Chapter 7 Implants and biomaterials (Titanium Metal)

Professor of Institute of Biomaterials and Bioengineering, Tokyo Dental and Medical University

Takao Hanawa

I. Properties of metallic materials

The term “metallic material” generally refers to a polycrystalline compound formed with metallic bonding*1. Metal oxides, metal salts, and metal complexes all contain metal ions, but these compounds are constructed with ionic or covalent bonds and thus display distinct properties from metallic material that is formed with metal bonding. For this reason, in the field of material engineering, ceramics and metals are differentiated from each other even though both utilize the same inorganic materials. The strengths of metallic material as a biomaterial are summarized in Fig. 3-7-1. These properties of metallic materials arise as the result of metal bonding interactions.

Their advantage is their strength and therefore their resistance to damage in comparison with ceramic and polymer materials. The ceramics is poor in toughness, particularly with regards to its notch toughness; therefore, ceramics are often result in a sudden fracture. Consequently, they are unsuitable for use in regions that are subject to heavy loads, repeated heavy loads, or parts where pressure is concentrated, for example, where a screw is used. The weakness of polymeric materials makes use inapplicable in locations where a large load is applied, and their inability to withstand heat limits sterilization methods.

*1 Electrostatic attractive forces occur between positively charged metal ions and delocalized electrons that are gathered in an “electron sea”. The delocalized electrons are shared within a lattice of positively charged ions. As an example, the ductile property of metals is explained by the way in which metals bond even if atoms slide off and break, as the “free” electrons are able to fix this deformation, reforming the metallic bonding.

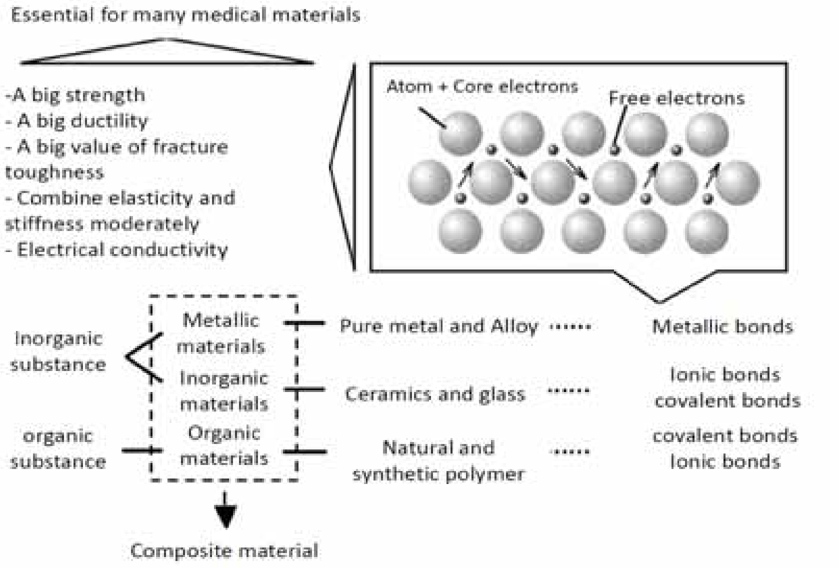


Fig. 3-7-1

The strengths of metal material that have been produced by the type of material that is largely dependent on the type of chemical bonding, and the metal bonding

II. Overview of titanium

The valuable properties of titanium (Ti)*2 and its alloys, such as corrosion resistance and a high strength-to-density ratio, have been applied to aerospace materials. Young's modulus*3 of Ti and Ti alloys is valued as it is half that of stainless steel or Co-Cr alloy, and is close to that of cortical bones. The excellence of Ti in mechanical biocompatibility has led to its widespread use in the area of biomaterial such as in fixtures of dental implants, fracture fixation materials and artificial joints. In addition, from past experience, it has become clear that its compatibility with both hard and soft tissues surpasses that of all other metal materials. Its compatibility with blood is not yet clear, however, its safety for application within the human body has been well established, and it lacks toxicity. Its safety and tissue compatibility are a result of the chemical characteristic of Ti, or more specifically, its surface properties, and knowledge of these properties is thus of extreme importance.

Ti is an extremely active element, and its standard electrode potential for the reaction,

Ti → Ti + 2 e- with regards to the standard hydrogen electrode is as low as -1.63V, indicating its high activity. According to Pourbaix1), of the metal elements that are in practical use, Ti is the most thermodynamically active (easily-ionized) metallic element after Mg and Be. This active property forms a basis for the chemical characteristics of Ti, such as the difficulty encountered in working the metal, its resistance to corrosion, and its safety (Fig. 3-7-2). Even though Ti as an element is extremely active, Ti as a metallic material is highly resistant to corrosion. This is because of the highly reactive nature of Ti. It reacts readily with water molecules in solution, or moisture in the atmosphere to form a thin layer of titanium oxide on the metal surface. Even when the surface is scratched, the newly exposed sub-layer rapidly becomes coated with oxide, appearing inert. For this reason, its resistance to corrosion is much higher than that of stainless steel or Co-Cr alloy. This nature not only provides resistance to corrosion, but also explains its ready incorporation into the body, and its lack of toxicity.

At room temperature, pure Ti exists as a hexagonal, close-packed (hcp) crystal structure (α- layer), however, at temperatures above 882°C, it becomes a body-centered cubic (bcc) crystal structure (β-layer). Ti often co-solubilizes with O, C, N, and therefore its pure form does not exist. Ti that includes these impurities is referred to as commercially pure titanium (cpTi). cpTi is classed into four different types according to the impurities contained and mechanical properties (Table 3-7-1). The increase in spec number corresponds to the increase in the concentration of impure elements, the tensile strength and 0.2%-proof stress, with a decrease in elasticity.

---

*2 Titanium is found in the mineral ores in the forms of rutile (TiO_2) and ilmenite ($FeTiO_3$) but cannot be extracted by a direct reduction of the naturally exsisting state due to the strong covalent bonding with the oxygen atoms. The development of metallurgy process was complicated but in 1946, the method to reduce $TiCl_4$ with magnesium was developed, enabling the industrial production of titanium metal. The name, "titanium" was derived from the Titans of Greek methology who were overthrown by the Gods of the Olympians, became imprisoned in the realms of the underworld, as an element which was enclosed within the mineral ores (rutile)

*3 In a typical metal material, a line appears initially on application of stress and strain. This is based on Hooke's Law. The gradient of the slope of stress-strain curve at any point, E, is defined by the formula:

$\sigma = E\varepsilon$ (σ = stress, ε = strain), which is typically the tangent modulus, but where this is it the initial linear portion of the curve, it is referred to as the Young's modulus.

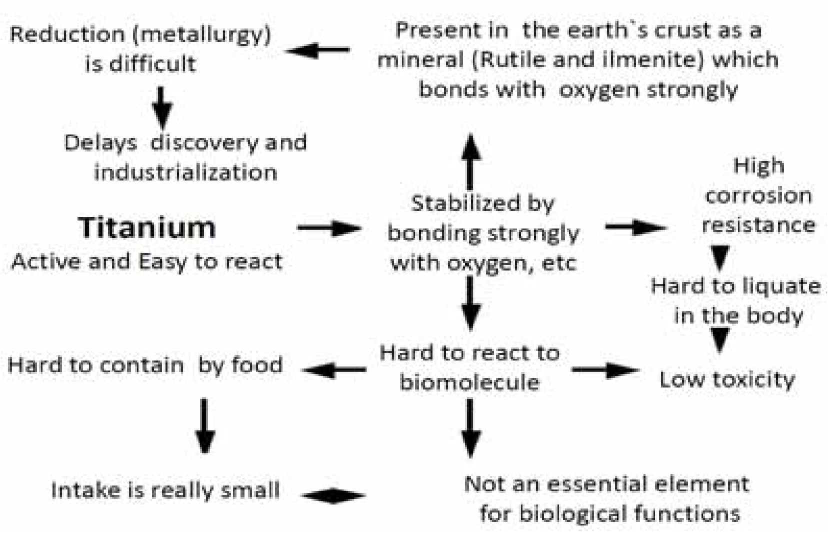


Fig. 3-7-2
The property of titanium that is determined by the degree of its activity

	Pure Ti 1 grade	Pure Ti 2 grade	Pure Ti 3 grade	Pure Ti 4 grade
Chemical element	Composition (mass%)			
Fe	<0.15	<0.2	<0.25	<0.3
O	<0.18	<0.25	<0.35	<0.45
N	<0.03	<0.03	<0.05	<0.05
H	<0.0125	<0.0125	<0.0125	<0.0125
C	<0.1	<0.1	<0.1	<0.1
Ti	Balance	Balance	Balance	Balance
	Mechanical properties			
Tensile strength	275-412	343-510	481-618	>550
0.2% proof stress MPa)	170	275	380	>440
Elongation (%)	>27	>23	>18	>15
Young's modulus (GPa)	114			

Table 3-7-1
The constituents of commercially pure titanium and their mechanical properties-JIS

III. Titanium alloy as a biological material

Ti alloy can exist at room temperature as an α-type alloy, an α+β-type alloy and a β-type alloy, depending on the type and the amount of alloy metals. Many Ti alloys have been developed for biomedical purposes (Table 3-7-2). Ti-6AI-4V alloy, a typical Ti alloy is an α+β-type, and used commonly in biological materials. This alloy has several properties that are superior to those of other Ti alloys, including processability, thermal processability, and weldability, in addition to its resistance to corrosion, strength, and biocompatibility. In biomaterials, ELI graded compounds with low interstitial impurity contents of elements such as O, C, N, H are used. The impurities lower the notch fatigue strength, in other words, lower the fatigue strength in circumstances of etching and damage, therefore ELI graded materials with low impurity contents are excellent in their degree of toughness. ELI graded materials are often used as fracture stabilizing plates, screws and artificial hip stems. A notable characteristic of

Ti-6AI-4V alloy is its 0.2% proof stress of 895 MPa, a value that is much higher than even that of stainless steel or Co-Cr-Mo alloys. This indicates that even under a high load, it is not easily plastically deformed. In Europe, due to the high toxicity of vanadium (V) included in Ti-6AI-4V alloy, Ti-6AI-7Nb[2] alloy is used. The V of this alloy has been substituted by the element niobium (Nb) that is also in group 5 of the periodic table, permitting substitution. Due to the lack of V, properties such as resistance to corrosion and safety exceed those of the Ti-6AI-4V alloy. Other alloys are already standardized, including Ti-5Al-2.5Fe developed in Europe, Ti-15-Zr-4Nb-4Ta (close to β- type) developed in the States, Ti-6Al-2Nb-1Ta and Ti-15-Al-4Nb-4Ta developed in Japan; Ti-5Al-3Mo-4Zr is currently under development. These Ti alloys are of the α+β- type. The β-types of Ti-alloys have lower values of Young's modulus, around 60 GPa (or 20 GPa in cortical bones)[3]. Within the β-type, Ti-15Mo, and Ti-12Mo-6Zr-3Fe alloys developed in the States, and the Ti-15Mo-5Zr-3Al alloy developed in Japan have now been standardized.

Development of Ti alloys for biological purposes have included the replacement of V or Al with safer elements such as Nb, Ta, Zr, and Hf, all Group 4 and 5 elements (Fig. 3-7-3). On the contrary, for fracture stabilization, use of alloys with a low value of Young's modulus is required to prevent stress shielding; for this, the β-type is effective. Ti-29Nb-3Ta-4.6Zr is currently undergoing development in this country as a β-type alloy [4, 5]. This alloy is converted into the β-type with heat treatment and forging, and is thought to exhibit the lowest value of Young's modulus. In comparison to the Ti-6Al-4V alloy, this has a faster rate of osteogenesis. The properties of Ti-16Nb-5.8Sn alloy, Ti-15Mo alloy, and Ti-10Fe-5Mo alloy are currently under investigation. Several heat processing treatments for alloys have been devised in the past. Heat processing has enabled the structural and mechanical properties be controlled.

Alloy composition (mass%)	Type	UNS	ASTM	ISO
Ti-3Al-2.5V	α+β	R56320	ASTM B 348	-
Ti-6Al-4V	α+β	R56400	ASTM F 1472	ISO 5832-3
Ti-6Al-4V ELI	α+β	R56401	ASTM F 136	ISO 5832-3
Ti-6Al-7Nb	α+β	R56700	ASTM F 1295	ISO 5832-11
Ti-15Mo	β	R58150	ASTM F 2066	-
Ti-13Nb-13Zr	β	R58130	ASTM F 1713	-
Ti-12Mo-6Zr-2Fe	β	R58120	ASTM F 1813	-
Ti-45Nb	β	R58450	AMS 4982	-
Ti-35Nb-7Zr-5Ta	β	R58350	-	-
Ti-55.8Ni	Intermetallic compound		ASTM F 2063	-

Table 3-7-2
Standardized Ti alloy

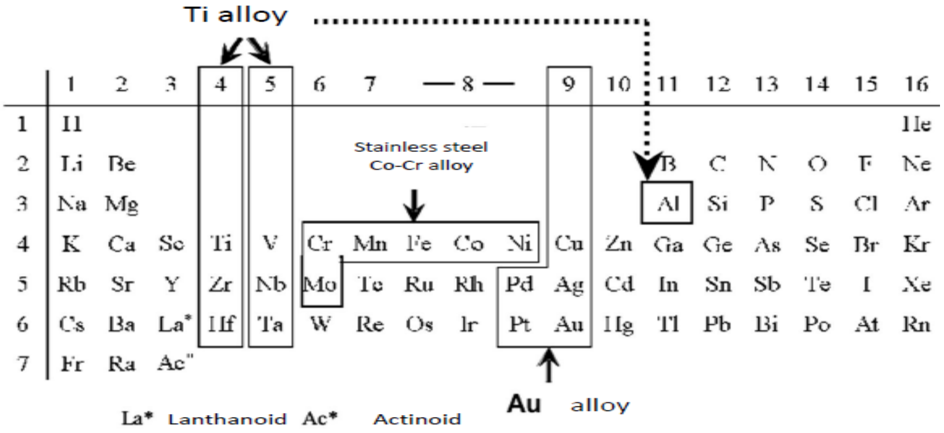


Fig. 3-7-3
The positions of the metal atoms in the periodic table that are utilized in biological activity

IV. The surface of titanium

Biomaterials function by interacting with biological tissues; therefore the reaction between the material surface and biological tissues needs to be fully understood. It is self-evident that reactions are determined by the properties of the few nm at the material surface, including resistance to corrosion and tissue biocompatibility. The metal surface under atmospheric conditions or in solutions always forms a layer of reactive film. The film that is formed in solution displays low solubility, and provided it has been formed without pores and is highly adhesive, it becomes resistant to corrosion (an inert/passive film). the passive film that is formed is transparent and is as thin as 1-5 nm. Metals such as Ti, Zr and Ta, that are essential as metal biomaterials, are easily oxidized (these are also known as valve metals). As this layer envelops the metal surface, it stops the progress of corrosion passed this point, therefore resulting in an apparently inert metal.

With regards to Ti, the reaction below occurs in solution at room temperature, resulting in its inert properties.

$$\mathrm{Ti + 2H_2O \rightarrow TiO_2 + 4H^+ + 4e^-} \text{ (anode reaction)} \qquad (1)$$

$$\mathrm{2H+ + 2e^- \rightarrow H_2} \text{ (cathode reaction, in acidic solution)} \qquad (2)$$

$$\mathrm{O_2 + H_2O + 4e^- \rightarrow 4OH^-} \text{ (cathode reaction, in neutral or alkaline solution)} \qquad (3)$$

The reaction described by equation (1) does not occur in one step, but in steps from:
$\mathrm{Ti \rightarrow Ti^{2+} \rightarrow Ti^{3+} \rightarrow Ti^{4+}}$.

The same reaction (1) can occur with atmospheric moisture; Ti as a biomaterial generally exists as an inert metal covered by an oxidative layer.

The passive film of Ti is made up of amorphous or low crystalline stoichiometric TiO_2. However, this is not completely amorphous, and includes lower oxide and crystalline grains6). A component of low crystalline rutile can also be identified in the passive film of Ti (surface oxide film) under the transmission electron microscope (TEM), but it remains fundamentally amorphous. The Ti2p and O1s spectra taken with X-ray photoelectron spectroscopy (XPS) of the Ti surface, after polishing, are shown in Fig. 3-7-4. From Ti2p spectra, the condition of the Ti metallic state can be detected through the surface oxidative layer, and the surface oxidative layer contains TiO_2 as the main constituent, as well as TiO and Ti_2O_3. The O1s spectra indicate the content of hydroxide, hydroxyl groups, bound water, and hygroscopic water. The presence of TiO_2 and hydroxyl radicals becomes more prominent nearer the metal surface 7).

Any damage to the surface can be self-repaired rapidly. As shown in Fig. 3-7-5, when the passive film is damaged, outflow of Ti ions and the anodic current that accompany regeneration of the film can be detected, however, this current declines within a short period, once the film has regenerated. This is why the corrosion resistance of these materials is high 8).

Even though an inert film is formed on the titanium surface, the surface remains active. It reacts with the moisture in air, forming hydroxyl groups on the surface. The passive layer of Ti as shown in Fig. 3-7-6-a, an inert film is formed in solution as well as on reaction with moisture in the atmosphere, resulting in the formation of hydroxyl groups9). The concentration of hydroxyl groups on the metal oxide surface has been investigated using various methods, and that of TiO_2 has been determined to be 4.9 – 12.5 nm^{-2} 10).

The hydroxyl groups on the oxide surface become ionized in solution, as shown in Fig. 3-7-6-b, thus forming an ionized solution [9)-12)]. The surface charge that arises from ionized hydroxyl groups depends on the surrounding pH of the solution; the positive and negative charges balance out at a given pH, with a net electrical charge of zero. This pH is known as the point of zero charge (pzc). The pzc value is specific for each oxide, and becomes an indicator of whether an oxide surface takes on acidic or alkaline properties. For example, in TiO_2, rutile has a value 5.3, and anatase as 6.2 [13)]. This means that in solutions with a pH above 6.2, the negative charges predominate over the positive charges, whereas in acidic solutions, the number of positive charges increases. The pzc values for each oxide are shown in Table 3-7-3 [11)]. The pzc of TiO_2, in comparison to SiO_2, Al_2O_3, and MgO, is closer to 7, and TiO_2 thus does not display a marked acidic or alkaline state under physiological pH.

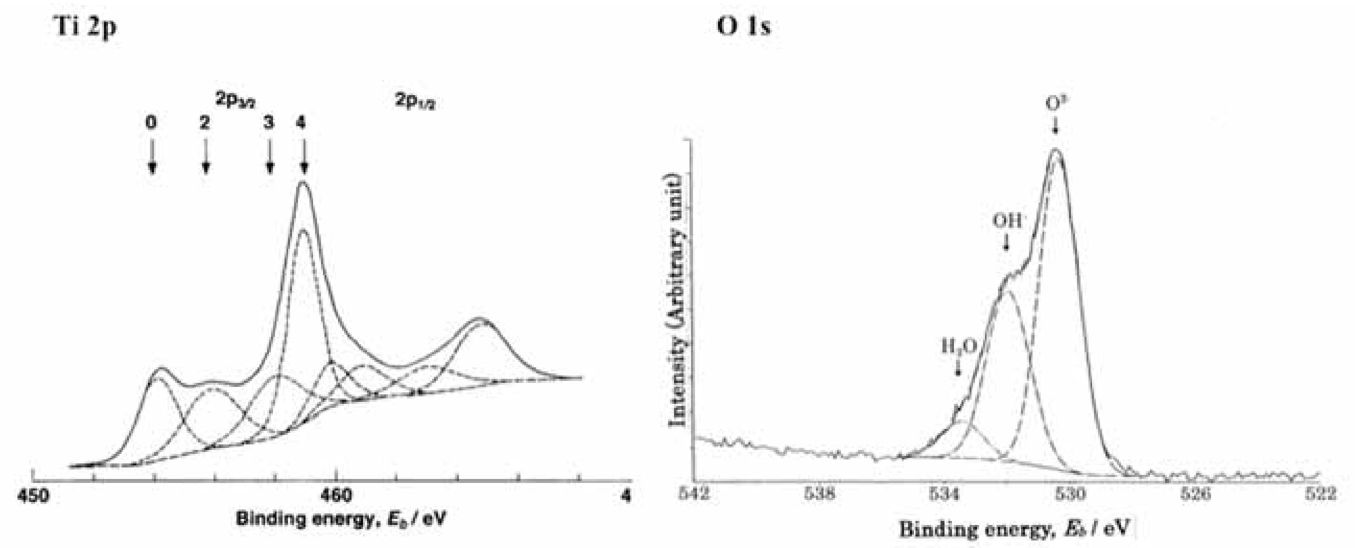


Fig. 3-7-4

The Ti2p and O1s spectra of the Ti surface taken with X-ray photoelectron spectroscopy (XPS)

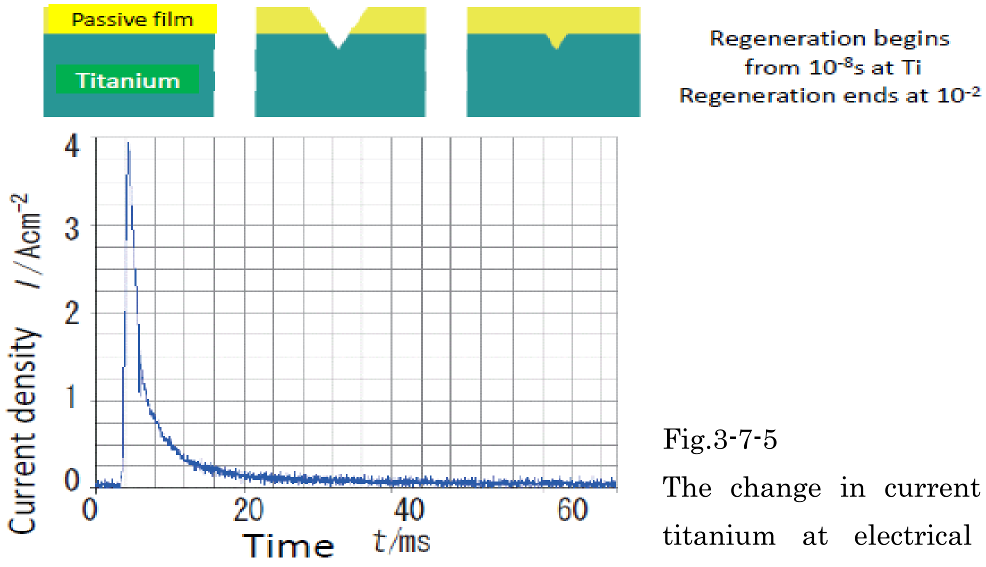


Fig.3-7-5

The change in current density in polishing the titanium at electrical potentials of 0 V and saturated calomel electrode (SCE) on the inert layer on the surface of titanium in Hank's solution

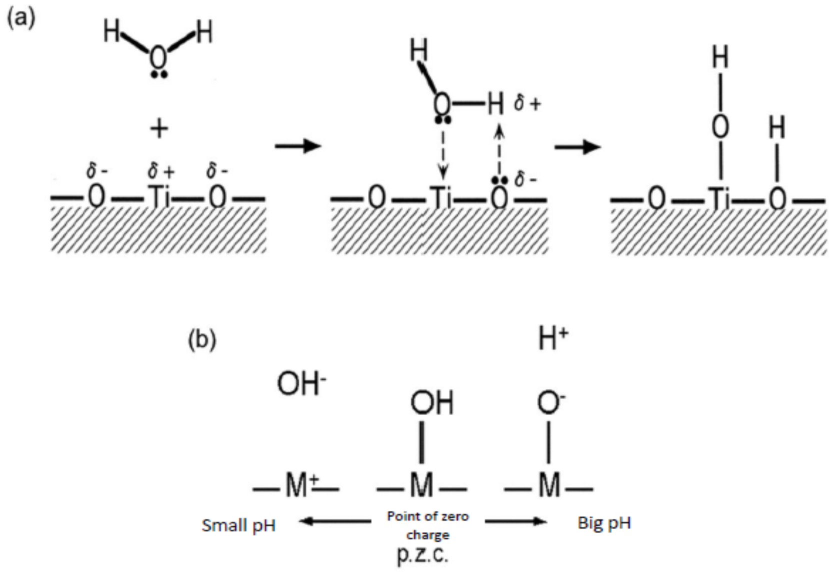


Fig. 3-7-6

a) The formation of hydroxyl group on the surface of titanium oxide
b) Ionization and point of zero charge of the surface hydroxyl group in solution

Oxidized compounds	Point of zero charge
SiO_2	2.0-3.7
SnO_2	4.7-5.3
TiO_2	6.7*
Fe_2O_3	6.6-6.7
Cr_2O_3	7.0
Al_2O_3	9.0

Table 3-7-3
Point of zero charge for oxidized compounds

V. Reconstruction of the oxide passivation film

The passive film appears to be in a stable state, however, a cycle of repetitive partial solubilization and re-deposition can be detected at the microscopic level. The structure is therefore constantly changing in accordance with its biological surroundings (Fig. 3-7-7). With regards to titanium dental implants, Ca, P and S are reconstituted into the surface oxidative film once it is inserted into the jaw bone [14), 15)]. Calcium phosphate is also formed on the surface of Ti-6Al-4V alloy when it is used for fracture fixation. In particular, calcium phosphate with a large [Ca/P] ratio can be detected on the surface of an intramedullary needle inserted into the medullary cavity. Furthermore, when Ti or Ti alloy is immersed in Hank's solution, calcium phosphate has been found to be deposited [16)-19)], and under conditions used for cell culture, formation of sulfite, or sulfide has also been found [20)]. These findings show that the physiological processes that occur in the body are well reflected by *in vitro* experiments. This formation of calcium phosphate is considered to be the reason why osteoporotic fractures occur at the time of intramedullary needle extraction.

Calcium phosphate can be formed on stainless steel [21)], and Co-Cr alloy [22)], however, its rate of formation and its [Ca/P] ratio is much higher on Ti or Ti alloy. On the contrary, calcium phosphate cannot be formed on Zr[23)]. Therefore, the ability of Ti to form calcium phosphate rapidly is the reason for its compatibility with the hard tissues.

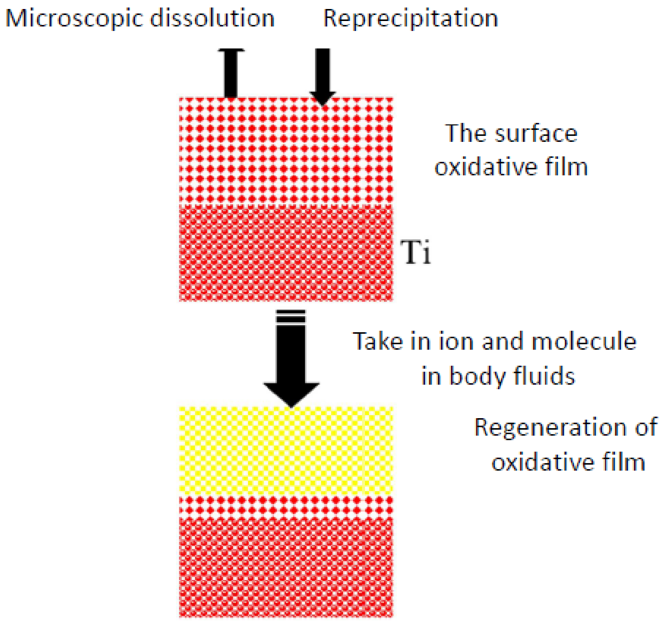


Fig. 3-7-7
Reconstruction of surface oxide (Inert) layer in the biological environment

VI. Corrosion-resistant properties of titanium

Metal itself does not exhibit toxicity in the form of allergic reactions or carcinogenic properties, but this can arise from the metal ions or derivatives of the metal including oxides, hydroxides, salts, or complexes that result as a product of rust or corrosion. These can subsequently bind biological molecules or cellular organelles, inhibiting their biological functions. Fig. 3-7-8 shows the anode polarization curve of pure Nickel (Ni), 316L stainless steel, Co-Cr-Mo alloy, Ti, and Ti-6Al-4V alloy in rabbits, or in Ringer's solution, respectively 24).The same polarization results were also obtained with Hank's solution (a solution that is used for cell culture, where the concentrations of inorganic ions are similar to those in extracellular fluid). The passive state maintaining current density is lower for Ti and Ti-6Al-4V than for other materials, suggesting relatively high resistance to corrosion. In order to evaluate the electrochemical influence of living cells that are cultured on the Ti surface, a unit for electrochemical measurement in cell-culture environment has been developed 25). Measurements taken with this unit showed that even though Ti shows a decline in corrosion potential, the presence of the living cells had no effect 26).

Ti-6Al-4V, on the other hand, showed charge stabilization after five days of being immersed in biological saline, but in Hank's solution, the charge continued to show a gradual increase 27). This suggests that the passive film of Ti is stable in saline, but the film grows in Hank's solution. As noted above, the reason for this is formation of a calcium phosphate film on the Ti surface in Hank's solution.

On investigation of the effect of uric acid and amino acids, immersion charges measured for a period of 150 days did not change, suggesting that these biological molecules had no effect on the corrosion of Ti alloys. Proteins are generally known to accelerate metal corrosion, but have no effect on Ti or Ti alloys. However, organic acids such as EDTA and sodium citrate have been shown to corrode Ti alloys 28). The reactive oxygen species produced by macrophages are also factors that promote corrosion of Ti 29). Introduction of materials into the body generally induces inflammation, causing macrophages to proliferate and interact with the material. Therefore corrosion of Ti introduced into the body is mostly likely to occur via this phenomenon. Recently, dental reparation devices constructed of Ti or Ti alloys have shown to erode due to the inclusion of fluorine in tooth paste and mouth washes 30), 31).

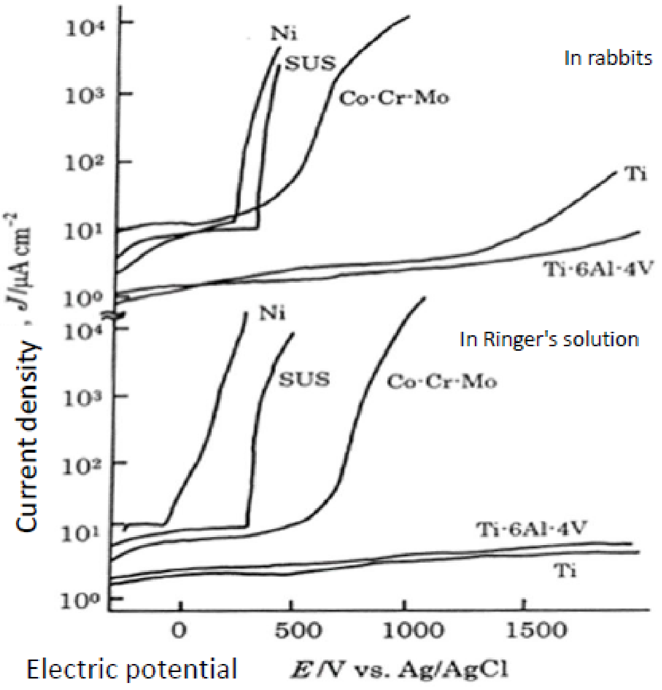


Fig. 3-7-8
The anode polarization curve of each of the metal materials in rabbits and Ringer's solution

VII. Properties and toxicity of titanium ions

In non-noble metals such as Ti alloys, the passive film can become disrupted due to friction, exposing the underlying metal, and causing elution of metal ions. The passive film can usually repair itself efficiently however, if this friction is continuous, specific types of metal ion become eluted. After performing fretting fatigue experiments in pseudo-body fluids, the filtrate of the resulting pseudo-body fluid was examined to quantify the metal elements: Ti, the main constituent, was not detected but elements such as Ni, were detected, or even minute quantities of Fe instead [32] (Fig. 3-7-9). These findings indicate that in environments where friction is a common occurrence, the fraction of the metal elements eluted from the alloys cannot be expected to reflect the composition of alloys, and the possibility of elution of elements present in minute amounts should not be ignored. Active substances that have similar properties to Ti ions are used up in the process of regeneration of the passive film, but inactive metal ions become the subject of elution. This implies that elements like Ti with high activity are less likely to be eliminated even in high friction environments. The reactivity of the eluted ions is also important. As shown in Fig. 3-7-10, metal ions can largely be divided into three types: ions that react readily with water molecules and anions to form stable oxides, hydroxides and salt compounds; those that do not react; and those that form unstable complexes. The first two types are less likely to react with the biological molecules, but the last type is more likely to do so. Examples of the first two types are, Ti and Zr ions, and examples of the last type are Ni and Cu ions.

Toxicity of metal materials is determined by: (1) the ease with which the ions are eluted out (susceptibility to corrosion), (2) the activity of the eluted metal ions, (presence of passivation ability, and reactivity with surrounding molecules) (3) toxicity of the metal ions and their derivatives. These factors should all be taken into consideration, in order to understand the toxicity of the material. The factors that have been stated above illustrate the very safe nature of titanium metal.

Even though Ti is abundant in the earth's crust, it is not an essential element for biological functions, and its presence in the body is minimal. This is probably due to the fact that Ti ions react readily with water molecules in solution, becoming stable, and therefore were not taken up by the body to be used for structural components during the developmental and evolutionary stages of our existence. This implies that bonding of the Ti ions with biological molecules is complicated, and thus its safety can be guaranteed even if Ti ions are eluted out.

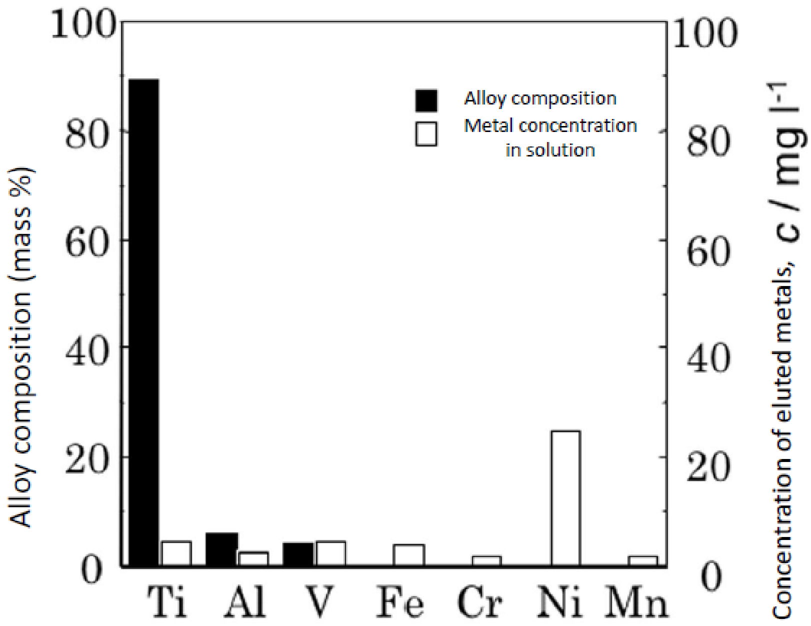


Fig. 3-7-9

The relationship between metal constituents of the alloys and their metal ion concentrations. The metal ion concentration of each alloys were measured from the filtrated PBS (-) (phosphate buffered saline that does not contain cations such as $Mg^{2+}$and Ca^{2+}) solution after undergoing fretting fatigue experiment in PBS (-), at 131 MPa, for 4.10 ×106 times.

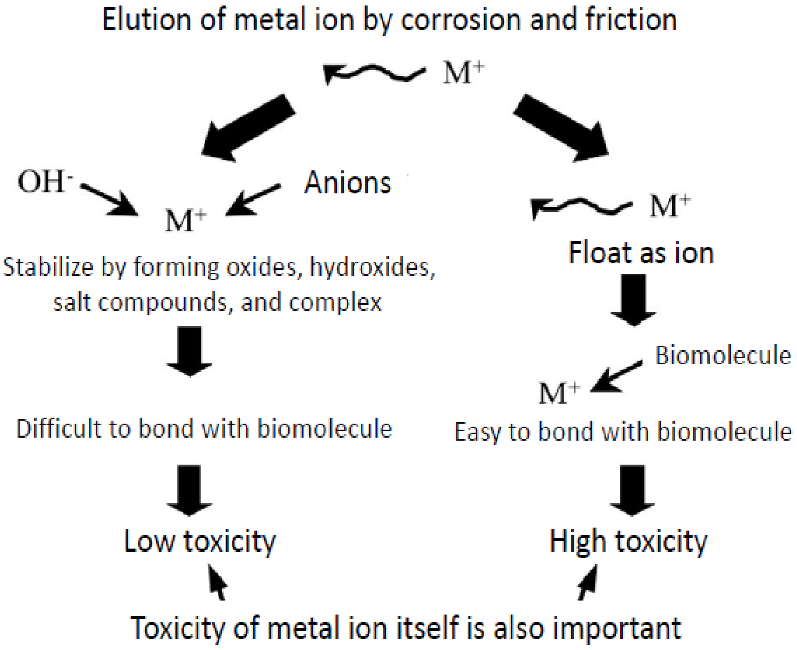


Fig. 3-7-10

The factors that determine the toxicity of metal implants

VIII. Protein Adsorption

Protein adsorption occurs at the interface, as soon as a material interacts with biological tissues. Protein adsorption affects subsequent adhesion to cells, and corrosion of metal materials. Protein adsorption to metals or their oxidized surfaces has been investigated with numerous analytical techniques 33).

The conformations of proteins are specific to their functions, therefore awareness of the conformational changes that occur as a result of adsorption with the material surface is essential for understanding their functions (Fig. 3-7-11). Proteins are charged compounds, thus the electrostatic charge of the metal surfaces influences the protein conformation. The strength of the static charge at adsorption is determined by the dielectric constant, where the larger the dielectric constant, the smaller the electrostatic charge. The dielectric constants of water and oxides, as well as those of Co-Cr-Mo alloys and stainless steel, calculated from the surface oxide structure, are shown in Table 3-7-4 34). The dielectric constant of TiO_2 is much closer to that of water in comparison with other compounds therefore the conformational changes of proteins that result from adsorption to Ti surface can be expected to be relatively small. In the case of fibrinogen adsorption to Au and Ti surfaces in solution, even though higher adsorption is seen to the Au surface, a thicker layer is formed on Ti 35). This is because the dielectric constant of the Ti is much higher due to the presence of a TiO_2 layer, while the surface of Au is not covered with an oxide layer, which can be seen from its low dielectric constant. The conformational change of fibrinogen is found to be far lower when adsorbed by a thicker layer.

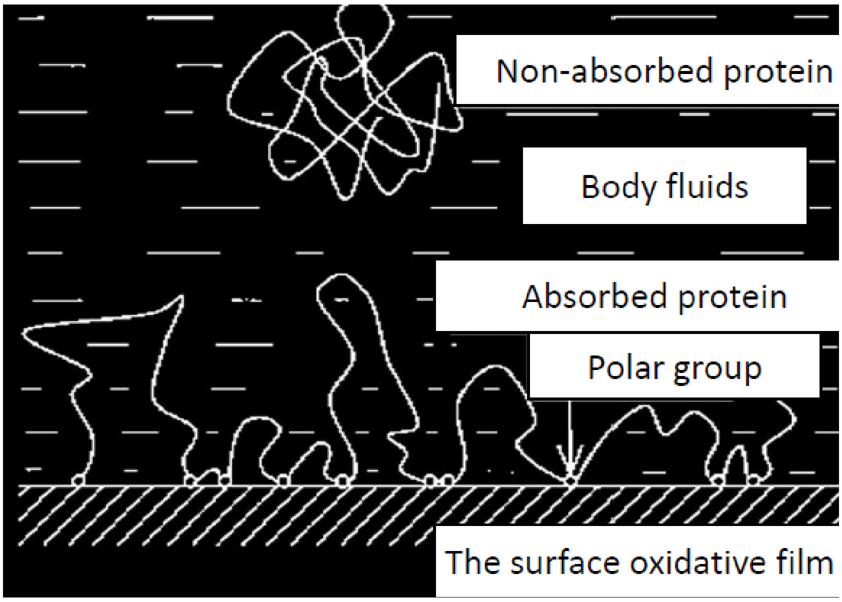
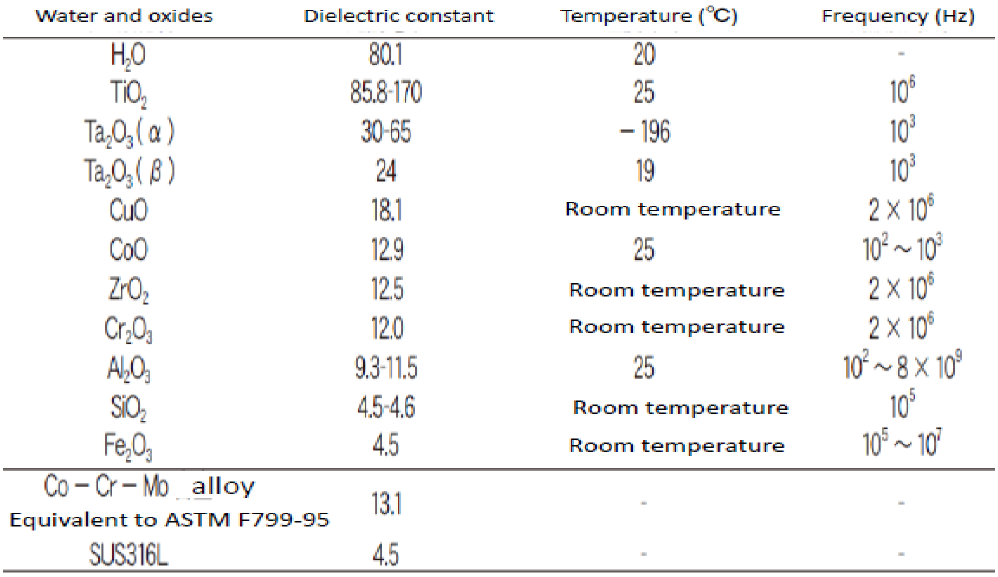


Fig. 3-7-11
Proteins change their conformation when adsorbing onto solid surfaces



Water and oxides	Dielectric constant	Temperature (°C)	Frequency (Hz)
H_2O	80.1	20	-
TiO_2	85.8-170	25	10^6
Ta_2O_3 (α)	30-65	− 196	10^3
Ta_2O_3 (β)	24	19	10^3
CuO	18.1	Room temperature	2×10^6
CoO	12.9	25	$10^2 \sim 10^3$
ZrO_2	12.5	Room temperature	2×10^6
Cr_2O_3	12.0	Room temperature	2×10^6
Al_2O_3	9.3-11.5	25	$10^2 \sim 8 \times 10^9$
SiO_2	4.5-4.6	Room temperature	10^5
Fe_2O_3	4.5	Room temperature	$10^5 \sim 10^7$
Co − Cr − Mo alloy Equivalent to ASTM F799-95	13.1	-	-
SUS316L	4.5	-	-

Table 3-7-4
Dielectric constants of water and oxides22)

References

1) Pourbaix M. Atlas of Electrochemical Equilibria in Aqueous Solution Houston, Texas: National Association of Chemical Engineers; 1974.

2) Semlitsch M, Staub F, Weber H. Titanium-aluminum-niobium alloy, development for biocompatible, high strength surgical implants.Biomed Technik.1985; 30: 334-339.

3) Narushima T. Titanium, titanium alloy.Biomaterials.2005; 23: 86-95.

4) Niinomi M, Akahori T, Nakai M, Hattori Y, Kasuga T. Multifunctional titanium alloys with low elastic modulus for biomedical applications. Kinzoku: Materials science & technology. 2007;77: 128-134. (in Japanese)

5) Kuroda D, Niinomi M, Morinaga M, Kato Y, Yashiro T. Design and mechanical properties of new b type titanium alloys for implant materials. Mater Sci Eng. 1988; A243: 244-249.

6) Kelly EJ. Electrochemical behavior of titanium. Mod Aspect Electrochem.1982; 14: 319-424.

7) Hanawa T, Asami K, Asaoka K. Repassivation of titanium and surface oxide film regenerated in simulated bioliquid. J Biomed Mater Res.1998; 40: 530-538.

8) Shimodaira S. Materials science of corrosion and corrosion prevention. Tokyo. Agne Gijutsu Center.1995: 29.

9) Okazaki S. Surface properties and surface modifications of TiO2. Journal of the Adhesion Society of Japan.1985; 21: 24-31. (In Japanese)

10) Tamura H, Furuichi R. Determination of the dissociation constants of surface hydroxyl groups-Application to the characterization of metal oxides-. Bulletin of the Japan Institute of Metals.1988; 27: 158-164. (in Japanese)

11) Healy TW, Fuerstenau DW. The oxide-water interface — Interreaction of the zero point of charge and the heat of immersion. J Colloid Sci.1965; 20: 376-386.

12) Boehm HP. Acidic and basic properties of hydroxylated metal oxide surfaces. Discuss Faraday Soc.1971; 52: 264-289.

13) Parfitt GD. The surface of titanium dioxide.Prog Surf Membrane Sci.1976; 11: 181-226.

14) Sundgren JE, Bodo P, Lundstrom I. Auger electron spectroscopic studies of the interface between human tissue and implants of titanium and stainless steel. J Colloid Interface Sci.1986; 110: 9-20.

15) Espostito M, Lausmaa J, Hirsch JM, Thomsen P. Surface analysis of failed oral titanium implants. J Biomed Mater Res Appl Biomater.1999; 48: 559-568.

16) Hanawa T, Ota M. Calcium phosphate naturally formed on titanium in electrolyte solution. Biomaterials.1991; 2: 767-774.

17) Hanawa T, Ota M. Characterization of surface film formed on titanium in electrolyte. Appl Surf Sci.1992; 55: 269-276.

18) Healy KE, Ducheyne P. The mechanisms of passive dissolution of titanium in a model physiological environment. J Biomed Mater Res.1992; 26: 319-338.

19) Serro AP, Fernandes AC, Saramago B, Lima J, Barbosa MA. Apatite desorption on titanium surfaces – the role of albumin adsorption. Biomaterials.1997; 18: 963-968.

20) Hiromoto S, Hanawa T, Asami K. Composition of surface oxide film of titanium with culturing murine fibroblasts L929.Biomaterials.2004; 25: 979-986.

21) Hanawa T, Hiromoto S. Yamamoto A, Kuroda D, Asami K. XPS characterization of the surface oxide film of 316L stainless samples that were located in quasi-biological environments. Mater Trans. 2002; 43:3088-3092.

22) Hanawa T, Hiromoto S, Asami K. Characterization of the surface oxide film of a Co-Cr-Mo alloy after being located in quasi-biological environments using XPS.Appl Surf Sci.2001; 183: 68-75.

23) Hanawa T, Okuno O, Hamanaka H. Compositional change in surface of Ti-Zr alloys in artificial bioliquid. Journal of the Japan Institute of Metals.1992; 56:1168-1173. (In Japanese)

24) Nakayama Y, Yamamuro T, Kotoura Y, Oka K. In vivo measurement of anodic polarization of orthopaedic implant alloys. Biomaterials 1989; 10,: 420-414.

25) Hiromoto S, Noda K, Hanawa T. Development of Electrolytic Cell with Cell-Culture for Metallic Biomaterials. Corros Sci. 2002; 44: 955-964.

26) Hiromoto S, Noda K, Hanawa T. Electrochemical properties of an interface between titanium and fibroblasts L929. Electrochim Acta. 2002; 48: 387-396.

27) Fraker AC, Ruff AW, Yeager MP. Corrosion of titanium alloys in physiological solutions. In: Titanium Science and Technology, Jaffee RI, Burte HM, editors. New York: Plenum Press; 1973. p. 2447-2457.

28) Bruneel N, Helsen JA. In vitro simulation of biocompatibility of Ti-Al-V. J Biomed Mater. Res. 1988; 22:203-214.

29) Mu Y, Kobayashi T, Sumita M, Yamamoto A, Hanawa T. Metal ion release from titanium with active oxygen species generated by rat macrophages in vitro. J Biomed Mater Res. 2000; 49: 238-243.

30) Nakagawa M, Matsuya S. The latest studies on the corrosion of titanium and titanium alloys for biomaterials. Materia Japan. 2002;41:802-809. (In Japanese)

31) Wilhelmsen W, Grande AP. The influence of hydrofluoric-acid and fluoride-ion on the corrosion and passive behavior of titanium. Electrochim Acta. 1987; 32: 1469-1474.

32) Yamamoto A, Kobayashi T, Maruyama N, Nakazawa K, Sumita M. Fretting fatigue properties of Ti-6Al-4V alloy in pseudo-body fluid. Journal of the Japan Institute of Metals. 1995;59:463-470. (In Japanese)

33) Ivarsson B, Lundström I. Physical characterization of protein adsorption on metal and metal oxide surfaces. CRC Critic Rev Biocompatibility. 1986; 2: 1-96.

34) Lide DR, editor. CRC Handbook of Chemistry and Physics 87th ed. Boca Raton, Florida: CRC Press; 2006.

35) Sundgren JE, Bodöö P, Ivarsson B, Lundström I. Adsorption of fibrinogen on titanium and gold surfaces studied by ESCA and ellipsometry. J Colloid Interf Sci. 1986; 113: 530-543.