ステンレス鋼の表面改質による生体適合性向上
Surface tailoring of stainless steels for biocompatibility

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1. Introduction

Biomaterials can be roughly classified into ceramics, polymers, and metals depending on their property, and used depending on the application [1-3]. Metals have been widely used in dental restorations, prostheses and fracture fixation. However, metallic materials in contact with living tissue may be toxic to the human body if they are eluted as metal ions due to corrosion or become wear powder due to frictional wear. Biomaterials are required to be composed by non-toxic metal elements and not to cause inflammation even when dissolved as ions [4]. Therefore, austenitic stainless steels, Co-Cr alloys, pure Ti, Ti alloys, etc. have been used as excellent high corrosion resistance. The corrosion resistance of these metals is maintained by the passive film [5]. Austenitic stainless steels are used as bone screws and bone nails in orthopedics because of their good mechanical properties and easy processing [6-8]. At present, stainless steel is used in Japan as a short-term implant material from the viewpoint of the risk of metal ion elution, and rarely used for long-term implantation. On the other hand, in Southeast Asia, it is used for long-term implants because it is inexpensive. There is little doubt that austenitic stainless steel is a promising implant material, but at present the surface modification is hardly performed for enhancing its corrosion resistance.

Apart from corrosion resistance, osteoconductivity is another vital index for the property evaluation of the implanted materials [9]. The relationship between surface properties and bone conductivity of metal implants have been reported by Yamamoto et al., where the hydrophilization of smooth TiO_2 film (Ra/μm < 0.1) prepared on Ti surface improved bone conductivity [10,11]. According to Schwartz et al.[12], bone conduction proceeds in the sequence of protein adsorption to the implant, cell adhesion and proliferation, and then bone remodeling. For example, osteoblast is considered to have favorable adhesion to fibronectin-adsorbed implant surfaces and the bone formation is prone to happen [13]. Hydrophilization is thought to facilitate adsorption of cell adhesion proteins and promote osteoblast adhesion. However, these are based on the results derived from the evaluation for Ti alloys and so on for biomaterials, and it has not been judged whether it can be applied to stainless steel. There have been few studies on the relationship between the surface properties of stainless steel and osteoconductivity when used as implant.

As a promising and inexpensive material for implant application, stainless steel needs to be studied in a more intensive way, particularly in its osteoconductivity and anti-corrosion properties. In other words, it is integral to improve osteoconductivity along with guaranteeing its anti-corrosion property. In a corrosive environment containing chloride ions in a living body, passive film formed in air may not be able to ensure sufficient corrosion resistance [14,15]. Metal ions may dissolve chemically or electrochemically in body [14]. Therefore, in order to improve osteoconductivity and corrosion resistance in stainless steel, it is considered to be necessary to create a stable passive layer on which cell adhesible proteins and osteoblasts are positively adsorbed as well.

In this paper, we tried to prepare a protective film that is highly hydrophilic and suppresses the elution of metal ions by surface modification, and aimed to improve bone conductivity and reduce inflammation. The samples used were 304 stainless steel which is used for short-term implantation *in vivo*, and 316L stainless steel with high corrosion resistance by adding Mo for long-term implantation. As the surface modification method, the protective film was prepared by electrochemical anodizing treatment, immersion treatment in an acid with oxidizer activity, and hydrothermal treatment immersed in distilled water at high temperature and high pressure [15,16]. The chemical composition, roughness, hydrophilicity, corrosion resistance, protein adsorption and so on were characterized for surface film formed by surface treatments. In addition, *in vivo* tests were employed to evaluate osteoconductivity and inflammation due to dissolved metal ions .

2. Materials and methods

2.1 Specimen

Two types of cold-rolled austenitic stainless steels with standard specifications were used as specimens. Table 1 shows the chemical composition of the 304 and 316 L steels.

Table 1 Chemical compositions of stainless steel (mass %)

	Cr	Ni	Mo	Mn	Si	P	S	C
304	18.00 – 20.00	8.00 – 10.50	–	< 2.00	< 1.00	< 0.045	< 0.030	< 0.08
316L	16.00 – 18.00	10.00 – 14.00	2.00 – 3.00	< 2.00	< 1.00	< 0.045	< 0.030	< 0.08

Plates with surface area of approximately 1 cm^2 were used for *in vitro* testing and rods were used for *in vivo* testing, i.e., 2 mm (φ) × 5 mm (L) for bone conductivity evaluation and 1 mm (φ) × 5 mm (L) for inflammatory test. All specimen surfaces were wet-polished

to #2000 with SiC abrasive paper, and buff-polished with 0.05 μm alumina powder to give surface roughness Ra/μm <0.1. After degreasing with acetone and washing with distilled water, water droplets on the surface were removed using an air blower.

2.2 Surface modifications

Anodizing treatment, acid immersion treatment, and hydrothermal treatment were used as surface treatments to improve the corrosion resistance. The anodic oxidation was performed by increasing the potential to 2 V (vs Ag / AgCl sat. KCl) at a rate of 0.01 Vs^{-1} in a 0.1 M aqueous solution of NaOH at room temperature. The acid immersion was performed by immersing the specimen in concentrated sulfuric acid (18 M) at room temperature for 10 s and then washing with water. After acid immersion, it was placed in a Teflon container containing distilled water (40 mL) for hydrothermal treatment, sealed in a stainless steel capsule, and kept at 230 °C or 180 °C for 3 h.

2.3 Surface characterization

The surface morphology was observed with a scanning electron microscope (SEM). Chemical compositions of the austenitic stainless steel were analyzed with an X-ray photoelectron spectrometer (XPS, Thermo Fisher Scientific Escalab 250). It is known that surface roughness at μm level affects bone conductivity [10]. The surface roughness (analytical range 150 μm × 112 μm) was measured with an ultra-deep profile measuring microscope and evaluated by the arithmetic mean roughness Ra that is less susceptible to local flaws.

Hydrophilicity and hydrophobicity were evaluated using a water contact angle, WCA, by static droplet method (sessile drop method), on a drop shape instrument (FTA1000, First Ten Angstroms). Immediately after preparation of the film, 2 μL of distilled water was deposited on the specimen, and WCA after 10 s was measured by the curve fitting method of elliptic approximation.

2.4 Protein adsorption test

As well as the previous protein adsorption test of Ti alloys [17], stainless steels were immersed for 60 min in 0.5 mg mL^{-1} fibronectin aqueous solution or 25 mg mL^{-1} albumin solution at 37 °C. The amount of adsorbed protein on the surface was calculated based on reflection ATR method of Fourier transform infrared spectrophotometer.

2.5 Corrosion test

The amount of metal ions dissolved from the surface-modified specimens was

evaluated by the static corrosion test (JIS T0304). After coating with insulating tape to expose the modified surface, the specimen was immersed into 5 mL test solution at 37 ± 0.5 °C and left to stand for 7 d. In addition, in order to calculate the change in metal ion amount over time, the specimen was immersed in the 3 mL test solution, and was removed from the test solution every 1, 4 and 7 d, washed with distilled water, and then immersed in the new test solution. Test solutions was 3 mass% NaCl for accelerated corrosion test and Phosphate buffered salts (PBS(-)) adjusted according to the chloride concentration in the living body fluid. PBS(-) consisted of 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.76 mM KH_2PO_4 at pH 7.4. Metal ions corroded into the solutions were analyzed by an ICP spectrophotometer.

On the other hand, the influence of protein pre-adsorption on corrosion behavior of the specimens was investigated. 200 µL aqueous solution of 25 mg mL^{-1} albumin was dropped on the hydrophobic surface and 100 µL dropped on the hydrophilic surface, and these droplets were kept for 60 min at 37 °C, to realize the saturated adsorption of albumin to the entire surface of the specimen. Then, the specimens were immersed in PBS (-) of 3 mL in volume for 7 d in a thermostatic chamber set at 37 ± 0.5 °C, and the dissolved ions were detected by ICP.

2.6 Evaluation of osteoconductivity

The *in vivo* test was carried out using the specimen of a round bar (Φ2 mm × 5 mm L) polished and then surface-modified. In order to control the hydrophilicity, the surface-modified specimen was stored in PBS(-) for 1 d, and the as-polished material was stored in a sterile bottle before implantation. The specimen was implanted for 2 weeks into tibial bones of rats that were 8 weeks age, SD (Sprague Dawley), IGS (International Genetic Standard), male SPF (Specific Pathogen Free), using normal rats acclimatized and quarantined for 4 d. The rats were reared at 24 ± 3 °C, humidity of 50 ± 20 %, ventilation frequency of about 10 to 15 times/h, and lighting time of 12 h/d.

After 2 weeks of implantation, the animals were bred and observed, and then the specimens were removed together with the tibia and fixed with 10 % neutral formalin solution. The specimens were polished to a thickness of 20 µm and stained with toluidine blue, and then the cortical bone area was observed using a microscope (Mega Light 100, Shot Japan Co., Ltd.). $R_{B\text{-}I}$ was used as an index of osteoconductivity. The $R_{B\text{-}I}$ value is defined as the percentage (%) of direct adhesion of hard tissue but not soft tissue at the interface between the embedded stainless steel sample and bone. All animal experiments were conducted at the facilities of AAALAC International (International Association for Accreditation of Experimental Animals).

2.7 Anti-inflammatory evaluation

To prepare a specimen for anti-inflammatory evaluation, a round bar (Φ 1 mm × 5 mm L) was polished and then surface-modified. All specimens were stored in PBS(-) to exclude initial dissolution of metal ions and to maintain hydrophilicity. The specimens were implanted to the back of rat subcutaneously with 6 to 8 specimens per animal using a needle and observed for 2 weeks. Other conditions were the same as proposed in osteoconductivity evaluation. The rats were reared at temperature of 24 ± 3 °C, humidity of 50 ± 20 %, ventilation frequency of about 10-15 times/h, and lighting time of 12 h/d. After 2 weeks of implantation, rearing and observation, the whole skin was removed and fixed in 10% neutral formalin solution.

The anti-inflammatory property was, firstly, evaluated from presence or absence of suppuration or hemorrhage around the implanting specimen, and neovascularization or hemorrhage from the blood vessel, in the naked eye findings. Secondly, it was evaluated from the presence of inflammatory cells such as lymphocytes and neutrophils, and the thickness of fibrous capsule in tissue observation using light microscopy (Mega Light 100, shot Japan)

3. Results and discussion

3.1 Change in film composition by surface treatment

For the steels treated by the usual surface modification methods, most of them maintained smooth surfaces with Ra/μm < 0.1. Only the smooth specimens with Ra/μm < 0.1 was used because the surface roughness affected osteoconductivity according to the previous researches. Figure 1 shows XPS profiles of as-polished 316L steel used in the research. The ratio of the components was calculated based on the de-convolution of Fe $2p_{3/2}$, Cr 2p, Ni 2p and Mo 3d spectras [18,19]. Figure 2 shows the relative ratio of each element in oxidized form present in the oxide film for the specimens as-polished, hydrothermally treated in distilled water at 180 °C or 230 °C for 3 h, hydrothermally

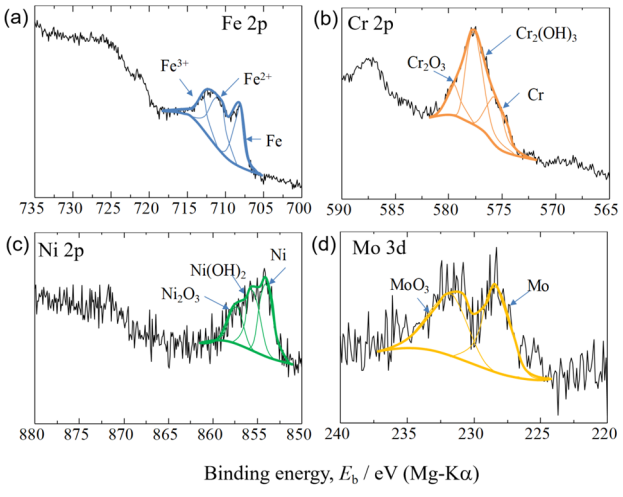

Fig.1 XPS profiles and peak analysis for 316L stainless steel as-polished

treated at 230 °C for 3 hours after immersing in concentrated sulfuric acid at 25 °C, and anodized in 0.1 M NaOH solution at 25 °C. The film formed by the surface modification was different in composition from the natural oxide film by polishing. Compared with the as-polished specimen, the percentage of Cr (III) in surface increased and Fe (sum of Fe(II) and Fe(III)) decreased by the hydrothermal treatment. Above all, when the hydrothermal treatment was performed at a higher temperature of 230 °C, the Cr (III) ratio in the film increased. Since film containing Cr (III) has high passive corrosion resistance, it is expected to suppress the corrosion *in vivo*. In the case of SUS316L, Molybdenum was present in the oxide film as hexavalent MoO_3 under all surface modification conditions. On the other hand, in the anodized specimens, the proportion of Cr (III) decreased and oxidized-state Ni and Fe tended to increase.

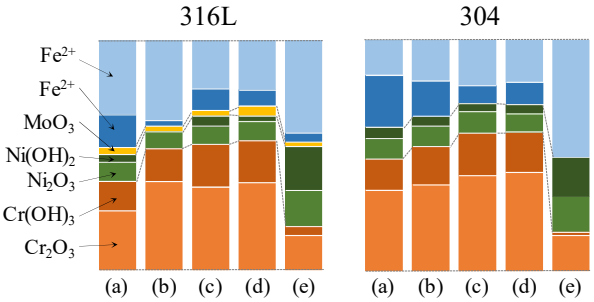

Fig.2 Metal ratio in XPS surface analysis for stainless steels of (a) as-polished, hydrothermal treated for 3 h in distilled water at (b) 180 °C and (c) 230 °C, (d) hydrothermal treated at 230 °C after immersing for 10 s in 18 M H_2SO_4 at 25 °C, and (e) anodized in 0.1 M NaOH at 25 °C

3.2 Corrosion behavior

Figure 3 shows the results of dissolution amount of metal ions for 7 d in 3 mass% NaCl and PBS(-) solutions at 37 °C under static dissolution test. The 316L steel had much less dissolution amount of total metal ions compared with that of 304 steel, in the NaCl aqueous solution, indicating 316L steel had higher corrosion resistance than 304 steel. Since the chloride ion concentration was low in PBS solution, the corrosion resistance was improved and the difference between 304 and 316 L became small. Corrosion resistance was most highly improved by hydrothermal treatment after sulfuric acid immersion. On the contrary, the corrosion resistance of the anodized specimen deteriorated and the dissolved amount of Fe and Cr ions increased greatly. Thus, metal ion dissolution was affected by the protective film formed by surface modification.

The static corrosion test with 3 mass% NaCl solution was different from the corrosive environment in the living body, so it is necessary to evaluate the metal ion amount dissolved from the stainless steels for biomaterial in the biological simulated environment. Surface morphology after the static dissolution test was not changed in comparison with that before the test, and no dissolution mark due to pitting corrosion was found. It is considered that the dissolved metal ions were not from the substrate but from the dissolution of the surface oxide film.

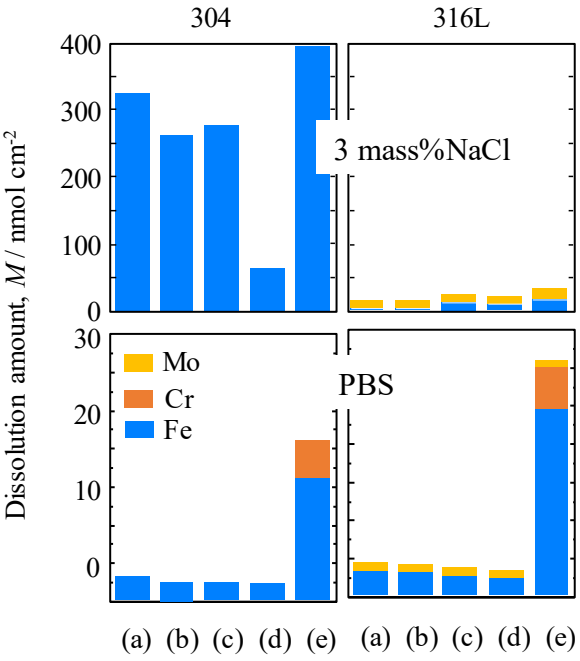

Fig.3 Dissolved ion for 7 d in 3 mass% NaCl and PBS solutions at 37 °C for 304 and 316L stainless steels stainless steels of (a) as-polished, hydrothermal treated for 3 h in distilled water at (b) 180 °C and (c) 230 °C, (d) hydrothermal treated after immersing for 10 s in 18 M H_2SO_4 at 25 °C, and (e) anodized in 0.1 M NaOH at 25 °C

Next, the time dependence of the dissolved metal ion amount was measured in the biological environment. From the results as shown in Fig.3, we targeted three specimens, i.e., specimen employed for hydrothermal treatment at 230 °C after immersion in 18 M H_2SO_4 which had the highest corrosion resistance, specimen anodized in a 0.1 M NaOH aqueous solution which was worst in corrosion resistance, and the as-polished. Pre-adsorption of albumin was also employed in the corrosion behavior investigations. Since *in vivo* there is protein, it is integral to analyze the effect of protein on corrosion resistance. Figure 4 shows the integration values of dissolved metal ions for 7 d. The initial amount after 1 d immersion was noticeable in 304 and 316L steels and the cumulative values increased gradually thereafter.

The Cr dissolution from the anodized material was confirmed only up to 1 d after immersion, and thereafter it was below the detection limit. From this fact, it is considered that Cr ions contained in the anodized film was corroded in the initial stage of immersion. The amount of dissolved Fe ions tended to increase in the albumin pre-adsorbed specimen compared with the specimen having no albumin. The promotion of

anti-corrosion property was realized through the formation of the oxygen concentration cell in the albumin adsorption part and the non-adsorption part or the formation of the oxygen deficient part. In addition, albumin could be polarized when it is adsorbed on a negatively charged surface such as hydroxyl group and it is thought that the specimen surface becomes acidic by electrically attracting surrounding protons [20-22]. These could be the reasons for the specimen modified by hydrothermal treatment after acid immersion having the lowest dissolution amount of metal ions.

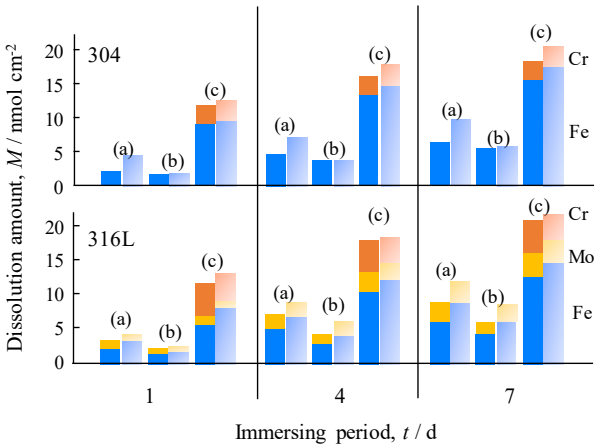

Fig.4 Change in dissolved ions by ICP analysis immersed in PBS solutions for 304 and 316L stainless steels of (a) as-polished, (b) hydrothermal treated for 3 h in distilled water at 230 °C after immersing for 10 s in 18 M H_2SO_4 at 25 °C, (c) anodized in 0.1 M NaOH at 25 °C. Left bar : albumin free, right bar : adsorbed albumin before immersion

3.3 Protein adsorption and osteoconductivity

The protein is firstly adsorbed on implant surface and then osteocyte is attracted to the protein layer, the osteocyte is attached to the protein layer, and finally the osteocyte is stretched out along the protein layer. So favorable protein adsorption was the prerequisite for good osteoconductivity. It is known that osteoconductivity of Ti alloy implants is related to hydrophilicity. This is because the more hydrophilic or hydrophobic the surface was, the more adsorption of proteins was promoted, according to our previous researches [10,11,17]. Therefore, the relationship between hydrophilicity and protein adsorption along with osteoconductivity were also investigated for stainless steel. A peak around 1650 cm^{-1} due to C = O stretching of the peptide bond contained in the protein was observed in the FT-IR results of the specimen immersed in albumin or fibronectin solution, as shown in Figure 5. The protein adsorption amount was calculated based on comparison of the peak area-with that of specimen with known adsorption value. The relationship with the water droplet contact angle, WCA, is shown in Figure 6 where the past results of Ti alloy are also shown as small marks. The V-shaped curves were obtained for Ti alloys with inflection points at 48 deg. for fibronectin and 65 deg. for albumin. The amount of protein adsorption increased as more

hydrophilic or hydrophobic for Ti alloy. Similar correlations were observed for stainless steels in which metal ions were more corrosive compared to Ti alloys. It was found that WCA was greatly affected by the surface treatment method of stainless steel. In particular, the hydrothermal treatment showed superhydrophilicity with WCA <10 deg. and the protein adsorption capacity was the highest.

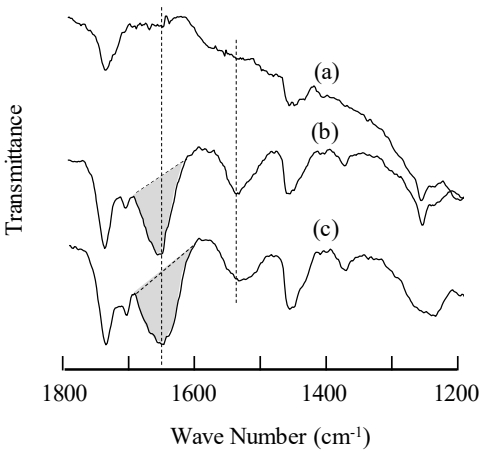

Fig.5 FT-IR spectrums for 304 stainless steel after immersing for 60 min at 298 K in distilled water (a), and in PBS solutions with 50 mgcm^{-3} albumin (b) and with 1 mg·cm^{-3} fibronectin (c)

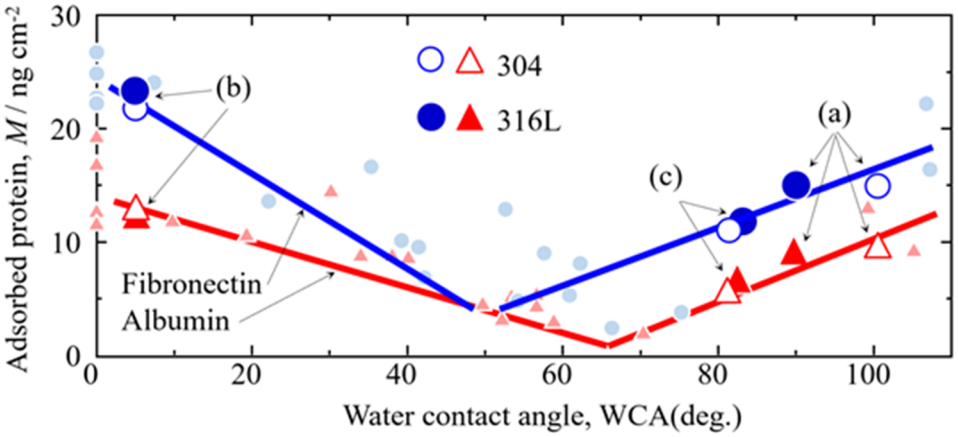

Fig.6 Amount of protein adsorbed on 304 and 316L stainless steels of (a) as-polished, (b) hydrothermal treated for 3 h in distilled water at 230 °C after immersing for 10 s in 18 M H_2SO_4 at 25 °C, and (c) anodized in 0.1 M NaOH at 25 °C. Small marks were results of Ti alloys in previous research

A typical picture of the interface between stainless steel embedded in a rat and cortical bone is shown in Figure 7. The interface was divided into parts in contact with the hard tissue or fibrous tissue of the new bone, and the proportion was affected by the surface treatment of the specimen to be embedded. Figure 8 shows the relationship

between the $R_{B\text{-}I}$ value indicating osteoconductivity and the WCA measured before implantation. The past results of other Ti alloys are also shown. For both 304 and SUS316L, the $R_{B\text{-}I}$ value was improved in the sample whose WCA was lowered by hydrothermal treatment at 230 °C after immersion in 18 M H_2SO_4 compared with the as-polished stainless steel. It was also found that plots of stainless steels was well located at the V-shaped curve obtained from the Ti alloy results. Therefore, it can be deduced that $R_{B\text{-}I}$ is closely correlated with WCA

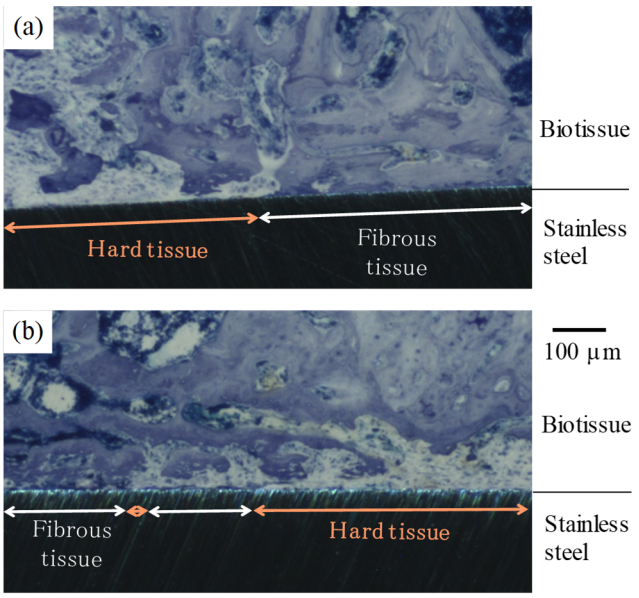

Fig.7 Photos of interface of 304 stainless steels implanted for 14 d in cortical bone of the tibiae of 8-week-old male rats
(a) as-polished specimen, (b) hydrothermally treated for 3 h in distilled water at 230 °C after immersing for 10 s in 18 M H_2SO_4 at 25 °C

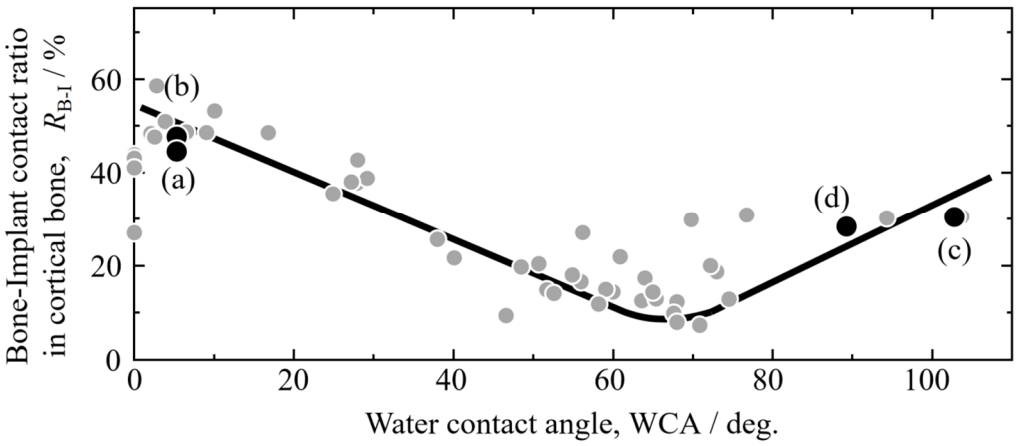

Fig.8 Osteoconductivity of 304 (a)(c) and 316L (b)(d) stainless steels of (c)(d) as-polished, (a)(b) hydrothermal treated for 3 h in distilled water at 230 °C after immersing for 10 s in 18 M H_2SO_4 at 25 °C, Small gray marks were results of Ti alloys in previous research

value, regardless of the type of metal, i.e., a certain WCA value would lead to almost the

same $R_{B\text{-}I}$. Stainless steel is more likely to corrode as metal ions than titanium, but no effect on bone conductivity was observed. The reason is considered to be that the protein adsorption ability is increased by hydrophilization and henceforth the bone conductivity is improved. From the above, protein adsorption capacity and osteoconductivity for stainless steel tended to be the same as other Ti alloys, indicating the surface hydrophilicity property of the implanted metals was the dominant factor to affect the osteoconductivity. Furthermore, the effect of metal ion elution was small compared with the effect of hydrophilicity property of implanted metals. So adjusting WCA value of implanted metals could be the primary consideration to improve bone conductivity.

The *in vitro* protein adsorption test had high consistency to the *in vivo* bone conductivity results. So it again proved that the protein adsorption on metal surface is the prerequisite for bone conductivity. The super-hydrophilicity could greatly increase the protein adsorption and enhance bone conductivity. Henceforth, t yhe surface modification by hydrothermal treatment after acid immersion was superior to as-polished specimen as control group, and the anodized specimen.

3.4 Inflammation

In gross observation, no inflammatory reaction such as suppuration or hemorrhage was observed around the stainless implant. Furthermore, no histological findings of hemorrhage and remarkable blood vessels were observed in the tissue observation of the specimens, and no cell necrosis was observed. Therefore, severe acute inflammation and chronic inflammation are considered not to occur. Therefore, we estimated the thickness of the fibrous capsule membrane formed around the implant as an indicator of inflammatory property as well as previous work [23]. When biomaterials are implanted in the body, acute inflammation occurs, which has a rapid and premature termination. At this time, the material with stronger recognition as a foreign substance is covered with a thick fibrous soft tissue for a long time. This fibrous soft tissue is a capsule membrane. Figure 9 shows an example of a photograph of the capsule membrane-sample interface

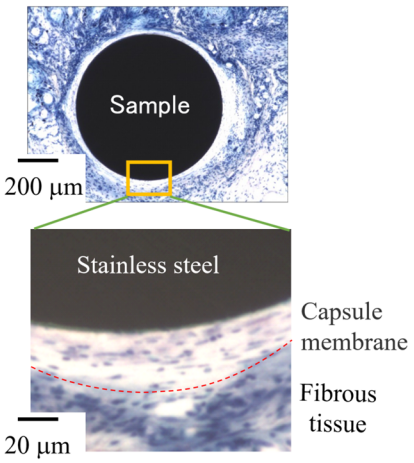

Fig.9 Photos of interface of 304 stainless steels implanted for 14 d in cortical bone of the tibiae of 8-week-old male rats

around the stainless steel specimen. The thickness of the capsule membrane was used as an index of inflammation.

Corrosion of implanted materials is considered to have an influence on inflammation. It is, however, difficult to evaluate the dissolution amount and metal ions actually inserted *in vivo*. Therefore, the amount of metal ions corroded *in vivo* was substituted by *in vitro* tests, i.e., the amount of metal ions from the specimen with adsorbed albumin in static corrosion test was used as an index indicating the case of corrosion *in vivo*. The specimen for evaluation of inflammation was surface-modified and stored in PBS (-) just before implantation to maintain hydrophilicity, so it is considered that initial corrosion within 1 d is finished before implantation in rats. Therefore, the amounts of Fe and Mo during immersion period 2 d to 4 d was used as an index. Figure 10 shows the relationship between the thickness of fibrous capsule and the amount of dissolved ions. It was found that the capsule membrane becomes thicker and the inflammation becomes stronger as the metal ions were easily dissolved. Considering the influence of ion species on inflammation, two strait lines, no proportional relation, are obtained corresponding to 304 and 316L steels respectively, in the case of Fe ions as shown in Fig.10 lower graph. The proportional relation between the thickness of capsule membrane and the dissolved molar amount of Fe and Mo can be obtained as upper graph. From the above results, corroded metal ions increases inflammation. The amount of eluted metal ions was positively related to the inflammation. Henceforth, this *in vivo* tests support that when evaluating the amount of metal corrosion *in vivo*, it is necessary to carry out in an

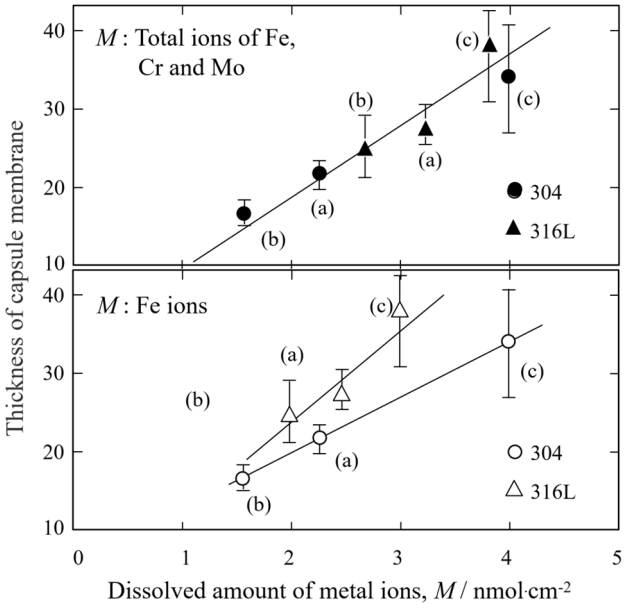

Fig.10 Relations between thickness of capsule membrane in *in vivo* test and amount of dissolved ions from stainless steels adsorbed albumin
(a) as-polished, (b) hydrothermal treated for 3 h in distilled water at 230 °C after immersing for 10 s in 18 M H_2SO_4 at 25 °C , (c) anodized in 0.1 M NaOH at 25 °C

environment where proteins closer to the *in vivo* environment exist. The 304 steel with relatively higher corrosion resistance showed the more suppressed inflammatory property. As mentioned in the corrosion test, the hydrothermal treatment for 3 h in distilled water at 230 °C after immersing for 10 s in 18 M H_2SO_4 at 25 °C had the lowest metal ion elution amount, suggesting the highest anti-corrosion property, which also meet well with the situation *in vivo* test that this specimen exhibited the lowest inflammation. So the inflammation test *in vivo* further proved the advantages of this particular surface modification to stainless steel. The protective film with low metal ion elution was prepared by surface modification of hydrothermal treatment after acid immersion, and the reduction of inflammation was realized by suppressing metal ion supply.

4. Conclusions

We aimed to improve osteoconductivity and reduce inflammation by surface treatment for two kinds of stainless steel [24]. Preparation of protective film by 230 °C hydrothermal treatment in distilled water after immersion in 18 M H_2SO_4 at 25 °C was a process effective in improving osteoconductivity and corrosion resistance. The percentage of Cr in the surface passivation coating increased and the percentage of Fe decreased by hydrothermal treatment. On the contrary, in the anodizing treatment, the proportion of Cr decreased and the proportion of Fe increased. As a result, the former showed higher osteoconductivity and higher corrosion resistance than the latter. Moreover, super-hydrophllic surface with water droplet contact angle, WCA, less than 10 deg. can be obtained by performing hydrothermal treatment. The relationship between the WCA and the osteoconductivity $R_{B\text{-}I}$ was similar to that of the titanium alloy etc. in our previous research. Effective surface treatment makes the surface super-hydrophilic, which results in an increase in protein adsorption, leading to an improvement in osteoconductivity. This study provided a close relationship between *in vitro* tests and *in vivo* tests.

Acknowledgements

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References

1) G. Wu, P. Li, H. Feng, X. Zhang, and P.K. Chu, Engineering and functionalization of biomaterials via surface modification. J. Mater. Chem. B 3 (2015) 2024-2042.
2) A.A. John, A.P. Subramanian, M.V. Vellayappan, A. Balaji, S.K. Jaganathan, H.

Mohandas, T. Paramalinggam, E. Supriyanto, and M. Yusof, Review: physico-chemical modification as a versatile strategy for the biocompatibility enhancement of biomaterials. RSC Adv. 5 (2015) 39232-39244.

3) M. Sumathra, K.K. Sadasivuni, S.S. Kumar, and M. Rajan, Cisplatin-Loaded Graphene Oxide/Chitosan/Hydroxyapatite Composite as a Promising Tool for Osteosarcoma-Affected Bone Regeneration. ACS Omega 3 (2018) 14620-14633.
4) X. Guan, M. Xiong, F. Zeng, B. Xu, L. Yang, H. Guo, J. Niu, J. Zhang, C. Chen, J. Pei, H. Huang, and G. Yuan, Enhancement of Osteogenesis and Biodegradation Control by Brushite Coating on Mg-Nd-Zn-Zr Alloy for Mandibular Bone Repair. ACS Appl. Mater. Inter. 6 (2014) 21525-21533.
5) S. Sathishkumar, K. Louis, E. Shinyjoy, and D. Gopi, Tailoring the Sm/Gd-Substituted Hydroxyapatite Coating on Biomedical AISI 316L SS: Exploration of Corrosion Resistance, Protein Profiling, Osteocompatibility, and Osteogenic Differentiation for Orthopedic Implant Applications. Ind. Eng. Chem. Res. 55 (2016) 6331-6344.
6) S. Bagherifard, D.J. Hickey, A.C. de Luca, V.N. Malheiro, A.E. Markaki, M. Guagliano, and T.J. Webster, The influence of nanostructured features on bacterial adhesion and bone cell functions on severely shot peened 316L stainless steel. Biomaterials 73 (2015) 185-197.
7) M.K. Lei, and X.M. Zhu, in vitro corrosion resistance of plasma source ion nitrided austenitic stainless steels. Biomaterials 22 (2001) 641-647.
8) H. Ceylan, A.B. Tekinay, and M.O. Guler, Selective adhesion and growth of vascular endothelial cells on bioactive peptide nanofiber functionalized stainless steel surface. Biomaterials 32 (2011) 8797-8805.
9) H. Quan, Y. He, J. Sun, W. Yang, W. Luo, C. Dou, F. Kang, C. Zhao, J. He, X. Yang, S. Dong, and H. Jiang, Chemical Self-Assembly of Multifunctional Hydroxyapatite with a Coral-like Nanostructure for Osteoporotic Bone Reconstruction. ACS Appl. Mater. Inter. 10 (2018) 25547-25560.
10) D. Yamamoto, I. Kawai, K. Kuroda, R. Ichino, M. Okido, and A. Seki, Osteoconductivity of Anodized Titanium with Controlled Micron-Level Surface Roughness. Mater. Trans. 52 (2011) 1650.
11) M. Okido, R. Ichino, K. Kuroda, I. Kawai, Y. Dai, and A. Seki, Osteoconductivity and Hydrophilicity of TiO2 Coatings on Ti Substrates Prepared by Different Oxidizing Processes. Bioinorg. Chem. Appl. 2012 (2012) 495218.
12) Z. Schwartz, and B.D. Boyan, Underlying mechanisms at the bone-biomaterial interface. Journal of Cellular Biochemistry 56 (1994) 340-347.

13)S.R. Sousa, M. Lamghari, P. Sampaio, P. Moradas-Ferreira, and M.A. Barbosa, Osteoblast adhesion and morphology on TiO2 depends on the competitive preadsorption of albumin and fibronectin. J. Biomed. Mater. Res. A 84A (2008) 281-290.

14)Y.S. Hedberg, and I. Odnevall Wallinder, Metal release from stainless steel in biological environments: A review. Biointerphases 11 (2015) 18901.

15)R. Singh, M. Martin, and N.B. Dahotre, Influence of laser surface modification on corrosion behavior of stainless steel 316L and Ti-6Al-4V in simulated biofluid. Surf. Eng. 21 (2005) 297-306.

16)C.C. Shih, C.M. Shih, K.Y. Chou, S.J. Lin, and Y.Y. Su, Stability of passivated 316L stainless steel oxide films for cardiovascular stents. J. Biomed. Mater. Res. A 80A (2007) 861-873.

17)K. Kuroda, and M. Okido, Osteoconductivity Control Based on the Chemical Properties of the Implant Surface. Journal of Biomaterials & Nanobiotechnology 9 (2017) 26-40.

18)W. Fredriksson, S. Malmgren, T. Gustafsson, M. Gorgoi, and K. Edstrom, Full depth profile of passive films on 316L stainless steel based on high resolution HAXPES in combination with ARXPS. Appl. Surf. Sci. 258 (2012) 5790-5797.

19)P. Jussila, K. Lahtonen, M. Lampimäki, M. Hirsimäki, and M. Valden, Influence of minor alloying elements on the initial stages of oxidation of austenitic stainless steel materials. Surf. Interface Anal. 40 (2008) 1149-1156.

20)S. Omanovic, and S.G. Roscoe, Electrochemical studies of the adsorption behavior of bovine serum albumin on stainless steel. Langmuir 15 (1999) 8315-8321.

21)S. Karimi, T. Nickchi, and A.M. Alfantazi, Long-term corrosion investigation of AISI 316L, Co-28Cr-6Mo, and Ti-6Al-4V alloys in simulated body solutions. Appl. Surf. Sci. 258 (2012) 6087-6096.

22)C.M. Pradier, D. Costa, C. Rubio, C. Compere, and P. Marcus, Role of salts on BSA adsorption on stainless steel in aqueous solutions. I. FT-IRRAS and XPS characterization. Surf. Interface Anal. 34 (2002) 50-54.

23)K. Kensuke, I. Kenta, K. Hiroyasu, and O. Masazumi, Surface Modification of PEEK and Its Osteoconductivity and Anti-Inflammatory Properties. Journal of Biomaterials and Nanobiotechnology 9 (2018) 233-243.

24)C. Peng, T. Izawa, L. Zhu, K. Kuroda, M. Okido, Tailoring Surface Hydrophilicity Property for Biomedical 316L and 304 Stainless Steels: A Special Perspective on Studying Osteoconductivity and Biocompatibility, ACS Appl, Mater. Interfaces, 11, 49, 45489-45497 (2019)