Surface Characterization of Amorphous Zr–Al–(Ni, Cu) Alloys Immersed in Cell-Culture Medium

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For the evaluation of zirconium-base amorphous alloys as biomaterials, the surface compositions of amorphous Zr–Al–Ni–Cu and Zr–Al–Cu alloys were characterized using X-ray photoelectron spectroscopy. The alloys were polished, autoclaved, and immersed in Hanks’ solution or Eagle’s minimum essential medium containing fetal bovine serum, MEM + FBS. Aluminum was enriched in the surface oxide film and the substrate just under the film of the alloys polished in water. After autoclaving, aluminum and copper were enriched in the substrate while zirconium was preferentially oxidized and incorporated into the surface oxide film. In Hanks’ solution, copper and nickel decreased in the substrate and surface oxide film, resulting in the enrichment of aluminum in the substrate. In MEM + FBS, zirconium preferentially decreased by the effects of amino acids and proteins while copper was enriched in the substrate. The surface composition of zirconium-base amorphous alloys was much influenced by amino acids and proteins in MEM + FBS.

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1. Introduction

The biomedical use of zirconium-base amorphous alloys is attempted to utilize the advantages of amorphous structure in biomaterials, such as bone plate, wires, and dental implants. The zirconium-base amorphous alloys show high tensile strength over 1500 MPa and low Young’s modulus under 100 GPa, which can improve mechanical biocompatibility of the biomaterials. On the other hand, all materials applied in human body must show high corrosion resistance as well as high strength and low Young’s modulus. Therefore, anodic polarization behavior in various biological environments and repassivation behavior of the amorphous Zr–Al–Ni–Cu alloy were examined to evaluate the corrosion behavior of the alloy. The anodic polarization curve of the alloy showed high polarization resistance and low passive current density as well as enough resistance to pitting corrosion in phosphate buffered solutions containing chloride ions. The total charge of repassivation reaction of the alloy was lower than that of crystalline pure zirconium. These results indicate that the zirconium-base amorphous alloy is sufficiently corrosion resistant in the human body.

On the other hand, the biocompatibility of zirconium-base amorphous alloy was not examined. The biocompatibility is defined as “the ability to exist in an in vivo environment for an acceptable period of time with no detrimental effect on the host”. Therefore, the alloy surface is required neither to contain nor to release any elements showing cytotoxicity, carcinogenicity, and so on. The surface characterization of zirconium-base bulk amorphous alloy is rarely performed as far as the author’s knowledge. Hence, surface composition of the alloy must be understood to discuss the corrosion resistance and biocompatibility of the alloy.

The surface of biomaterial contacts directly to body fluid containing various inorganic ions such as chloride and phosphate ions and organic compounds such as amino acids and proteins. The amino acids and proteins are adsorbed by metals and sometimes accelerate the dissolution of metals. Therefore, the alloys, whose surface has been characterized for the evaluation of the corrosion resistance and biocompatibility, are necessary to be exposed to the biological environment.

In this study, the surface characterization was performed on amorphous Zr–Al–Ni–Cu and Zr–Al–Cu alloys immersed in Hanks’ solution, or a cell-culture medium. Effect of inorganic ions on the surface reaction was evaluated using the Hanks’ solution and that of amino acids and proteins was evaluated using the cell-culture medium.

2. Experimental Methods

2.1 Specimens

Master alloys with the nominal atomic composition of Zr₆₅Al₇.₅Ni₁₇.₅Cu₁₇.₅ and Zr₆₅Al₇.₅Cu₂₇.₅ were prepared by arc melting of pure zirconium, aluminum, nickel, and copper in an argon atmosphere. Weight of the resultant master alloy was > 99.9% of the gross weight of source metals. The master alloys were re-melted in a quartz nozzle and rapidly quenched by casting the melt on the rotating copper wheel with argon gas pressure. Ribbon alloys with a width of 2–5 mm and a thickness of 50–55 μm were obtained. The structures of the ribbon alloys were determined to be amorphous by X-ray diffraction method with Cu Kα radiation as shown in elsewhere.

Free- and roll-sides of the ribbon alloys were polished with 600 grid SiC paper in distilled water and cut into pieces with a length of 15–20 mm. The specimens were ultrasonically rinsed in distilled water and acetone and dried for a few days to generate air-formed passive film and kept in a dessicator. As-polished specimens are named “Polished”. The “Polished” specimens were sterilized in an autoclave under a saturated vapor pressure at 393 K for 1.8 ks. The specimens after autoclaving are named “Autoclaved”. Subsequently, the “Autoclaved” specimens were immersed in 5 ml of Hanks’
solution\textsuperscript{13} (Hanks) or Eagle’s minimum essential medium\textsuperscript{14} containing 10 vol\% fetal bovine serum (MEM + FBS). The immersion was performed in the air containing 5\% CO\textsubscript{2} and a saturated water vapor at 310 K for 2.4 Ms (28d) in an incubator. The specimens after immersion in Hanks and MEM + FBS are named “Hanks” and “MEM + FBS”, respectively. The composition of Hanks’ solution and MEM + FBS are summarized in Table 1.

### 2.2 SEM and EDS

The free-side surfaces of “Polished” and “MEM + FBS” of the Zr–Al–Ni–Cu alloy were observed by a scanning electron microscope, SEM, (HITACHI, S-3100HT) with accelerating voltage of 25 kV.

Bulk composition of “Polished” of the Zr–Al–Ni–Cu alloy was measured using an energy dispersive X-ray spectrometer, EDS, (OXFORD MODEL 7021, LINK ISIS) with accelerating voltage of 20 kV. Three different regions on the free-side surface of the specimen were characterized. The composition of the Zr–Al–Ni–Cu alloy determined by EDS was shown in Table 1. Concentration of aluminum was smaller than the nominal concentration. Aluminum seemed to be slightly underestimated because of its low nominal concentration. Aluminum was conventionally treated as metallic state peak. This will cause overestimation of the alloy substrate just under the surface oxide film of the alloy during immersion in MEM + FBS.

The composition of the Zr–Al–Ni–Cu alloy determined by EDS is shown in Table 3. Concentration of aluminum was smaller than the nominal concentration. Aluminum seemed to be slightly underestimated because of its low nominal concentration. The concentrations of other alloying elements were almost same as the nominal concentration. Thus, the bulk composition was assigned as the nominal composition.

### 2.3 XPS

X-ray photoelectron spectroscopy, XPS, was performed with an electron spectrometer (SSI-SSX100). X-ray source was the monochromatized Al K\textsubscript{α} line (1486.61 eV). Take-off angle of photoelectron was 35° to specimen surface. All binding energies in this paper are relative to the Fermi level. The spectrometer was calibrated against Au 4f\textsubscript{7/2} (binding energy, 84.07 eV) and Au 4f\textsubscript{5/2} (87.74 eV) of pure gold and Cu 2p\textsubscript{3/2} (932.53 eV), Cu 2p\textsubscript{1/2} (952.35 eV), and Cu Auger L\textsubscript{3}M\textsubscript{4,5}M\textsubscript{4,5} line (kinetic energy, 918.65 eV) of pure copper. The energy values were based on published data.\textsuperscript{15} The free-side surface of the specimen was characterized. The composition and thickness of the surface oxide film and composition of the substrate just under the surface oxide film were simultaneously calculated according to the method described elsewhere.\textsuperscript{16,17} Empirical\textsuperscript{18–21} and theoretically calculated\textsuperscript{22} data of relative photoionization cross-sections was used for quantification. The relative photoionization cross-sections, $\sigma_j/\sigma_0$, are summarized in Table 2, where relative photoionization cross-section of a level $j$ electron of an element $i$ to that of O 1s electrons.
Bulk compositions of the alloys are also shown as a reference. The composition of “MEM + FBS” of the Zr–Al–Ni–Cu alloy was not determined because photoelectrons in metallic state were not detected probably due to the thick layer of adsorbed amino acids and proteins. Concentrations of zirconium, [Zr], of “Polished” decreased to zero after an autoclave treatment, indicating that zirconium is preferentially oxidized and probably incorporated into the surface oxide. Concentrations of aluminum, [Al], of “Polished”, “Autoclaved”, and “Hanks” were higher than those in the bulk composition, indicating the enrichment of aluminum in the substrate. Concentrations of copper, [Cu], of “Autoclaved” and “MEM + FBS” were higher than those in the bulk composition. Enrichment of copper in the substrate of amorphous Zr–Al–Ni–Cu alloy also occurs in a deaerated phosphate buffered saline solution.5) In addition, the enrichment of copper at interface between film and substrate is also observed on bulk amorphous Zr₅₅Al₁₀Cu₃₀Ni₅ alloy anodized at 0.84 V(SCE) for 20 h in air-saturated 0.1-M Na₂SO₄ with pH 8.8) Thus, the enrichment of copper in the substrate seems to occur when the alloy is oxidized. Concentrations of nickel, [Ni], of “Polished” and “Autoclaved” were similar to that in the bulk composition and decreased to zero after immersion in Hanks.

The [Al] and [Cu] increased and [Zr] decreased after autoclaving. Thus, the enrichment of aluminum and copper in the substrate was enhanced with the preferential oxidation of zirconium. After immersion in Hanks’ solution, the [Al] increased while the [Cu] and [Ni] decreased. After immersion in MEM + FBS, the [Al] decreased to zero while [Cu] increased up to 100 at% on the Zr–Al–Cu alloy, while substrate composition of “MEM + FBS” of the Zr–Al–Ni–Cu alloy was not obtained.
adsorption layer on the surface oxide film and additions. Therefore, oxidation of the alloys proceeded in a saturated water vapor and aqueous solution.

The thickness, \( t \), of the surface oxide film of the Zr–Al–Ni–Cu and Zr–Al–Cu alloys is shown in Fig. 3, wherein the \( t \) on “MEM + FBS” of the Zr–Al–Ni–Cu alloy could not be determined because no signal originating from metallic state were detectable. The \( t \) was large in this order: “MEM + FBS”, “Hanks”, and “Autoclaved”. Therefore, oxidation of the alloys proceeded in a saturated water vapor and aqueous solutions.

In “MEM + FBS”, the amino acids and proteins form an adsorption layer on the surface oxide film and the ad- sorption layer was covered with so-called contaminant carbon layer. However, the quantification method for three layers on the substrate was not developed. Then, to apply the conventional quantification method in which the contaminant carbon and surface oxide layers are assumed to exist on the substrate, the following two models were assumed. One is that amino acids and proteins are uniformly contained in the surface oxide film and the contaminant carbon apparently does not exist, where the amount of adsorbed amino acids and proteins is estimated. The other is that all amount of carbon is originated from the contaminant carbon, although some part of the carbon should be coming from adsorbed amino acids and proteins in MEM + FBS. In any case, it should be mentioned that both models are too simplified, however, the latter model is reasonable to quantify the surface oxide film because the amino acids and proteins in the surface oxide film on “MEM + FBS” is negligible if they were incorporated.

Based on the former model, the compositions of the surface oxide film of “MEM + FBS” of the Zr–Al–Ni–Cu and Zr–Al–Cu alloys were calculated as listed in Table 4. Nickel was not incorporated into the film of the Zr–Al–Ni–Cu alloy. Concentrations of zirconium, aluminum and copper of both alloys were similar each other. Differences in these concentrations between both alloys was reduced because concentrations of carbon and nitrogen were too large. Concentrations of carbon and nitrogen of both alloys were also similar each other. Therefore, both alloys adsorb almost the same amount of amino acids and proteins. In any case, this model could not be used for the discussion on the composition of the surface oxide film.

Based on the latter model with the contaminant carbon covering surface oxide film, compositions of the surface oxide film of all specimens were calculated as shown in Fig. 4. In addition, relative concentration of aluminum to zirconium, [Al]/[Zr] was calculated as shown in Fig. 5. Phosphorus, sodium, and chlorine contained in Hanks’ solution and MEM + FBS were detected in “Hanks” and “MEM + FBS” of the Zr–Al–Ni–Cu alloy whereas sulfur was not detected. Phosphorous, sodium, chlorine, and sulfur were detected in “Hanks” and “MEM + FBS” of Zr–Al–Cu alloys, except that sodium in “MEM + FBS” and chlorine in “Hanks” were not detected.

The binding energies of P 2p electrons were 132.6–134.5 eV, indicating that phosphorus existed as phosphate ions, \( \text{H}_3\text{PO}_4^{3−} \), and phosphate salt, such as zirconium phosphate. Concentration of phosphorous of “Hanks” was larger than that of “MEM + FBS” of both alloys, indicating that the adsorbed amino acids and proteins probably in-

Table 4 Composition of the surface oxide film on “MEM+FBS” assuming that adsorbed amino acids and proteins dispersed in the film.

<table>
<thead>
<tr>
<th>Alloy</th>
<th>Zr</th>
<th>Al</th>
<th>Cu</th>
<th>Ni</th>
<th>P</th>
<th>Na</th>
<th>Cl</th>
<th>S</th>
<th>O</th>
<th>C</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zr–Al–Ni–Cu</td>
<td>2.9</td>
<td>1.2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.3</td>
<td>0.7</td>
<td>0.3</td>
<td>0.0</td>
<td>26.0</td>
<td>58.5</td>
<td>10.1</td>
</tr>
<tr>
<td>Zr–Al–Cu</td>
<td>3.9</td>
<td>1.0</td>
<td>0.1</td>
<td>—</td>
<td>0.4</td>
<td>0.0</td>
<td>0.3</td>
<td>0.7</td>
<td>23.4</td>
<td>58.5</td>
<td>11.8</td>
</tr>
</tbody>
</table>

Fig. 3 Thickness, \( t \), of the surface oxide film on the amorphous (a) Zr–Al–Ni–Cu and (b) Zr–Al–Cu alloys. Uncalculated: \( t \) were not calculated due to the lack of signals from elements in metallic state.

Fig. 4 Compositions of the surface oxide film on the amorphous (a) Zr–Al–Ni–Cu and (b) Zr–Al–Cu alloys. Undetected: Photoelectrons in metallic state of the element were not detected. No existence: The element did not exist in the environment.
hbit the incorporation of $\text{H}_3\text{PO}_4^{(3-)}$ into the surface oxide film. The binding energies of $\text{Na}$ $1\text{s}$ electrons were 1072.0–1072.1 eV, originating from $\text{Na}^{+}$, and those of $\text{Cl}$ $2\text{p}$ electrons were 197.8–199.2 eV, originating from $\text{Cl}^{-}$ as a chloride. Sodium and chloride ions were possibly located over the surface oxide film because sodium chloride is not incorporated in the surface oxide film on Zr–Ti alloy. The binding energy of $\text{S} 2\text{p}$ electrons was 169.1 eV in “Hanks” and 164.1 eV in “MEM + FBS” of the Zr–Al–Cu alloy, and those were originating from $\text{SO}_4^{2-}$ and sulfide, respectively. Therefore, the $\text{SO}_4^{2-}$ is incorporated from Hanks’ solution into the surface oxide film. On the other hand, the sulfide is originated from the adsorbed amino acids and proteins.

The ratio $[\text{Al}] / [\text{Zr}]$ in the bulk composition was 0.12 that was smaller than those in the “Polished” of both alloys (Fig. 5), indicating that aluminum was enriched in the surface oxide film. After autoclaving, during which the surface is oxidized without releasing any alloying elements, the [Zr] in the surface oxide film of the Zr–Al–Ni–Cu alloy increased, whereas that of the Zr–Al–Cu alloy decreased (Fig. 4). On the other hand, the ratio [Al]/[Zr] in the surface oxide film of both alloys decreased (Fig. 5) and [Zr] in the alloy substrate decreased (Fig. 2), indicating that zirconium is preferentially oxidized against aluminum and incorporated into the surface oxide film. Concentrations of copper and nickel, [Cu] and [Ni], in the surface oxide film increased after autoclaving as shown in Fig. 4, and [Cu] especially increased as much as [Al].

After immersion in Hanks’ solution, the [Zr] and [Al] in the surface oxide film decreased (Fig. 4). The ratio [Al]/[Zr] of the Zr–Al–Ni–Cu alloy did not change (Fig. 5). On the other hand, that of the Zr–Al–Cu alloy decreased (Fig. 5), indicating that aluminum preferentially dissolves against zirconium. The [Ni] decreased to zero and the [Cu] also decreased after immersion in Hanks’ solution (Fig. 4). As mentioned previously, the [Cu] and [Ni] in the alloy substrate also decreased after immersion in Hanks’ solution (Fig. 2). Therefore, copper and nickel dissolve from the alloy in aqueous solutions containing inorganic ions.

After immersion in MEM + FBS, the [Zr] in the surface oxide film decreased (Fig. 4), leading to the increase in the ratio [Al]/[Zr] as shown in Fig. 5. Therefore, the preferential dissolution of zirconium from the surface oxide film can be promoted by amino acids and proteins. The [Cu] and [Ni] in the surface oxide film decreased after immersion in MEM + FBS (Fig. 4), indicating that copper and nickel also dissolve in the solution containing amino acids and proteins. On the other hand, copper dissolves in the solution containing amino acids and proteins less than in the solution consisting of inorganic ions because copper was enriched in the alloy substrate (Fig. 2). Stability of metal ions in solution containing organic compounds depends on the stability of complex of the metal ions and organic compounds. Since the magnitude of chelate formation constant of alloying elements with ethylene diamine tetra acetic acid, EDTA, is larger in the order of $\text{Zr}^{4+} > \text{Cu}^{2+} > \text{Ni}^{2+} > \text{Al}^{3+}$, stability of complex formed between alloying elements and amino acids and proteins in MEM + FBS is possibly larger in the same order. As the result, zirconium dissolved preferentially to copper and nickel in MEM + FBS.

### 3.4 Oxygen in surface oxide

Concentrations of oxygen species in “MEM + FBS” were larger than those of “Polished”, “Autoclaved”, and “Hanks” of both alloys as shown in Fig. 4, because some part of oxygen species of “MEM + FBS” came from the adsorbed amino acids and proteins.

The XPS spectrum of O $1\text{s}$ was decomposed into three spectra originating from $\text{O}^{2-}$, hydroxide or hydroxyl group, $\text{OH}^-$, and hydrate and/or adsorbed water according to the binding energy. Relative concentrations of $\text{OH}^-$ to $\text{O}^{2-}$, $[\text{OH}^-] / [\text{O}^{2-}]$, are shown in Fig. 6. The ratio $[\text{OH}^-] / [\text{O}^{2-}]$ decreased after autoclaving, indicating that dehydration of the surface oxide proceeded at 393 K in a saturated water vapor. On the other hand, the ratio $[\text{OH}^-] / [\text{O}^{2-}]$ increased after immersion in Hanks’ solution and in MEM + FBS, respectively. Therefore, the hydration reaction of the surface oxide seems to be preferential to the dehydration reaction in aqueous solutions. The $[\text{OH}^-]$ was much larger than $[\text{O}^{2-}]$ on “MEM + FBS”, indicating that the hydration reaction may be preferential to the dehydration reaction with the existence of amino acids and proteins. In this case, some part of $[\text{OH}^-]$
originated from the adsorbed amino acids and proteins.

3.5 Surface composition of the zirconium-base amorphous alloy in human body

Dissolution of copper and nickel from the Zr–Al–Ni–Cu and Zr–Al–Cu alloys in Hanks’ solution and MEM + FBS is indicated in this study. However, copper was enriched in the substrate after immersion in MEM + FBS though nickel and copper in the surface oxide film dissolved. Therefore, the dissolution of nickel and copper possibly completes after releasing all amount in the film. Hence, the continuous dissolution of copper and nickel through the implantation in the human body may not occur.

The phosphate salt such as zirconium phosphate was formed on the Zr–Al–Ni–Cu and Zr–Al–Cu alloys, indicating that the surface of the zirconium-base amorphous alloy is inert in body fluid.25)

4. Conclusions

In the Zr–Al–Ni–Cu and Zr–Al–Cu alloys polished in water, aluminum is enriched in the surface oxide film and substrate just under the film. After autoclaving, zirconium is preferentially oxidized and aluminum and copper are thus enriched in the substrate. By immersion in Hanks’ solution, copper and nickel dissolve from the alloy, leading to enhance the enrichment of aluminum in the substrate. In MEM + FBS, amino acids and proteins cause the preferential dissolution of zirconium and aluminum in the substrate disappears, causing the significant enrichment of copper in the substrate. Phosphorous in the solutions is incorporated in the surface oxide film and forms phosphate salts, such as zirconium phosphate. On the other hand, the incorporation of phosphorous is inhibited by amino acids and proteins.

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