Evaluation of Biocompatibility for Titanium-Nickel Shape Memory Alloy
in Vivo and in Vitro Environments

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This study was conducted to evaluate the biocompatibility of titanium-nickel shape memory alloy used as a medical implant material. The authors carried out the following electrochemical corrosion test and in vivo and in vitro biological tests for the alloy and some metal and alloys clinically used previously to compare the intensities concerned with the biological reactions, that is, (1) anodic polarization test for the alloy in a quasi-body fluid, (2) cell proliferation tests for pure titanium (cp Ti), pure nickel (cp Ni), SUS316L stainless steel, titanium-6 mass% aluminium-4 mass% vanadium (Ti-6Al-4V), and titanium-55 mass% nickel (Ti-55Ni) by using of L929 fibroblastic cells, (3) Lactate dehydrogenase (LDH), human interleukin-1β (hIL-1β), and human tumor necrosis factor-α (hTNF-α) biochemical assays by using of U937 human macrophages administered the corrosion products of these alloys for the cells, (4) measurement of the amount of excretions of the metallic corrosion products of Ti-55Ni, SUS316L stainless steel, and Ti-6Al-4V with urine and feces injected into the abdomen cavity of Wistar rats, and (5) tissue reaction observations for SUS316L, Ti-55Ni, and cp Ni wires implanted along the femoral bone axis of the rats.

The following results were obtained. (1) The pitting corrosion potentials of Ti-55Ni alloy was drastically improved by the aging treatment. (2) In the case of Ti-55Ni alloy, the inflammatory cytokines, hIL-6β and hTNF-α, were suppressed to lower levels compared with Ti-6Al-4V alloy. (3) Corrosion products prepared from the titanium alloys were stable in the body. Then it is very hard to eliminate the titanium ions with urine and feces. (4) Ti-55Ni alloy was shown an excellent biocompatibility evaluated by the in vivo implantation test, because of the stable passive film formed on the surface and protected the metal ion release to the surrounding tissue.

Keywords: titanium-nickel shape memory alloy, implant metal, corrosion, cytotoxicity, biomaterial, biocompatibility

1. Introduction

Titanium-nickel (TiNi) alloy is suitable for a metallic biomaterial with numerous favorable properties, such as super-elasticity, shape memory, high corrosion resistance, and others. Ti-Ni alloy having super-elasticity is typically used as intravascular stents and orthodontic wires, while those having shape memory are used experimentally as dental implant and fracture fixation plates.¹⁻⁴ Thus, TiNi alloy has unique characteristics that are not available in other materials, and has great potential as a metallic biomaterial. However, because nickel is potentially allergenic and carcinogenic, the clinical applications of TiNi alloy are limited.

To evaluate the biocompatibility of metallic biomaterials, ISO10993 details a suitable method, and the safety standards of many nations are based on this method. Many investigators were reported about the potential uses of TiNi alloy, but reports have focused on its safety.

Such implants are directly contacted to the surrounding tissue in the body. In many clinical studies, metal ion releases were confirmed in tissues adjacent to metallic implants in human bodies.⁵⁻⁻⁷ Then, we designed an electrochemical and biological evaluations and comprehensively evaluated the biocompatibility of TiNi alloy by referring to conventional biocompatibility evaluation standards. The interaction between tissue and the metal surface is important to study of the biocompatibility of the implant. The electro-chemical interaction is considered adverse if there is evidence of specific damage by the metallic corrosion products or released metallic ions in the tissue.⁸⁻¹⁰ Thus, the biocompatibility of the implant is depended on the electro-chemical stability in the body. In previous cell culture evaluations for nickel-chromium dental alloys, the metal ions released from the alloy caused no evidence effects on the cellular morphologies or variability. However, decreases in cellular proliferation and phagocytosis were observed in cells exposed to nickel-based and nickel containing alloys.¹¹⁻¹³ For this reason, it is regarded that the intensity of the inflammatory or toxic reaction caused by nickel ion release or compounds though it is expected to use as implant material.

The present study was conducted to evaluate the biocompatibility of TiNi shape memory alloy as an implant medical material. As the first, the electro-chemical stability of passive film on Ti-55 mass% Ni (Ti-55Ni) alloys was evaluated by anodic polarization test in a quasi-biological fluid and the corrosion resistance of the alloy was compared to more conventional SUS316L stainless steel and Ti-6Al-4V alloys. As the second, in vitro and in vivo toxicity tests were carried out to evaluate the quality of the biocompatibility for the alloy. We also performed the same evaluation on SUS316L and Ti-6Al-4V, which were used clinically, and we discuss the problems associated with biomaterials implanted in the human body by comparing biocompatibility.

2. Experimental Procedures

2.1 Test materials

In order to facilitate comparison of the alloys currently in use for the manufacture of implants, a specimen of each type was selected for this investigation. Ti-55Ni wires were used for the electrochemical corrosion test. Ti-55Ni, Ti-6Al-4V, SUS316L stainless steel, commercial based pure nickel (cp Ni) and pure titanium (cp Ti) were used for in vitro
cytotoxicity and in vivo animal implantation tests. The Ti-55Ni wires used for the corrosion test were finished by cold-drawing with 30% reduction and intermediate annealing to make a wire with a diameter of 1.0 mm.10) The oxidized surface layer of each wire was removed by polishing with emery papers. The specimens were annealed and followed by quenching in water. Wire shape specimens were cut to papers. The specimens were annealed and followed by resin coating.

### 2.2 Electro-chemical corrosion test

Anodic polarization test was conducted using potentiostat (Seiko EG&G Model-327 Japan) according to the three electrodes method with test specimen (working electrode, WE), platinum wire (counter electrode, CE), and saturated calomel reference electrode (RE) as shown in Fig. 1. Salt bridge probe was used to connect the reference electrode. The probe tube was filled with saturated potassium chloride (KCl) solution. Anodic polarization test was conducted with the sweep rate of 0.5 mV/sec from −1.0 to +2.0 V (vs. SCE) for Ti-55Ni alloys and SUS316L specimens and from −1.0 to +6.0 V (vs. SCE) for Ti-6Al-4V and cp Ti specimens to observe the current density behavior of each material. 1.0 cm² of specimen surface was exposed in PBS(−) and other portion was isolated electrically from the electrolyte solution by epoxy resin coating.

### 2.3 Preparation of corrosion products

Corrosion product for each metallic specimen was prepared to use in vivo and in vitro toxicity tests. The potentiostat mentioned above was used to obtain metallic corrosion products. Fixed anodic potential was applied to each metallic specimen in 200 mL of PBS(−) solution. Each applying anodic potential was maintained for 20 minutes at +2.0 V (vs. SCE) for Ti-55Ni alloy, pure nickel, and SUS316L stainless steel and at +5.5 V (vs. SCE) for pure titanium and Ti-6Al-4V alloy respectively. Concentration of dissolved metallic ions in the solution were arranged to be carried out in PBS(−) solution. Anodic polarization test was conducted with the sweep rate of 0.5 mV/sec from −1.0 to +2.0 V (vs. SCE) for Ti-55Ni alloys and SUS316L specimens and from −1.0 to +6.0 V (vs. SCE) for Ti-6Al-4V and cp Ti specimens to observe the current density behavior of each material. 1.0 cm² of specimen surface was exposed in PBS(−) and other portion was isolated electrically from the electrolyte solution by epoxy resin coating.

### 2.4 In vitro toxicity test

#### 2.4.1 Preparation of Cell culture medium

L929 (Mouse Fibroblastic Cell) and U937 (Human Leukemic Monocyte Lymphoma Cell) were used for in vitro cytotoxicity tests.

Culture mediums for L929 and U937 were prepared as follows: 10.2 g of powdered synthetic mediums RPMI-1640® (Nissui 2, Nissui Pharmaceuticals Japan) and Eagle’s MEM® (Nissui 1, Nissui Pharmaceuticals Japan) and Eagle’s MEM® (Nissui 1, Nissui Pharmaceuticals Japan) were dissolved into 1 L of DDW, respectively. After 30 min of autoclave sterilization at 393 K, 0.3 g of L-glutamic acid, 1.0 × 10⁻⁴ units of penicillin and 100 mg of streptomycin, and 10 vol% of fetal bovine serum (FBS) were added to the medium. To control the hydrogen ion concentration in medium, 10 vol%
aq was intermixed to make pH 7.4 under atmospheric environment containing 5 vol% CO₂ in air. Both Cells were cultured in CO₂ Incubator at 310 K, 100% of humidity environments.

2.4.2 Procedures of cell toxicity test

(1) Measurement of cell proliferation rate

The evaluation of cytotoxicity for the corrosion products is based on the fundamental principle that the growth rate of living cells through the prolific activity is governed by their living environment. When the cells are incubated in a controlled suitable environmental medium, they are increased exponentially through the incubation period. However, when foreign substances are thrown into the medium, the prolific activity in the cells would be decreased, if the substance have toxicity for the cells. From comparison of these two cell proliferation rates, we can evaluate the cytotoxicity of the substance for the cells experimentally. The cell proliferation rate, \( R \), was defined as follows,

\[
R = \log_2 \frac{N}{N_0} / T
\]

where, \( N_0 \) and \( N \) mean the initial sowing cell density and the density after cultured for \( T \) days on each metallic specimen surface, respectively.

(2) LDH assay

U937 cells were used to perform the LDH assay for the metallic implant. 0.32 μg/mL of piromidic acid (PMA) was added in the culture medium, the cells could be differentiated in macrophage.

Metallic corrosion compound adjusted to the known concentrations, 2, 4, 8, 16, 32, 64, and 128 ppm in medium, was administrated to the cultured U937 macrophage. After the incubation period for 48 hours, each culture medium was collected to measure LDH revitalization of cells. The strength of toxicity is closely depended on the amount of detected lactate dehydrogenase (LDH) concentration in the medium, because of the LDH inside of the cell is flowed out through the damaged cell membrane. Hence, the concentration of LDH in the medium is considered as a toxic factor indicated the degree of damage for cells. To evaluate the degree of damage of cell membrane, the LDH activity in the medium was measured. Also, to know the total concentration of LDH of the cultured cells, the cultured cells were completely destroyed by a supersonic wave homogenizer for 3 minutes at 277 K.

The damage of cell membrane (\(*D*) was defined as the following equation.

\[
*D = \frac{\text{Objective LDH av.} - \text{Negative LDH av.}}{\text{Positive cont. LDH av.} - \text{Negative LDH av.}} \times 100(\%)
\]

where, the objective LDH av. means the concentration of LDH released to the medium from the inside of cells. The positive LDH av. means the concentration of LDH in medium, which cells were destroyed by the homogenizer. And the negative control LDH av. means the concentration of LDH in the incubated medium without cells. All the experiments were iterated five times. The mean value was assumed to be LDH av.

LDH concentration in each sample was measured using a LDH Assay kit (LDH UV Test Wako®, Wako Pure Chemical Industries Japan) and assayed it according to the instruction.

(3) Inflammatory test

Macrophage are derived from myeloid stem cells in the bone marrow and represent the final stage of differentiation of the mononuclear phagocyte cell line. They play a role in inflammation, host defence, and reactions against a range of autologous and foreign substances.\(^{11}\) Their functions involve the release of inflammatory mediators, including the production of cytokines, such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). The amount of the production of these cytokines is proportional to the degree of inflammation reaction of the macrophage. Then, the concentrations of cytokines, IL-1β and TNF-α, produced with macrophage were assayed by enzyme linked immuno-sorbent assay (ELISA) method to evaluate the toxicity of the metal ions.

1.0 x 10³ cells/well of U937 macrophages were cultured in 2 mL of 10 vol% fetal bovine serum (FBS) added Eagle’s MEM including metallic corrosion products for 48 hours. After the incubation period, the supernatants of medium were corrected and hIL-1β and hTNF-α were measured employing ELISA method using testing kits (h-Interleukin-1β ELISA® and hTNF-α ELISA® Roche Diagnostics in Germany).

2.5 In vivo toxicity test

2.5.1 Metal ion injection and measurement of recovery with urine and feces

In vivo studies were performed with specific pathogen-free (SPF) adult female Wister rats, the body weights were ranged from 2.34 to 2.44 N, mean 2.37 ± 0.04 N, obtained from the Animal Laboratory Center in CLEA Japan Inc. Seven rats in each experimental group were used to measure the amount of metal ions in urine excretion and internal organs storage.

1.195 mg of corrosion products of Ti-55Ni, Ti-6Al-4V, and SUS316L Stainless Steel including in 5 mL of lactate Ringer’s Solution were prepared for the metal ions excretion test. The each corrosoidal sample adjusted at pH 8 with 1 N hydrochloric acid (HCl) and NaOH, was injected to the abdomen cavity of rat. Rats were anaesthetized with pentobarbital (0.7 mg/kg) intraperitoneally for all experiments. They were housed individually in metabolic cages for a week and urine and feces samples were collected daily. After the breeding period, 1 mL of venous blood was collected and turning flow treatment with 500 mL of heparinized normal saline solution for 30 minutes to remove the blood in the organs and tissues. After then, the animals were sacrificed, the internal organs, i.e. liver, kidney, pancreas, lungs, and 1 mL of blood, were collected. All the corrected urine samples and organs were diluted with 63 vol% HNO₃ and heated to 393 K to complete dissolve and evaporated until the volume was less than 1 mL on a hot plate. Then, the solutions were adjusted at 5 mL accurately to 20 vol% of HNO₃ with addition of DDW. Metal ion concentrations for each sample were analyzed individually using the ICP analyzer mentioned above.

2.5.2 Preparation for fluorescent microscopy observation

Wister rats were anaesthetized with pentobarbital intraperitoneally. Periosteal implantation was accomplished using Ti-55Ni alloy, pure Ni, and SUS316L wires. The hair was shaved around the implantation site of the right lower leg and
the skin was disinfected with povidone iodine (Isodine®, Meiji Pharmaceuticals Co., Japan). A 20 mm of skin incision was made along the lateral side of the right femur. The muscles were bluntly separated to disclose the femoral bone periostium membrane. The periostium membrane was kept intact as much as possible to avoid the scar formation effects of surgical trauma. Test specimen was implanted in direct contact with the membrane along to the bone axis as shown in Fig. 2. 5 days before the rat was sacrificed, 0.7 mg/mL of an antibiotic, Tetracycline, was injected to the abdomen cavity of the rat to label the bone newly formed around the implant specimen. The administered Tetracycline® (Pfizer Japan Inc.) is confined in the bone newly formed. After breeding the rats for 2 and 8 weeks, they were sacrificed and the both side of femurs were corrected from the body.

The bones were fixed with 99.5 vol% ethanol and stained with Villanueva bone stain solution (Villanueva bone stain powder® Maruto Inc. Japan) for 24 hours. Then, the samples were rinsed with water for 24 hours. After the treatment, samples were dehydrated with 100% ethanol and substituted with acetone. Finally, the samples were embedded with PMMA resin. The PMMA block was sliced off to be 50 m in thickness with a diamond wheel cutter and polished until to be 10–20 m in thickness. After being polished, the hard tissue film like sample was put on flat glass plate to observe with a fluorescent microscope. Remodeling activity of bone could be observed quantitatively using a bone labeling technique with Tetracycline.

3. Results

3.1 Electro-chemical corrosion test

The anodic polarization characteristics of TiNi alloy were measured in PBS(−) solution. As controls, we used Ti-6Al-4V and SUS316L stainless steel, which were already used in clinically. Figure 3 shows the results of the electro-chemical tests. In Fig. 3(a), the untreated and solution-treated specimens of Ti-55Ni alloy changed to the trans passive state, showing a pit corrosion potential of approx. +0.5 V (vs. SCE). In Fig. 3(b), the aged specimens group of TiNi alloy showed a pitting corrosion potential of approx. +1.2 V (vs. SCE). Aging treatment improved the corrosion resistance in a similar manner as electro-polishing, as reported previous-
The current density of aged specimens showed the same level in the passive area compared with not aging specimens and electro-polishing specimen. We also performed similar polarization tests on SUS316L, cp Ti, and Ti-6Al-4V as controls. The results were shown in Fig. 3(c). The pit corrosion potentials of SUS316L and Ti-6Al-4V were +0.4 and +5.4 V (vs. SCE), respectively. The current density of aged TiNi alloys in the passive state was similar level to Ti-6Al-4V. The pit corrosion potential of aged Ti-55Ni alloys was remarkably higher than that of SUS316L. The current density of SUS316L in the passive state, however, was much lower than that of Ti-55Ni alloy. Pure titanium showed the most low level of current density in all test materials.

### 3.2 In vitro toxicity test

1.0 × 10^5 cells/cm² of L929 adhesion cells were seeded on soaked metallic specimens and incubated for 3 days, after which the cell density change was measured. The influence of metal type on cell proliferation was used as a method of evaluating cytotoxicity. The results were shown in Fig. 4(a). Non heat treated Ti-55Ni alloy specimens were used in this experiment. There were no significant differences between cell proliferation for any of the materials tested. In addition, the proliferation did not differ from the controls, which cells seeded on culture dishes not containing any metal.

The corrosion products of Ti-55Ni alloy, SUS316L, and Ti-6Al-4V were added to culture medium at metal ion concentrations of 2, 4, 8, 16, 32, 64, and 128 mass ppm. L929 cells were added to 8-well culture plates at 1.0 × 10^5 cells/well and were incubated in 1 mL of metal ion-containing culture medium for 3 days. After the incubation period, number of cells was counted and the proliferation rate was evaluated for each culture dish. Fig. 4(b) shows the results. For the Ti-55Ni alloy corrosion products, the proliferation rate decreased at a metal ion concentration of 64 ppm, however, the rate was recovered at 128 mass ppm. It was uncertain why the rate was decreased only at 64 mass ppm. Pure titanium had no effect on proliferation at metal ion concentrations of up to 32 mass ppm, but the number of cells decreased at higher concentrations. Ti-55Ni alloy showed a similar trend to Ti-6Al-4V without at 64 mass ppm.

We measured the LDH concentrations in the culture medium in order to quantify cell membrane damage attributable to the phagocytosis of Ti-55Ni alloy corrosion products by U937 macrophages. By measuring the concentration of enzyme released into the culture medium, the degree of cellular damage can be evaluated. The metallic corrosion products of Ti-55Ni alloy and Ti-6Al-4V were administered to U937 cells at concentrations of 2, 4, 8, 16, 32, 64, and 128 mass ppm. The amount of released LDH was measured as LDH activity. The measurement results were shown in Fig. 5. The damage level of cell membrane with Ti-6Al-4V peaked at 32 mass ppm. At higher metal ion concentrations, the damage level remained high. For NiTi alloy, the damage levels were lower than that of Ti-6Al-4V in all metal ion concentrations and simply increased with metal ion concentration.

IL-1β and TNF-α are representative cytokines indicating inflammatory reaction. As cells produce these cytokines, inflammatory reactions increase in intensity. Figure 6 shows the production of both cytokines after exposure to the corroidal solution of Ti-55Ni alloy and Ti-6Al-4V. Each cytokine was measured by the ELISA. For Ti-6Al-4V, both

![Fig. 4 (a) Prolifcations of L929 cells cultured on metal plates (b) Comparison of the cell proliferation rates, R, depending on the metal ion concentrations for cp Ti, cp Ni, Ti-55Ni, and Ti-6Al-4V.](image)

![Fig. 5 Damage degrees of cell membrane by corrosion extractions exposure tests. LDH assay was carried out for Ti-55Ni alloys and Ti-6Al-4V.](image)
cytokine productions peaked at a metal ion concentration of 16 and 20 mass ppms and decreased at higher concentrations. For Ti-55Ni alloy, both cytokine productions increased with metal ion concentration. At all metal ion concentrations tested in this study, cytokine production with Ti-55 Ni alloy remained lower than that with Ti-6Al-4V.

3.3 *In vivo* toxicity test

1.16 mg of corrosion products for each alloy were diluted in 5 mL of physiological saline solution and injected into the abdominal cavities of Wistar rats. Rats were then kept in metabolic cages for one week. Urine from the rats was collected daily and feces excreted was collected at the end of the week. Figure 7 shows the amount of metal ions excreted in the collected daily urine. For all alloys, the chemical compositions at injection into the abdominal cavities as shown in Table 1 did not correspond to the metal ion contents. In Fig. 7(a), Ti-55Ni alloy released very little titanium, but large amounts of nickel. Ferrite, the main component of SUS316L, was excreted only 8 μg with the urine sample at the first day as shown in Fig. 7(b). The release of chromium was low and did not correspond to the chemical composition in Table 1. The discharge of nickel and molybdenum decreased with time but they were eliminated smoothly. With regard to Ti-6Al-4V, very little release was noted. Titanium and Aluminum remained undigested in the abdomen cavities. Table 3 listed the metal concentrations accumulated in internal organs and the
amount of metals excreted in urine and feces. Titanium from Ti-55Ni and Ti-6Al-4V were not recovered from urine or feces, but large amounts remained present in the body.

Metal wires were implanted into Wister rat femurs for two or eight weeks, and tissue reactions were studied histologically. Figure 8 shows Villanueva stain tissue images of a tetracycline-labeled bone. The representative cp Ni wire in the tissue image was covered with a hypertrophied fibrous pseudo membrane, thus indicating strong toxicity. Slight intervention of fibrous tissue was observed where Ti-55Ni wires contacted surrounding tissues. The yellow color image indicates the tetracycline-labeled woven bone and actively the new bone formation at the labeled area. After eight weeks, the inflammatory ossification reaction observed at two weeks had ceased completely and the wires were covered with mature bone tissue. SUS316L wires showed similar fluorescent images as the Ti-55Ni wires. Table 4 gives the thicknesses of pseudo films formed with each metal wire.

### Table 3 Excretion and storage of metallic ions.

<table>
<thead>
<tr>
<th>Material</th>
<th>Element</th>
<th>Excretion with</th>
<th>Storage in</th>
<th>Recovery rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Urine (µg)</td>
<td>Feces (µg)</td>
<td>Organs (µg)</td>
</tr>
<tr>
<td>316L Cr</td>
<td>Fe</td>
<td>61.4 ± 19.5</td>
<td>1038.5 ± 201.7</td>
<td>Incapable*</td>
</tr>
<tr>
<td>S.Steel</td>
<td>Cr</td>
<td>2.9 ± 1.3</td>
<td>7.3 ± 6.4</td>
<td>13.0 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>Ni</td>
<td>75.4 ± 17.2</td>
<td>19.4 ± 12.9</td>
<td>4.3 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>Mo</td>
<td>17.0 ± 1.7</td>
<td>6.4 ± 2.6</td>
<td>10.4 ± 1.8</td>
</tr>
<tr>
<td>Ti-6Al-4V</td>
<td>Ti</td>
<td>6.9 ± 1.2</td>
<td>12.3 ± 2.9</td>
<td>47.5 ± 18.8</td>
</tr>
<tr>
<td></td>
<td>Al</td>
<td>20.2 ± 2.1</td>
<td>41.8 ± 23.4*</td>
<td>94.4 ± 23.1</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>28.2 ± 4.2</td>
<td>2.9 ± 0.7</td>
<td>5.7 ± 1.6</td>
</tr>
<tr>
<td>Ti-55Ni</td>
<td>Ti</td>
<td>3.1 ± 0.5</td>
<td>5.6 ± 1.1</td>
<td>21.6 ± 8.5</td>
</tr>
<tr>
<td></td>
<td>Ni</td>
<td>221.7 ± 50.6</td>
<td>56.9 ± 37.9</td>
<td>12.6 ± 4.9</td>
</tr>
</tbody>
</table>

*High levels of metallic ion were detected in control rats.

### Table 4 Pseudo membrane thickness formed around the alloys.

<table>
<thead>
<tr>
<th>Material</th>
<th>Pseudo membrane thickness (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 weeks</td>
</tr>
<tr>
<td>cpNi</td>
<td>360 ± 40</td>
</tr>
<tr>
<td>SUS316L</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>Ti-55Ni</td>
<td>24 ± 1</td>
</tr>
</tbody>
</table>

### 4. Discussion

#### 4.1 Corrosion resistance of TiNi alloy

The corrosion resistance of each metal was evaluated electrochemically by anode polarization method. Metal ion release from metals with excellent corrosion resistance is smaller. Therefore, the maintenance of corrosion resistance in a living body is an important requirement for metallic biomaterials.

All materials provided for the experiment obtain electro-
chemical stability by forming a passive film on the metal surface. Therefore, the electro-chemical strength of the oxidized metal surface determines the corrosion resistance of the metal.\textsuperscript{13} Ti-55Ni wires of 1 mm diameter were produced by hot forging an ingot, followed by cold drawing. The untreated wires received no treatment beyond this and possess an oxidized surface similar in appearance to black leather. Other Ti-55Ni wires received solution treatment after the oxidized surface was removed with emery paper, and solution-treated wires were aged by 30 minute annealing at each temperature. As shown in Fig. 2, the aged wires showed marked improvements in corrosion resistance when compared with untreated and solution-treated wires.

4.2 \textit{In vitro} evaluation of biocompatibility

In order to evaluate cytotoxicity, various biocompatibility and safety test methods have been devised. ISO10993 also gives representative test methods. Cells are exposed to a specimen or a specimen extract and toxicity is quantified based on cell proliferation rate. We evaluated the cytotoxicity of Ti-55Ni alloy by the direct contact method and the extract exposure method. The direct contact method showed no significant differences in cell proliferation between cp Ti, cp Ni, Ti-55Ni, SUS316L, and Ti6Al4V. In addition, cell proliferation rate did not differ significantly from the culture dish used as a control. Most metals used for metallic biomaterials are covered with passive films and cells only contact the metal oxide films. Because passive films are electrochemically stable, they are considered inactive to cells and have little effect on cell proliferation, irrespective of base metal type. The direct contact method is the most orthodox method, but may not allow appropriate toxicity evaluation. The extract exposure method allows cytotoxicity evaluation with high sensitivity. In our cytotoxicity test using extracts, we administered the same amounts of extract as present in each alloy to cells and compared the toxicity. We found that Ti-55Ni alloy showed better biocompatibility than Ti-6Al-4V. However, because the chemical components of PBS(\textsuperscript{1–}) are not same to the actual body fluid, the corrosion characteristic of the alloy in PBS(\textsuperscript{1–}) might not be strictly shown similar behavior in actual body fluid.

LDH activity directly indicates cell damage. In addition, cytokines can be regarded as an index for degree of inflammation. As shown in Figs. 5 and 6, LDH activity and cytokine production peaked with 20 to 30 mass ppm of corrosion products concentration of Ti-6Al-4V and decreased at greater concentrations. Both values became maximal at the same metal ion concentration not because toxicity decreased at 30 ppm but because cytotoxicity increased further at higher metal ion concentrations, and thus the reduced proliferation or extinction of cells lowered LDH and cytokine production. Because Ti-55Ni alloy did not show such peaks in LDH activity and cytokine production, Ti-55Ni alloy does not apparently cause cell extinction. Therefore, it is considered that Ti-55Ni alloy possesses better biocompatibility than Ti-6Al-4V.

As Fig. 3(c) shows, Ti-6Al-4V has the high corrosion resistance. Thus, if the alloy is implanted in a living body, the metal ion release is predicted to be low. If corrosion resistance is taken into account, the positions of Ti-55Ni and Ti-6Al-4V may be reversed in terms of biocompatibility. Considering the above results, electrochemical and cytotoxicity testing are both essential for biocompatibility evaluation, as cytotoxicity is determined by the toxic intensity and elution of the chemical composition.

4.3 Accumulation and excretion of metal ions

If metals eluted in a living body are accumulated in surrounding regions or internal organs, various functional disorders may occur. Implanted materials also require evaluation regarding general bio-systems. When evaluating this type of safety, we cannot rely solely on cytotoxicity testing; animal experiments are required. ISO10993 also indicates the necessity of animal experiments.

In order to clarify the tendency of metal extracts remaining in internal organs, we injected corrosion products into the abdominal cavities of rats and measured the metal concentrations in urine and feces, as well as the amounts remaining in internal organs. As Fig. 7 shows, nickel from Ti-55Ni alloy and SUS316L stainless steel was excreted smoothly in urine beginning immediately after administration. However, titanium from Ti-55Ni and Ti-6Al-4V was scarcely discharged in urine. Because the titanium content is about 45 mass\% for Ti-55Ni but 90 mass\% for Ti-6Al-4V, an implant made of Ti-6Al-4V will remain almost completely intact if corroded in a living body. The results of cytotoxicity tests also indicate that Ti-6Al-4V corrosion products are more toxic than Ti-55Ni alloy corrosion products, and may seriously impair biocompatibility if exposed to friction in an internal environment, as the passive film is subject to damage. Because Ti-55Ni alloy has greater wear resistance than Ti-6Al-4V, metal ion release may be lower, thus impairing biocompatibility to a lesser degree.

4.4 Evaluation of biocompatibility by histological observation

Histological observation is the most practical evaluation method for metallic biomaterials. This method allows evaluation of the bio-tissue reactions generated by direct contact with metals. From cp Ni, nickel ions are easily eluted into a living body, and these caused strong inflammatory reactions in the surrounding tissues. Thus, nickel wires are covered with fibrous pseudo-membranes. To evaluate the inflammatory reactions of an implanted metal, the Japanese Orthopedic Association prescribes a thickness-measuring standard for fibrous membranes. Based on the evaluation method, we measured the thicknesses of inflammatory membranes around Ti-55Ni and evaluated biocompatibility. As controls, pure Ni and SUS316L were measured in the same way. As Table 3 shows, Ti-55Ni alloy was found to have biocompatibility equal to or greater than that of SUS316L stainless steel. Similar results were also obtained in hard-tissue specimens, as shown in Fig. 8. After two weeks, bone tissues contacting Ti-55Ni showed strong inflammatory ossification around the metal. After eight weeks, however, the inflammatory ossification settled and osteoid tissues became woven bone. SUS316L showed similar bone formation. Based on these results, Ti-55Ni alloy has equivalent or more excellent biocompatibility as SUS316L stainless steel.
5. Conclusions

The interaction between tissue and the metal surface is influenced on the biocompatibility evaluation. Especially, the problem of metallic ion release with metal corrosion in the body is important. In this paper, we conducted electrochemical corrosion test and some biological tests for Ti-Ni alloy and investigated on of the biocompatibility. The following results were obtained:

(1) The potential changed from passive to the trans passive state, showing a pit corrosion for the solution-treated Ti-55Ni alloy was approx. +0.5 V (vs. SCE). The aging treatment was improved the corrosion resistance and the potential was risen to approx. +1.2 V (vs. SCE).

(2) In the L929 cell direct contact test for Ti-55Ni alloy, there were no significant differences between cell proliferations compared with that of SUS316L and Ti-6Al-4V. Because passive films are electrochemically stable, they are considered inactive to cells and have little effect on cell proliferation, irrespective of base metal type.

(3) In the L929 cell exposure test for Ti-55Ni alloy corrosion products, we found that Ti-55Ni alloy showed better biocompatibility than Ti-6Al-4V.

(4) The corrosion extractions exposure test, LDH activity and IL-1β and TNF-α production peaked with 30 mass ppm for Ti-6Al-4V. However, Ti-55Ni alloy did not show such peaks. Therefore, it is considered that Ti-55Ni alloy possesses better biocompatibility than Ti-6Al-4V.

(5) In histological biocompatibility evaluation for Ti-55Ni alloy, it was found to have biocompatibility equal to or greater than that of SUS316L stainless steel.

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