for use.

2.2. Structure Characterization. Nano Indenter DCM system test analyzed mechanical performance to be on submicron scale on the surface of NiTi memory alloy, taken off the nickel by process of dealloying. Test adopted the pyramid pressure head and pressed in the maximum depth of 500 nm. The maximum load was 500 mN, but the displacement and loading precision are 0.01 nm and 50 nN, respectively. The place of 70% maximum load maintained 60 s of the thermal drift correction after unloading. The Oliver and Pharr method was used to calculate H and E value.

2.3. Cellular Compatibility Experiments. The New Zealand rabbit, 2–5 days old, was twisted off neck to lead to death and was put on asepsis platform after body disinfection with 75% alcohol. After clipping the whole layer of the backside skin, we removed the subcutaneous tissue by sterile blade and operated separation dermis by the mechanical method (biopsy). DMSCs were separated and subcultured by the traditional program successfully. DMSCs in passage 3 were seeded at a final concentration of 1×10^5 cells per mL to the glass slides coated with polylysine in a 6-well plate. Cells detected positively of Vimentin, cytokeratin, nestin, CD34, and FVIII factors by the SABC method were considered as DMSCs.

The NiTi memory alloy after surface treatment by crystallization at 400°C for 1.5 h was group A, the untreated alloy was group B, and pure DMSCs was control group.

The sample specifications of NiTi memory alloy were width 10 mm, length 15 mm, and height 2 mm, then 40 pieces sample of group B or 40 pieces sample of group A, and DMSCs were developed together in vitro. Compared with the control group, NiTi memory allos from group A and B were put on the preset against taking off microslides within 12-well plate, respectively. Every hole was covered by cell suspension with the same volume and density. DMSCs were counted by the flow cytometry, and cell growth curves were drawn after cell growth situation was observed for 1 d, 5 d, and 8 d. The hydroxyproline and alkaline phosphatase of cells with group A or B were measured for 1 d, 5 d, 8 d (the kit was provided by the Nanjing Jiancheng Biotechnology Company). The content of nickel ion in cell culture medium. The content of nickel ions in cell culture medium from three groups was determined by AA-800 type graphite furnace atomic absorption spectrophotometer.

3. The Results and Discussion

3.1. Porous Structure and Properties of TiO₂ Coating. The surface morphology of NiTi alloy after the different treatment time via amino oxidation method at 55°C is shown as Figure 1. It clearly shows that NiTi memory alloy began gradually to form the porous structure in the dealloying process for 4 h, at last in the porous alloy surface won a porous nanogrid structure, which is a typical characteristics after dealloying. The grid structure is constituted by the rest undissolved element after one-element selectivity dissolution from the alloy components. XRD research shows that because of the thinner membrane formation after dealloying the diffraction peak of alloy substrate phase remained predominant, but having the titanium dioxide diffraction peak of faint sharp titanium ore phase and rutile phase appeared after processing, then surface formed the titanium oxide.

Figure 2 shows mechanical properties of NiTi alloy after dealloying. We can see that the elastic modulus of 120 nm depth frontal film is lower, and the elastic modulus of 50 nm depth frontal film is lower than 20 GPa, which is almost equal to the elastic modulus of human body cortical bone. Hence, this layer film with low modulus of elasticity will benefit to weaken stress shelter effect from alloy in the course of the bone tissue growth. The elastic modulus of 120 nm depth has already reached the highest 71 Gpa, after that basically remained level, which illustrates that it is already the elastic modulus value of matrix NiTi austenitic parent phase. The nanohardness-displacement change on the surface layer of test sample is seen Figure 2(b). It can be seen that the nanohardness of alloy increases gradually from the exterior to the interior and mutations does not appear, beginning to appear slow flat after a depth of about 300 nm and a maximum value 4.7 GPa. From the outside to the inside, hardness value with the slow continuous increase the gradient transition is between that membrane layer, and matrix, and the stress is smaller with good combination. In the load-displacement curve as shown in Figure 2(c), both loading curve and unloading curve are nonlinear, then the

FIGURE 1: SEM images of the surface morphologies of NiTi alloy dealloyed for 0 h, 4 h, 6 h, 9 h, and 15 h.

maximal displacement of the pressure head including both elasticity and plastic deformation parts, while unloading produces elastic recovery and elastic recovery reaches 44.4%, the residual displacement depends on the elastic deformation mainly, which shows obvious characteristics of ceramic material. Electrochemical test results in Hank's simulation body solution (pH = 7.45) are shown in Figure 3. Compared to the NiTi memory alloys without surface treatment, the self-corrosion potential of dealloying NiTi sample gets bigger enhancement from 0.05 V to 0.92 V.

3.2. Cells Compatibility

3.2.1. Cell Proliferation. DMSCs grow well in control group, but DMSCs cultured in groups A and B have difference forms as shown in Figure 4. DMSCs in group A have uniform, long fusiform and their cytoplasm is abundant, then cell culture flask is almost full of DMSCs after two days, which is close to control groups. However, most of the DMSCs in group B are polygonal, with significant morphological differences and less cytoplasm. In Figure 5, the amount of DMSCs increases sharply in two days and begins to reduce after two days gradually; moreover all data are significantly less than those of control group or group A which have less influence on DMSCs proliferation.

3.2.2. Determination of Hydroxyproline and Alkaline Phosphatase in Cell Culture Medium. Hydroxyproline content analysis results as Table 1 shows. The content of hydroxyproline in the culture medium of group B reduced gradually, which has close relationship to DMSCs growth. It shows that cell growth is slow, so absorption of hydroxyproline is also

TABLE 1: The content of hydroxyproline in cell culture medium (mg/L).

Group	Initial content	1 d	5 d	8 d
B	20.00	12.07 + 0.13 39.6%	11.40 + 0.08 43%	6.47 + 0.04 67.6%
A	20.00	5.34 + 0.21 73.3%	2.89 + 0.29 85.5%	2.51 + 0.35 87.5%

slow which led to the content decrease slow. However, the content of hydroxyproline in group A declined dramatically, which shows that cell growth is quick. The decrease of hydroxyproline results from its consumption of the collagen synthesis process.

The results of alkaline phosphatase content analysis as Table 2 shows. The content of alkaline phosphatase in the culture medium of group A declined gradually, and in the culture medium of group B the content of alkaline phosphatase also gradually declined, but all data of group B are lower than those of group A, because, after the surface modification the NiTi memory alloy is the slow release of the nickel ions which have less influence on cell metabolism.

3.2.3. Determination of Nickel Ions in Cell Culture Medium. As indicated in Table 3, the content of nickel ions release in cell culture medium increases with time; the amount of nickel ion in group A after 5 days is greatly below the amount of group B. The change is slight after 8 days, and the incremental quantity is smaller. In addition, in cell culture medium of group B, the concentration of nickel ion is about 3.7 times higher than that of the group A significantly after

FIGURE 2: NiTi memory alloy surface dealloying and surface nanocreeching test.

TABLE 2: The content of alkaline phosphatase in culture medium (U/g prot).

AKP	1 d	5 d	8 d
Group A	8.35 + 0.62	5.71 + 0.43	4.25 + 1.30
Group B	7.06 + 1.13	4.27 + 1.09	3.61 + 1.52

5 days, which is a leading cause of affecting cell proliferation. The much more nickel ions from NiTi shape-memory alloy in group B may occur in corrosion within the body and have biological system damage on the body.

3.3. Discussion. After calculated the dealloying conditions of thermodynamics, a dealloying treatment method was applied to nearly equiatomic NiTi alloy so as to remove the harmful element of nickel selectively from NiTi alloys and form a Ni-free titanium oxide layer on the surface [23]. The results show that the dealloying treatment at

TABLE 3: The content of nickel ions in cell culture medium (ng/mL).

Group	Time		
	1 d	5 d	8 d
Group A	28.7	46.8	50
Group B	31.1	173	177.5
Control	1.97	1.97	1.97

low temperature leads to 130 nm Ni-free surface layer that possesses a nanometer structure and in situ formation of titania surface that possesses a degree of bioactivity because of the combination of hydroxyl (OH^-) group in the process of dealloying treatment simultaneously. This dealloying treatment can avoid sensitization and allergies and improve biocompatibility of NiTi shape-memory alloys. It will be a good news to biomedical application. DMSCs can timely, accurately reflect the early nickel ions adverse effects which are the influence of growth and cell metabolism

FIGURE 3: Electrokinetic potential polarization curve of NiTi after dealloying treatment in Hank's simulation body fluids (pH = 7.45) and crystallization in 500°C for 1 h.

FIGURE 4: NiTi memory alloy and DMSCs culture in vitro.

nickel ions give to. The NiTi memory alloys after surface modification or without surface modification and leather stem cells in culture in vitro observe the influence on leather stem cells and determin concentration of nickel ions, the content of hydroxyproline and alkaline phosphatase in cell culture medium, thus compare the cell compatibility difference between surface modified NiTi memory alloy and NiTi memory alloy without surface modification.

From cell count detection result in the incubation, logarithmic growth, and the platform period of the cells, NiTi memory alloy without the surface modification does have influence on the proliferation of the cells, but the NiTi memory alloy after surface modification reduced greatly the influence of cell proliferation. The content of alkaline phosphatase in cell culture medium of group A declined gradually and in group B has same trend, but all of values are below those of group A. The pure TiO_2 coating free of nickel ions within the hundreds of nanometers of surface of NiTi memory alloy after surface modification blocked the

diffusion of nickel ions in the alloy matrix. The low level of nickel ions in cell culture medium has less effect on cell metabolism. It has been proved that the NiTi memory alloy after surface modification at low temperature process can effectively prevent the spread, dissolution, and release of the nickel ions.

Hydroxyproline is the main raw material of collagen synthesis in cells, so the number of hydroxyproline can reflect the collagen synthesis. In the cell culture medium of group B, the content of hydroxyproline is slow decline, which is in proportion to cell growth to explain the slow growth of cells and less collagen synthesis. So the slow absorption of hydroxyproline in culture liquids results in hydroxyproline content dropping gradually. The level of hydroxyproline in group A dropped sharply; it shows the rapid growth of cells and the more amount of collagen, which also prove the fact that the modified surface can effectively improve the tissue compatibility of alloy.

FIGURE 5: Flow cytometric cell count analysis.

4. Conclusions

Nanoporous TiO₂ coatings on NiTi alloy were prepared via dealloying method. The hydroxyl (OH⁻) group was introduced into the surface of TiO₂ during the process of dealloying treatment simultaneously. The direct contact method was used for the evaluation and comparison of biological compatibility between NiTi alloy and samples after dealloying treatment. Research results indicate that the dealloying treatment method at low temperature could be of interest for biomedical application, as it can avoid sensitization and allergies and improve biocompatibility of NiTi shape memory alloys. This paper led to the foundation of the clinical trials for surface modification of NiTi memory alloy.

Acknowledgments

The work is supported by the National Basic Research Program of China (973 program, 2011CB710901), the National Natural Science Foundation of China (Grants no. 11120101001, 10925208, 51072012), and the Fundamental Research Funds for the Central Universities (Grant no. YWF-1-03 Q- 079).

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