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Evaluation of Compatibility of Nickel-Titanium Shape Memory Alloy with Femur Surrounding Tissue

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Nickel-titanium shape memory alloy (NiTi alloy) has unique mechanical properties such as shape memory effect and super elasticity along with good wear and corrosion resistances. For this reason NiTi alloy is expected to use as implant material in the biomedical field. However, the biocompatibility of NiTi alloy is not fully examined. The purpose of this research to evaluate in vitro cytocompatibility and the biological response of NiTi alloy, especially to evaluate inflammatory response by in vivo animal implantation assays and improvement of corrosion resistance for NiTi alloy by heat-treatment. NiTi alloy showed the good cytocompatibility compared with that of Ti-6Al-4V alloy, judging from the small amount of hIL-1β and LDH generations. The fibrocellular layer generated by NiTi alloy implantation resembles the one produced by stainless steel rather than Ni implantation. Biocompatibility of NiTi alloy is superior compared with pure nickel. Also, it was made clear that the biocompatibility of NiTi alloy showed almost the same level as that of the stainless steel, which gave a lot of actual results as a biomaterial until now. Therefore, it was understood that there is no problem in the biocompatibility of NiTi alloy for applying as a biomaterial. And corrosion resistance of in air oxidized NiTi alloy was recognized to be improved.
Key words: Nicke-Titanium shape memory alloy, Cytocompatibility, Biocompatibility, Biomaterial

1. INTRODUCTION

NiTi alloy shows unique mechanical properties namely shape memory effect and super elasticity [1] [2] along with good wear resistance and good corrosion resistance [3]. It provide a possibility for making self-locking, self-expanding and self-compressing implant. For this reason NiTi alloy is expected to use as implants material in the biomedical field [4]~[7]. Howevers, questions may arise because of its high nickel content. Even though Ni is essential element in the human body, its excess concentration may cause allergic reactions and promote carcinogenesis and toxic reaction [8]. Biocompatibility seems to vary with local Ni ions release and this release is closely related to corrosion behavior. The key of NiTi alloy's biocompatibility resides in improvement of the corrosion resistance of the material. Different kinds of surface treatments were applied to protect the surface of the alloy from the corrosion. The purpose of this research is to evaluate corrosion resistance and cytocompatibility of NiTi alloy and inflammatory response issues by in vivo animal implantation assays. And also, the goal is trying to improve corrosion resistance of NiTi alloy by surface modification.

2. EXPERIMENTAL PROCEDURES

2.1 Test materials

The test materials were NiTi alloy (44% titanium, 56% nickel by weight), AISI 316L (12% nickel, 16% chromium, 2% molybdenum and 70% iron by weight), Ti-6Al-4V alloy (90% titanium, 6% aluminium and 4% vanadium by weight) and pure nickel (99% nickel by weight). The NiTi alloy which were treated by acid pickling, electro-polished and then passivated in a nitric acid solution at room temperature was refered as electro-polished (EP) specimen. The NiTi alloy which were treated by acid pickling, electro-polished, passivated in a nitric acid solution at room temperature and then oxidized at 773K in air was refered as heat treated (HT) specimen. The surface of variously treated specimens were observed by FE-SEM (S-4200 Hitachi Japan). Passive film of various treated specimens were analyzed by XPS (Q-2000 Physical electronics U.S.A.). Implant materials of ϕ 1.0×15mm were taken from a longer wire by mechanical cutting. The cut end of the implant materials were rounded using water-cooled grinding. Then, the implant materials were degreased by ethanol, washed with an ultrasonic vibrobath, and autoculaved (30min, 393K).

2.2 Creation of corrosion products

Through dissolving NiTi alloy and Ti-6Al-4V alloy in lactic Ringer solution (Lactec Injection® Otsuka pharmaceuticals Japan) test solution with various ions concentration were prepaired. Anodic dissolution time for NiTi alloy and Ti-6Al-4V alloy was selected as 60 min. Concentration of dissolved ion in the solution were arranged to be from 2 to 128 ppm through analyzing them by ICP (SPS7700 Seiko Japan). The steam sterilization

was applied to all solutions under the conditions of 120℃ for 20min.

2.3 Culture solution

10 grams of RPMI (Roswell Park Memorial Institute)-1640 (Nissui 2®Nissui Pharmaceuticals Japan) are added to 1 litter of ultra pure water. The solution was sterilized 20min under the temperature condition of 120℃. Then, 0.3 grams of L-glutamic acid ten thousand unit of penicillin (Crystalline Penicillin G Potassium® Banyu Pharmaceuticals Japan) and 10 milligram of streptomycin (Streptomycin Sulfate® Meiji Conectionary Japan) were added. To control hydrogen ion concentration, 40 milliliters of sodium hydrogen carbonate was added. Finally, by adding fetal bovine serum (FBS) the nutrient medium was made. The cell was cultivated by using the nutrient medium in germ-free room. Then, it was applied for the experiment.

2.4 Cell membrane damage

Corroded solution was administered to the macrophage. After incubating it for 48 hours, the culture fluid is withdrawn. The absorbance of 50μl of the skimmed liquid was measured during 6 minutes using testing kit (LDH uv Test Wako® Wako Pure Chemical industries Japan). Depending upon the degree of damage of the organization cell membrane LDH inside of the cell was discharged outside of the cell. To evaluate the degree of damage of the organization cell, the LDH activity was measured. Also, to evaluate the total activity of LDH of organization cell, the cell was completely destroyed by the supersonic wave using cell smash machine. Then, the damage of cell membrane (D*) was calculated from following equation.

$$*D=\frac{\text{Objective LDH av.}-\text{Negative cont. LDH av.}}{\text{Positive cont. LDH av.}-\text{Negative cont. LDH av.}}\times 100$$

where, objective LDH av. is lactate dehydrogenase which solve out from a cell by corrosion products. Positive control LDH av. is lactate dehydrogenase which solve out from a cell by using supersonic wave using cell smash machine. Negative control LDH av. is lactate dehydrogenase which exists in medium.

2.5 Inflammation reaction of macrophage

Cytokine measurement was conducted as follows. The skimmed liquid was obtained conducting 10min centrifugal separation of culture fluid 48 hours after adding corrosion product to it under condition of 1400rpm. Using 50ml of skimmed liquid hIL-1βvalue was measured employing ELISA method [9] using testing kid (h-Interleukin-1β ELISA® Roche Diagnostics Germany).

2.6 Anodic polarization measurement

Electrochemical measurement was conducted using three electrode method (HZ-3000 Hokutodenkou Japan) composed of specimen (working electrode), Pt (counter electrode) and the saturated calomel electrode (S.C.E.) (reference electrode). All the test were prefomed in lactic Ringer's solution of 310K. Potentio dynamic corrosion test was conducted with the sweep rate of 20mV/min from –1000 mV to 2000 mV (vs. S.C.E.).

2.7 Test animals and surgical procedure

The test animals were female Wister rats from the laboratory Animal Center (University of Kitasato). Their weights range from 239g to 249g. Periosteal implantation was accomplished using 9 NiTi alloy and 9 Pure Ni and 9 AISI 316L implants by applying one specimen per rat. The animals were anaesthetized with a blend anesthetic injected intraperitoneally. The hair was shaved around the implantation site and the skin was sterilized. A 20 mm skin incision was made with a knife along the lateral side of the right femur. The muscles were bluntly separated to disclose the femoral bone periosteum. The periosteum was kept intact, to avoid the scar formation effects of surgical trauma. The test implant was placed in direct contact with the intact femur periosteum. 2, 4 and 8 weeks after implantation, the implants were dissected from three rats in each group together with the femur and 5-10mm surrounding soft tissue.

3. EXPERIMENTAL RESULTS AND DISCUSSIONS

3.1 Evaluation of damage of cell membrane

Influence of material difference upon the damage of cell membrane was shown in Fig.1. Remarkable damages of macrophage were recognized under the corrosion product concentration conditions up to 32ppm. Most serious damages in the macrophage were obtained under the concentration of corrosion product from 64ppm to 128ppm. Damage of cell membrane (D) for both NiTi alloy and Ti-6Al-4V alloy obtained under various corrosion product concentration conditions were summarized. The damage of cell membrane (D) showed the tendency of increase as the increase of concentration of corrosion product for both NiTi alloy and Ti-6Al-4V alloy. However the degree of damage of cell was saturated even under the condition of 32ppm for Ti-6Al-4V alloy due to fatal damage of cell. Therefore, the damage degree of cell membrane showed relatively low for NiTi alloy. For this reason, NiTi alloy has better cytocompatibility compared with Ti-6Al-4V alloy. Even though it was said that Ti-6Al-4V alloy had better biocompatibility, the corrosion product of it damaged cell membrane to relatively high degree.

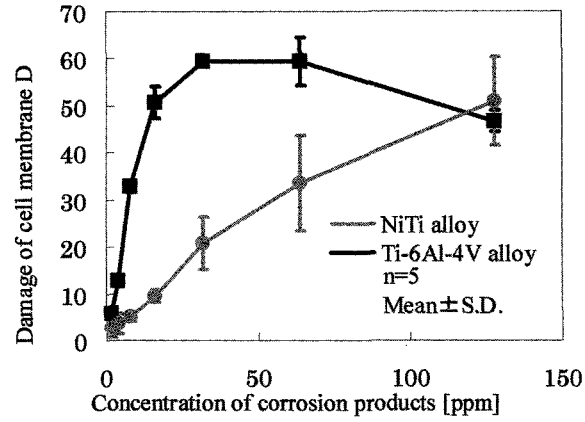

Fig.1 Influence of material difference upon the damage of cell membrane.

3.2 Evaluation of inflammatory reaction of macrophage

To evaluate the degree of the inflammatory reaction of cell, hIL-1β value was measured. These measurements

were conducted under the same concentration condition of both NiTi alloy and Ti-6Al-4V alloy. Then, the amount of hIL-1β generated by macrophage was determined employing ELISA method and shown in Fig.2. This figure showed that in case of Ti-6Al-4V alloy corrosion product administered hIL-1β value increased abruptly as the increase of concentration of corrosion product. After hIL-1β showed the peak value under the condition of 32ppm, then that value decreased again gradually. Therefore, the corrosion product of Ti-6Al-4V alloy caused serious inflammatory reaction in the macrophage even under the concentration condition of 32ppm. The re-decrease in hIL-1β value under the corrosion product concentration conditions up to 32ppm may imply that the damage of cell is so serious as the macrophage being lost its function. On the other hand, in case of NiTi alloy, the hIL-1β value was suppressed compared with Ti-6Al-4V alloy. That increased gradually as the increase of corrosion product concentration and no peak was recognized in hIL-1β value. Therefore, judging from the data of inflammatory reaction of macrophage, NiTi alloy shows better biocompatibility compared with Ti-6Al-4V alloy.

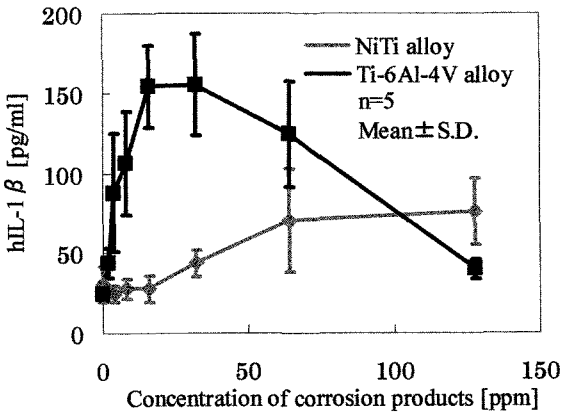

Fig.2 Dependency of hIL-1β value upon the concentration of corrosion products.

3.3 In vivo evaluation of biocompatibility of NiTi alloy

Periosteal implantation was accomplished using electro-polished NiTi alloy, Pure Ni and AISI 316L by applying one specimen per rat. After implantation of 2, 4 and 8 weeks, in vivo evaluation of biocompatibility was conducted. No osseointegration was seen in any of the samples, but fibrous capsule with various thickness was recognized. Fibrous capsule thickness was largest at all implantation period in the pure Ni group. In NiTi group, the fibrous capsule thickness value was found to be smaller than that of Pure Ni group. The fibrous capsule generated by NiTi implants resembles more the one produced by AISI 316L implants than Ni implants. Dependency of fibrous capsule thickness upon material difference and implantation period were summarized and shown in Fig.3. Biocompatibility of NiTi alloy is superior compared with that of Pure Ni. No pitting was recognized by FE-SEM observation to both of NiTi alloy and AISI 316L specimen surface after 8weeks implantation. Also, it was made clear that the biocompatibility of NiTi alloy showed almost the same level as that of the stainless steel, which gave a lot of actual results as a biomaterial until now. Therefore, it was understood that there is no problem in the biocompatibility of NiTi alloy for applying as a biomaterial.

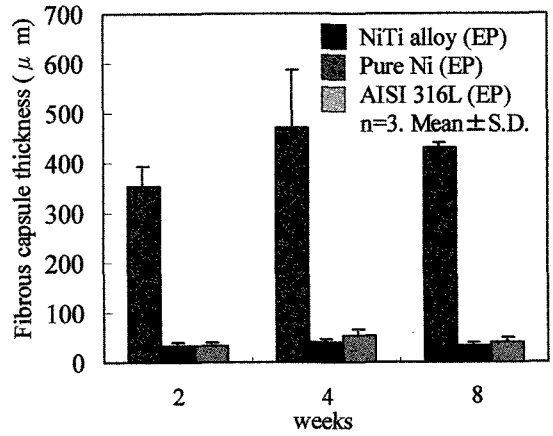

Fig.3 Dependency of fibrous capsule thickness upon materials and implantation period.

3.4 Anodic polarization behavior of NiTi alloy

Anodic polarization measurements were conducted in lactic Ringer's solution at 310K for NiTi alloy, Ti-6Al-4V alloy and AISI 316L stainless steel. The obtained results were shown in Fig.4. This figure indicated that current density of AISI 316L is increased at about 500mV[vs. S.C.E] and corrosion is acceleratedly progressed. It was made clear that current density of NiTi alloy and Ti-6Al-4V alloy were shown to be suppressed. These materials showed superior corrosion resistance compared with that of AISI 316L. NiTi alloy showed superior corrosion resistance compared with Ti-6Al-4V alloy in passivated area, judging from its low current density. For that reason NiTi alloy showed superior corrosion resistance compared with AISI 316L and Ti-6Al-4V alloy which gave a lot of actual results as a biomaterial until now. From the view point of corrosion resistance NiTi alloy was made clear to be applicable to orthopedic field.

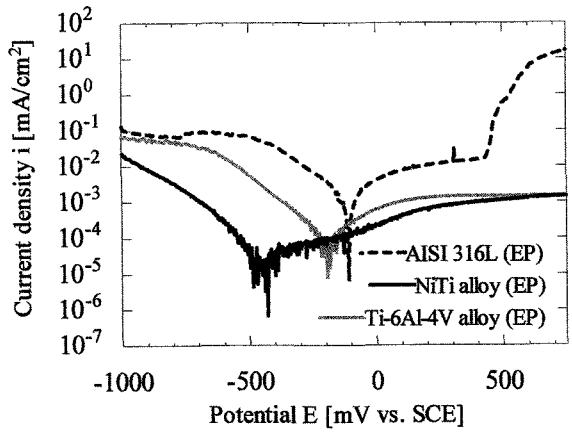

Fig.4 Polarization curves of NiTi alloy, Ti-6Al-4V alloy and AISI 316L in lactic Ringer's solution of 310K.

3.5 Improvement of corrosion resistance by oxidazation

Although the NiTi alloy shows superior corrosion resistance it was anxious about the toxicity of Nickel content. It is necessary to improve corrosion resistance of NiTi alloy by surface treatment. To specify optimum oxidation time for passivity film formation on NiTi alloy surface various heat treatments in air were conducted. The anodic polarization curve of NiTi alloys with different surface finish, i.e. EP and HT (heating time was 1,3 and 5 hours) were obtained and shown in Fig.5. The NiTi alloy specimen whose surface were heat treated showed superior corrosion resistance compared with that of electro-polished NiTi alloy, judging from the low current density in anodic area. It was made clear that optimum oxidation time was 1 hour, judging from low current density in passivity area. In this case, the specimen with finer finish and with TiO_2 thin surface layer investigated by XPS (Q-2000 Physical electronics U.S.A.) showed superior corrosion resistance. Therefore, not only the corrosion resistance of NiTi alloy but also the safety and reliability of that to a living body may be extremely improve. Biocompatibility of heat treated NiTi alloy implantation in the living body is going to be evaluated in near future.

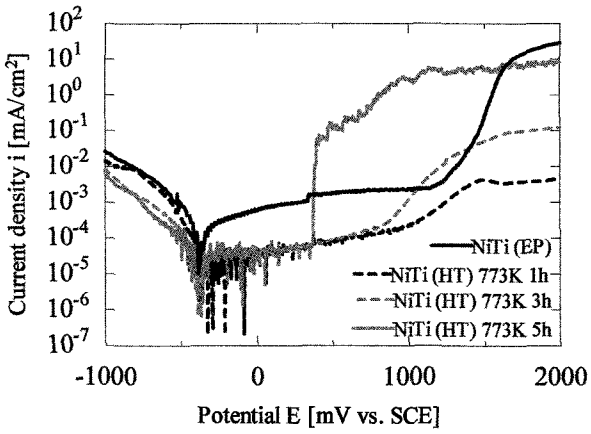

Fig.6 Polarization curves of NiTi alloy with different surface finish: in lactic Ringer's solution of 310K after electro-polishing and heat treatments.

4. CONCLUSIONS

NiTi alloy used for stent and bone nail material is existing for a long time in living body. Excess amount of dissolved Ni ion may cause allergic reactions and toxic reactions. Accordingly it is important to make clear the influense of the corrosion products of NiTi alloy upon the living body. Therefore in this paper, corrosion resistance and biocompatibility of NiTi alloy were investigated. Also, for improving the biocompatibility of NiTi alloy various in air oxidation treatments were conducted. Results obtained are summarized as follows:

1.The LDH activity value of NiTi alloy that means the physical damage to the cell membrance obtained to be moderate values compared with that of Ti-6Al-4V alloy.

2. Concerning inflammatory reaction of macrophage, NiTi alloy shows better biocompatibility compared with Ti-6Al-4V alloy judging from value of hIL-1β.

3.Fibrocellular capsule thickness was largest at all implantation period in the pure Ni group. In NiTi group the fibrocellular capsule thickness value was found to be lower than Pure Ni group. The fibrocellular capsule generated by NiTi implants resembles more the one produced by stainless steel than Ni implants.

4.NiTi alloy showed better corrosion resistance compared with AISI 316L stainless steel and Ti-6Al-4V alloy.

5.Corrosion resistance of NiTi alloy was improved when the specimen surface was in air oxidized and optimum heat treating time was 1hour.

Therefore, the solve out of nickel ion can be suppressed by applying such surface treatment. Therefore, NiTi alloy shows good corrosion resistance and good biocompatibility and can be applied to an orthopedics field.

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