Surface treatment and modification of metals to add biofunction

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To add biocompatibility or biofunction to metal surface, an intelligent interface between metals and tissues must be acquired. Tremendous surface modification techniques are currently studied to create the intelligent interface. In particular, bone formation or bone bonding is major purpose of the surface modifications. Time transient of surface modification techniques are summarized and the importance of roughened or porous surface to combine materials with bone tissue is demonstrated. As an example of surface modification, electrodeposition of poly(ethylene glycol) to inhibit biofilm formation is introduced. A dual-functional surface is formed on titanium by micro arc oxidation. In addition, the effect of topography on the elongation and differentiation of human mesenchymal stem cells was confirmed on the hybrid micrometer-level and nanometer-level grooves of titanium surface. Metal surface is possibly biofunctionalized by various surface modification techniques.

Keywords: Surface modification, Titanium, Osseointegration, Micro arc oxidation, Biofunction

INTRODUCTION

Metals show high strength, elongation, fracture toughness, and durability, which are specific advantages against ceramics and polymers. Therefore, metals are widely used for medical devices, especially implants to prevent the fracture or degradation of devices during use in the human body. In dentistry, inlay, crown, bridge, partial denture with clasp, orthodontic wire, implant anchor, reamer, file, dental implant, and treatment tool and apparatus consist of metals. A disadvantage of using metals as biomaterials is that they are typically artificial materials and have no biofunction. Therefore, metals require additional properties before they can be used as functional biomaterials. Requirements of specific properties to metals used for dental devices are summarized in Table 1. To respond to these requirements, new alloy designs and many techniques for the surface modification (including surface treatment) have been investigated and some of them are already commercialized. This paper presents a concept of an intelligent interface between metals and tissues. In addition, time transient of surface modification techniques to accelerate bone formation are explained and some of the examples are shown.

INTELLIGENT INTERFACE AND SURFACE MODIFICATION

When a metallic material is implanted into a human body, immediate reaction occurs between its surface and the living tissues, while titanium shows osseointegration. An artificial material usually makes clear interface against a tissue: The interface works as a barrier for transportation of molecules and conduction

of biofunction, as shown in Fig. 1. On the other hand, if we could create unclear and graded interface at which molecules smoothly transport, both material and tissue are integrated together, and biofunctions are conducted, this interface may be defined as intelligent interface. In addition, this interface is expected to be a field not only for chemically biofunctional conduction, but also for mechanically stress conduction. Of course, one of the solutions is surface modification of materials.

Surface modification is a process that changes a material's surface composition, structure, and morphology, leaving the bulk mechanical properties intact. Surface modification can improve the tissue compatibility of the surface layer. Category of surface modification changing the chemical property of the surface is shown in Fig. 2. Dry processes (performed in a vacuum or in air) and wet processes (performed in aqueous solutions) are the conventional and predominant surface modification techniques used to accelerate bone formation. The surface modification techniques of metals in the field of biomedical materials are reviewed¹⁻³⁾.

A tremendous number of surface modification techniques to improve the hard tissue compatibility of titanium have been developed. Among these techniques, I summarized techniques investigated in our laboratory in Fig. 3. Most researches in the figure were performed to accelerate bone formation on titanium, while some of them were performed to inhibit biofilm formation and platelet adhesion. Calcium phosphate formation on titanium was the origin of our studies on surface modification. Composition of surface oxide film on titanium varies according to environmental changes, though the film is macroscopically stable. When titanium which has been surgically implanted into the human jaw is characterized using Auger electron spectroscopy, its surface oxide film reveals constituents of calcium,

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Table 1 Requirements of specific properties to metals used for dental devices

Required property	Target medical device	Effect
Non-fracture	All devices	Durability
Elastic modulus	Dental implant	Prevention of bone absorption by stress shielding
Superelasticity Shape memory effect	Multi-purpose	Improvement of mechanical compatibility
Bone formation Bone bonding	Dental implant; implant anchor	Fixation of devices in bone
Prevention of bone formation	Implant anchor	Prevention of assimilation
Adhesion of soft tissue	Dental implant	Fixation in soft tissue; prevention of inflectional disease
Inhibition of biofilm formation	All devices; treatment tools and apparatus	Prevention of infectious disease
Low magnetic susceptibility	All devices; treatment tools and apparatus	No artifact in MRI

Fig. 1 Clear interface works as a barrier for transportation of molecules and conduction of biofunction. Unclear and graded interface at which smooth transportation of molecules occurs, both material and tissue are integrated together, and biofunctions are conducted.

phosphorus, and sulfur^{4,5}). By immersing titanium and its alloys in Hanks' solution and other solutions⁶⁻⁸, preferential adsorption of phosphate ions occurs. Even during cell culture on titanium, calcium phosphate is formed on it⁹. According to the above results, calcium-ion-implantation into titanium to accelerate bone formation was investigated first. Recently, we are concentrating the development of electrochemical techniques such as cathodic polarization, micro arc

Properties required to surface of metals

Bone formation; Bone bonding
Adhesion to soft tissue
Inhibition of biofilm formation

Properties required to metal itself

Fig. 2 Category of surface treatment of metallic materials to add biocompatibility and biofunctions remaining good mechanical property.

oxidation (MAO), and electrodeposition of biofunctional molecules.

EVOLUTION OF SURFACE MODIFICATION FOR BONE FORMATION

In dental implants, the chemical bonding of metal surfaces with bone is not expected, while titanium shows osseointegration. In other words, it is impossible for metals as typical artificial materials to chemically and naturally bond with bone as living tissue, especially in the human body with body fluid. Therefore, the surface morphology is sometimes controlled, and rough and porous surface is formed in titanium. Living tissue,

Fig. 3 Surface modification researches published from our laboratory.

such as bone, is expected to grow into the rough or porous surface, and the materials and bone are strongly connected as a result of the so-called anchoring effect. Figure 4 shows chemical bonding and a mechanical anchoring connection between bone and material. Therefore, popular commercial brand of dental implant employed surface modification techniques to form porous or rough surface: For example, TiUnite® by MAO and Straumann® by blast and acid etching.

Osseointegration is the first definition of the interface between a metallic material and living tissue. The definition of osseointegration is as follows: The formation of a direct interface between an implant and bone, without intervening soft tissue. No scar tissue, cartilage or ligament fibers are present between the bone and implant surface. The direct contact of bone and implant surface can be verified microscopically¹⁰⁾. This “osseointegration” concept was immediately accepted by dentists and dental materials researchers in the world to show biocompatible advantage of titanium among metals that makes it possible that titanium occupies major position in dental implant bodies.

Figure 5 shows the evolution of surface treatment techniques to improve hard tissue compatibility at the research level:

- First generation: Grind machining of the surface.
- Second generation: Grooving, blast, acid etching, anodic oxidation, and laser abrasion.
- Third generation: Chemical treatment and hydroxyapatite coating.
- Fourth generation: Immobilization of biofunctional molecules (collagen, bone morphogenetic

Fig. 4 Mechanical anchoring and chemical bonding between bone and material.

Fig. 5 The evolution of surface treatment techniques to improve hard tissue compatibility at the research level.

protein, and peptide).

Fifth generation: Coating of stem cells and tissues?

The bone formation of the materials surface is accelerated when biomolecules concerning bone formation are immobilized on the material surface, such as in the fourth generation. Therefore, many studies have achieved good results in this direction. However, to increase the popularity of the immobilization of biofunctional molecules, it is necessary to ensure the safety, quality maintenance during storage, and dry-conditioned durability of the immobilized layer. Therefore, it is difficult for manufacturers to commercialize the fourth generation implants. As a result, most of commercialized goods are categorized into the second generation, a few belong to the third generation. The commercialization went faster for the second than third generation possibly because materials employing mechanical anchoring are more practical than materials employing chemical bonding with bone.

MAO FOR DUAL-FUNCTIONAL SURFACE

By applying a positive voltage to a titanium immersed

in an electrolyte, anodic oxidation (or anodizing) of titanium occurs to form a TiO_2 layer on the surface. When the applied voltage is increased to a certain point, a micro-arc occurs as a result of the dielectric breakdown of the TiO_2 layer. At the moment the dielectric breakdown occurs, titanium ions in the implant and OH^- ions in the electrolyte move very quickly in opposite directions to form TiO_2 again. This process is generally referred to as MAO, or plasma electrolysis¹¹⁾.

After MAO, bone formation on titanium is accelerated as shown in Fig. 6. Silver is easily contained in the surface oxide layer by the addition of silver in the electrolyte for MAO. In this case, live bacteria decreased with increasing the amount of silver in the electrolyte and killed over 0.4-mmol L^{-1} silver amount (Fig. 7) and simultaneously calcification occurred in this silver amount. This result reveals that bone formation and antibacterial property are simultaneously performed by addition of calcium, phosphate, and silver to surface oxide layer by MAO: A dual-functional surface was formed on titanium by MAO, as shown in Fig. 8. It is possible to obtain and control dual-functional property by this technique.

Fig. 6 Acceleration of calcium phosphate formation by MAO and concept of antibacterial property by the release of silver ions.

Fig. 7 Viable bacteria counts on titanium untreated and treated by MAO in electrolytes with various concentrations of silver.

Fig. 8 Dual-functional window appears according to the calcification test and antibacterial test.

MAO is also effective to titanium alloys and zirconium. MAO treatment helps form a porous surface with a biologically active bone-like apatite layer on the Ti-24Nb-4Zr-7.9Sn alloy (TNZS) specimens, which may improve the biological response of MAO-TNZS implants¹²⁾. On the other hand, ZrO₂ layer resembling the color of teeth with good mechanical properties was obtained by the combination of sputter deposition of metal zirconium and subsequent MAO treatment¹³⁾. Surface treatments with various combinations of MAO and chemical treatments were performed on zirconium disks to enhance the bioactivity of zirconium¹⁴⁾.

ELECTRODEPOSITION OF BIOFUNCTIONAL MOLECULES

Poly(ethylene glycol) (PEG) is a biofunctional molecule on which the adsorption of proteins is inhibited. Therefore, immobilization of PEG to a metal surface is important for biofunctionalizing the metal's surface. Both terminals of PEG are terminated with –NH₂. The cathodic potential is charged to titanium with a potential of –0.5 V_{SCE} and is maintained at this potential for 300 s. During charging, the terminated PEGs electrically migrate to and are immobilized on the titanium cathode^{15–17)}, as shown in Fig. 9. The PEG-immobilized

Fig. 9 Electrodeposition of PEG to titanium surface and its process.

Fig. 10 Aligned hMSCs on groove surfaces and differentiation of hMSCs on the surfaces.

surface inhibits the adsorption of proteins and cells, as well as the adhesion of platelets¹⁸⁾ and bacteria^{19,20)}, indicating that this electrodeposition technique is useful for biofunctionalizing metal surfaces. It is also useful for all electroconductive materials, even materials having a complex surface topography.

MICROMETER/NANOMETER-SCALE TOPOGRAPHY

To succeed the 5th generation surface modification shown in Fig. 5, the coatings of tissue or stem cells on titanium is necessary. Micrometer-level, nanometer-level and hybrid of both level grooves were fabricated on titanium using a femto-second laser²¹⁾. Human mesenchymal stem cells (hMSCs) cultured on specimens showed a high degree of alignment on micrometer-level and hybrid groove surfaces after 6 h of incubation, whereas cells attached to nanometer-level and hybrid groove surfaces were elongated (Fig. 10). An examination of vinculin-positive adhesion plaques indicated that micrometer-level grooves influenced cellular alignment by modifying initial cell polarization, whereas nanometer-level grooves influenced cellular extension. In addition, the hybrid grooves sometimes accelerated osteogenic and chondrogenic differentiation of hMSCs. These findings provide a basis for the design of novel biomaterial surfaces that can control specific cellular functions.

CONCLUSIONS

Metallic materials are widely used in medicine not only for dental implants, but also for orthopedic and cardiovascular devices. Implant devices are always used in close contact with living tissues. Therefore, interactions between material surfaces and living tissues must be well controlled. Metal surface is possibly biofunctionalized by various surface modification techniques. These techniques make it possible to apply metals to a scaffold in tissue engineering and regenerative medicine.

REFERENCES

- 1) Hanawa T. An overview of biofunctionalisation of metals in Japan. *J Royal Soc Interface* 2009; 6: S361-S369.
- 2) Hanawa T. Biofunctionalization of titanium for dental implant. *Jpn J Dent Sci Rev* 2010; 46: 93-101.
- 3) Hanawa T. Metal-polymer composite biomaterials. In: Dumitriu S, Popa V, editors. *Polymeric Biomaterials Vol. 1, Structure and Function*. Boca Raton, FL, USA: CRC Press; 2013. pp. 343-375.
- 4) 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.
- 5) 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.
- 6) Hanawa T, Ota M. Characterization of surface film formed on titanium in electrolyte. *Appl Surf Sci* 1992; 55: 269-276.
- 7) Hanawa T. Titanium and its oxide film: a substrate for formation of apatite. In: Davies JE, editor. *The Bone-Biomaterial Interface*. Toronto: University of Toronto Press; 1991. pp. 49-61.
- 8) Hanawa T, Okuno O, Hamanaka H. Compositional change in surface of Ti-Zr alloys in artificial body fluid. *J Jpn Inst Met* 1992; 56: 1168-1173.
- 9) Hiromoto S, Hanawa T, Asami K. Composition of surface oxide film of titanium with culturing murine fibroblasts L929. *Biomaterials* 2004; 25: 979-986.
- 10) Brånemark PI, Hansson BO, Adell R, Breine U, Lindström J, Hallén O, Ohman A. Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. *Scand J Plast Reconstr Surg Suppl* 1977; 16: 1-132.
- 11) Yerokhin AL, Nie X, Leyland A, Matthews A, Dowey SJ. Plasma electrolysis for surface engineering. *Surf Coat Technol* 1999; 122: 73-93.
- 12) Tsutsumi Y, Niinomi M, Nakai M, Tsutsumi H, Doi H, Nomura N, Hanawa T. Micro-arc oxidation treatment to improve the hard-tissue compatibility of Ti-29Nb-13Ta-4.6Zr alloy. *Appl Surf Sci* 2012; 262: 34-38.
- 13) Nyan M, Tsutsumi Y, Oya K, Doi H, Nomura N, Kasugai S, Hanawa T. Synthesis of novel oxide layers on titanium by combination of sputter deposition and micro-arc oxidation techniques. *Dent Mater J* 2011; 30: 754-761.
- 14) Ha JY, Tsutsumi Y, Doi H, Nomura N, Kim KH, Hanawa T. Enhancement of calcium phosphate formation on zirconium by micro-arc oxidation and chemical treatments. *Surf Coat Technol* 2011; 205: 4948-4955.
- 15) Tanaka Y, Doi H, Iwasaki Y, Hiromoto S, Yoneyama T, Asami K, Imai H, Hanawa T. Electrodeposition of amine-terminated poly(ethylene glycol) to titanium surface. *Mater Sci Eng* 2007; C27: 206-212.
- 16) Tanaka Y, Doi H, Kobayashi E, Yoneyama T, Hanawa T. Determination of the immobilization manner of amine-terminated poly(ethylene glycol) electrodeposited on a titanium surface with XPS and GD-OES. *Mater Trans* 2007; 48: 287-292.
- 17) Tanaka Y, Saito H, Tsutsumi Y, Doi H, Imai H, Hanawa T. Active hydroxyl groups on surface oxide film of titanium, 316L stainless steel, and cobalt-chromium-molybdenum alloy and its effect on the immobilization of poly(ethylene glycol). *Mater Trans* 2008; 49: 805-811.
- 18) Tanaka Y, Matsuo Y, Komiya T, Tsutsumi Y, Doi H, Yoneyama T, Hanawa T. Characterization of the spatial immobilization manner of poly(ethylene glycol) to a titanium surface with immersion and electrodeposition and its effects on platelet adhesion. *J Biomed Mater Res* 2010; 92A: 350-358.
- 19) Tanaka Y, Matin K, Gyo M, Okada A, Tsutsumi Y, Doi H, Nomura N, Tagami J, Hanawa T. Effects of electrodeposited poly(ethylene glycol) on biofilm adherence to titanium. *J Biomed Mater Res* 2010; 95A: 1105-1113.
- 20) Kwabe A, Nakagawa I, Kanno Z, Tsutsumi Y, Hanawa T, Ono T. Evaluation of biofilm formation in the presence of saliva on poly(ethylene glycol)-deposited titanium. *Dent Mater J* 2014; 33: 638-647.
- 21) Chen P, Aso T, Sasaki R, Tsutsumi Y, Ashida M, Doi H, Hanawa T. Micron/submicron hybrid topography of titanium surfaces influences adhesion and differentiation behaviors of the mesenchymal stem cells. *J Biomed Nanotechnol* 2017; 13: 324-336.