An overview of biofunctionalization of metals in Japan

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Surface modification is an important and predominant technique for obtaining biofunction and biocompatibility in metals for biomedical use. The surface modification technique is a process that changes the surface composition, structure and morphology of a material, leaving the bulk mechanical properties intact. A tremendous number of surface modification techniques using dry and wet processes to improve the hard tissue compatibility of titanium have been developed. Some are now commercially available. Most of these processes have been developed by Japanese institutions since the 1990s. A second approach is the immobilization of biofunctional molecules to the metal surface to control the adsorption of proteins and adhesion of cells, platelets and bacteria. The immobilization of poly(ethylene glycol) to a metal surface with electrodeposition and its effect on biofunction are reviewed. The creation of a metal–polymer composite is another way to obtain metal-based biofunctional materials. The relationship between the shear bonding strength and the chemical structure at the bonding interface of a Ti-segmentated polyurethane composite through a silane coupling agent is explained.

Keywords: metal; biofunction; surface modification

1. INTRODUCTION

The technological evolution of ceramics and polymers during the last three decades makes it possible to apply these materials to medical devices; in fact, many metal devices have been replaced with those made of ceramics and polymers. In spite of this event, over 70 per cent of implant devices still consist of metals because of their high strength, toughness and durability. At present, it is difficult to replace the metals in medical devices with ceramics or polymers.

A disadvantage of using metals as biomaterials is that they are typically artificial materials and have no biofunction. To add biofunction to metals, surface modification is necessary because biofunction cannot be added during manufacturing processes such as melting, casting, forging and heat treatment. Surface modification is a process that changes a material’s surface composition, structure and morphology, leaving the bulk mechanical properties intact. With surface modification, the tissue compatibility of the surface layer can be improved. Dry processes (using ion beams) and wet processes (performed in aqueous solutions) are conventional and predominant surface modification techniques used to accelerate bone formation.

On the other hand, immobilization of biofunctional molecules to metal surfaces is effective. Stents are placed in stenotic blood vessels for dilatation, and blood compatibility or prevention of adhesion of platelets is necessary. In addition, lubrication of the blood vessels is necessary for proper sliding and insertion of guide wires and catheters. When metals are used as sensing devices, cell adhesion must be controlled. The major cause of the retrieval of implants is infection owing to biofilm formation. What is required is a biofilm-inhibiting surface, the fundamental property of which is to control the adsorption of proteins, cells, platelets and bacteria. This functional surface may be created by the immobilization of biofunctional molecules.

Polymers are widely used as biomaterials because of their high degree of flexibility and biofunction, but their structures give them insufficient strength and durability for some purposes. If a polymer and a metal could be bonded, a new composite material with good biofunction and high mechanical strength could be created.

In this paper, research on the above three approaches in Japan is reviewed, and work on the biofunctionalization of metals through various techniques is introduced.
2. HARD TISSUE COMPATIBILITY

2.1. Surface treatment for bone formation

Titanium (Ti) and its alloys, which show good hard tissue compatibility, are used for dental implants and artificial hip joints. However, the hard tissue compatibility of these materials is poorer than that of bioactive ceramics such as hydroxyapatite and bioactive glasses. Therefore, a tremendous number of surface modification techniques to improve the hard tissue compatibility of Ti have been developed, as shown in figure 1, and some have been commercialized. In figure 1, surface treatment techniques are categorized according to dry or wet processes. One of the several purposes of surface treatment is to improve hard tissue compatibility (bone formation). Other categories are feasible for hard tissue compatibility. The research to improve hard tissue compatibility involves two approaches based on the resultant surface layer: a calcium phosphate layer with the thickness measured in micrometres and a surface-modified layer with the thickness measured in nanometres. Most of these processes have been developed in Japan since the 1990s. Figure 2 shows the history of the surface treatment technique to improve hard tissue compatibility.

2.2. Hydroxyapatite coating

Currently, plasma spraying ofapatite on metallic materials is widely used to form the apatite layer. In the case of plasma-sprayed apatite, however, the apatite–Ti interface or the apatite itself may fracture under relatively low stress because of low interface bonding strength and low toughness of the sprayed layer. To overcome this weakness, dynamic ion mixing is applied to form an apatite with high interface bonding strength.

Calcium ions are implanted during the mixing process to induce strong bonding between the apatite film and the Ti substrate, with implanted calcium ions serving as binders (Yoshinari et al. 1994). Hydroxyapatite and calcium phosphate coatings with RF magnetron sputtering have been applied (Yoshinari et al. 1997, 1998; Ozeki et al. 2002). Electrochemical treatment (Ban et al. 1997; Ban & Maruno 1998) is used commonly to form an apatite layer on Ti. Through an electrochemical process, carbonate-containing apatite with a desirable morphology, such as plate, needle and particle, could be precipitated on a Ti substrate, which is sometimes heated to obtain a better coating layer (Kuroda et al. 2008). Low-voltage alternating current is also effective to precipitate calcium phosphate on Ti (Tanaka et al. 2007c), as shown in figure 3. This technique is useful for the treatment of thin wire and fibre without the dissolution of Ti.

2.3. Surface modification without calcium phosphate

Hard tissue compatibility can be improved by modifying the Ti surface instead of the apatite coating. Many surface modification techniques with neither a hydroxyapatite coating nor a calcium phosphate coating have been developed.
When calcium ions were implanted into Ti, calcium phosphate precipitation in an electrolyte was speeded up, and new osteoid tissue was formed earlier—as early as 2 days after implantation into rat tibia—on calcium-ion-implanted Ti than on unimplanted Ti (figure 4; Hanawa et al. 1997a; Ichikawa et al. 2000). This superiority of calcium-ion-implanted Ti was due to a modification of the surface by calcium-ion implantation. The modified surface of calcium-ion-implanted Ti contained Ti oxide containing calcium in the chemical state of calcium titanate (Hanawa et al. 1993). A calcium titanate coating to control thickness and crystallinity was also effective for bone formation (Ohtsu et al. 2007).

When Ti is immersed in NaOH or KOH alkaline solution, a hydrated Ti oxide gel containing alkaline ions (gel-like Ti oxide containing hydroxyl groups) 1 μm in thickness is formed on the Ti substrate (Kim et al. 1996). Upon heating, the gel layer condenses, and the gel bonds to the substrate strongly. When Ti with the gel is immersed in simulated body fluid, alkaline ions are released from the layer to the solution. Simultaneously, hydronium ions are soaked up by the layer, eventually forming a titania hydrogel, which increases the magnitude of the supersaturation of hydroxyapatite in the solution near the surface. The gel induces apatite nucleation, and the apatite layer is rapidly formed. This process is already commercialized for the stem of an artificial hip joint.

Immersion of Ti in TaCl₅-containing H₂O₂ accelerates apatite formation in simulated body fluid (Ohtsuki et al. 1997). A pull-out test of specimens from rat tibia revealed increased bonding strength of Ti to the bone.
A titanium oxide layer, which contains calcium hydroxide, is formed whenever Ti is immersed in calcium-containing solutions. The oxide layer catalyses the precipitation of calcium phosphate on Ti when immersed in Hanks’ solution (Hanawa et al. 1997b). The most effective means to precipitate apatite is immersion in an alkaline solution. While in identical calcium-containing solutions, hydrothermal modification of Ti was performed using an autoclave (Hamada et al. 2002). Apatite precipitation in Hanks’ solution was greatest on Ti modified in the calcium hydroxide solution. Surface modification of Ti in calcium hydroxide was more effective with an increase in temperature or pressure. Even regeneration of the surface oxide film on Ti in Hanks’ solution, i.e. repassivation, is effective to form calcium phosphate (Hanawa et al. 2002a,b).

2.4. Inhibition of assimilation

When Ti alloys are used for bone fixators, such as bone screws and bone nails implanted in bone marrow, Ti alloys form calluses on themselves and sometimes assimilate with bone. Therefore, bone may be refractured when the fixators are retrieved after bone healing because Ti easily forms calcium phosphate on itself (Hanawa & Ota 1991; Hiromoto et al. 2004). Stainless steel (SS) is used for complete retrieval after healing. Therefore, surface treatments that do not cause callus formation are necessary for the safe use of Ti alloy devices. It has been reported that zirconium (Zr) forms zirconium phosphate but not calcium phosphate (Hanawa et al. 1992, 2002b).

The coating of Zr inhibits the formation of calcium phosphate on Ti (Kobayashi et al. 2007). Figure 5 shows the relative concentration of elements detected from Zr-coated Ti, Zr and Ti. Calcium was not detected from Zr-coated Ti and Zr. According to the binding energies of the Ca 2p3/2 and P 2p electrons, Zr-coated Ti and Zr surfaces precipitate zirconium phosphate but not calcium phosphate. Therefore, Zr coating is a useful technique to inhibit the assimilation of Ti alloys with bone. Another approach is the development of a Zr-based alloy, which is being actively studied in Japan (Kobayashi et al. 1995; Ikarashi et al. 2005).

3. IMMobilization of functional molecules

3.1. Immobilization of poly(ethylene glycol)

The immobilization of biofunctional molecules on noble metals, such as gold, is usually conducted by using the bonding −SH or −SS− group. The adhesion of platelets and the adsorption of proteins, peptides, antibodies and DNA are controlled by modifications of the above technique. However, this technique can be used only for noble metals. On the other hand, 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 in the biofunctionalization of the metal surface. While many effective but complicated PEG immobilization techniques have been studied, few promising techniques for the immobilization of PEG to a metal surface have been developed.

3.2. Electrodeposition of PEG

Both terminals of PEG (MW: 1000) were terminated with −NH₂ (B-PEG), and only one terminal was
terminated with –NH₂ (O-PEG) (Tanaka et al. 2007a).

The chemical structures of the PEGs are shown in figure 6. These terminated PEGs were dissolved in a NaCl solution, which was used as an electrolyte for electrodeposition at 310 K. The cathodic potential was charged to Ti from the open circuit potential to \(-0.5 \text{ V} \) versus a saturated calomel electrode and was maintained at this potential for 300 s. During charging, the terminated PEGs electrically migrated to and deposited on the Ti cathode, as shown in figure 6. Figure 7 shows the thicknesses of the PEG deposition layers determined by ellipsometry. The thicknesses of the deposition layers, i.e. the amounts of deposited PEG, are listed in descending order: immersion of B-PEG for 24 hours; electrodeposition of B-PEG for 300 s; electrodeposition of O-PEG for 300 s; and immersion of B-PEG for 2 hours. More PEGs were immobilized for 300 s with electrodeposition than for 2 hours with immersion, as shown in figure 7, indicating that electrodeposition is more effective than immersion for the deposition of PEG on the Ti surface.

The bonding manner of PEG to a Ti surface is much more significant in the design of PEG-immobilized materials than are characterization techniques to determine the manner of PEG immobilization. The manner of PEG immobilization was characterized using X-ray photoelectron spectroscopy (XPS) with an angle-resolved technique and glow discharge optical emission spectroscopy (GD-OES). Not only electrodeposition but also immersion led to the immobilization of PEG onto a Ti surface. However, more terminated amines combined with Ti oxide as ionic NH–O by electrodeposition, while more amines randomly existed as NH\(\text{C}_3\) in the PEG molecule by immersion. Moreover, the differences in amine termination led to different bonding manners: U-shaped in B-PEG and brush-shaped in O-PEG (Tanaka et al. 2007b; figure 8).

The concentrations of hydroxyl groups located inside and on the surface oxide films of Ti, a type 316L austenitic SS and a cobalt–chromium–molybdenum alloy (Co–Cr–Mo) were evaluated using XPS and a zinc-complex substitution technique. As a result, the concentration of the active hydroxyl groups on Co–Cr–Mo was significantly larger than those on Ti.
and SS: the largest amount of PEG was immobilized onto the Co–Cr–Mo alloy. The amounts of the PEG layer immobilized onto the metals were governed by the concentrations of the active hydroxyl groups on each surface oxide in the case of electrodeposition, which was governed by the relative permittivity of the surface oxide in the case of immersion (Tanaka et al. 2008).

The PEG-immobilized surface inhibited the adsorption of proteins and cells, as well as the adhesion of platelets (figure 9) and bacteria (figure 10), indicating that this electrodeposition technique is useful for the universal biofunctionalization of metal surfaces. It is also useful for all electroconductive and morphological materials. In addition, biomolecules such as collagen can be easily electrodeposited onto metal surfaces with electrodeposition because biomolecules contain many electrical charges.

3.3. Immobilization of functional peptide through PEG

Peptides containing Arg–Gly–Asp (RGD) accelerate cell attachment and extension of bone cells on Ti (Rezania et al. 1997). RGD is a peptide known to involve cell adhesion, which is involved in many extracellular matrix proteins (Pierschbacher & Ruoslahti 1984). Bone formation is accelerated by immobilizing RGD on a Ti surface (Schliephake et al. 2002). To immobilize RGD to the electrodeposited PEG on Ti, PEG with an –NH₂ group and a –COOH group (NH₂–PEG–COOH) must be employed. One terminal group, –NH₂, is required to bind stably with a surface oxide on a metal. The other terminal group, –COOH, is useful to bond biofunctional molecules such as RGD. NH₂–PEG–COOH works as a binder of RGD to metal surfaces.
surfaces and molecular structures, except terminals that are hydrophobic and inhibit the adsorption of proteins. RGD probably works as a bone formation site.

4. METAL–POLYMER COMPOSITE

The unequivocal relationship between the shear bonding strength and the chemical structure at the bonding interface of a Ti-segmented polyurethane (SPU) composite through a silane coupling agent (γ-mercaptopropyltrimethoxysilane (γ-MPS)) was investigated (Sakamoto et al. 2007). GD-OES analysis showed that the intensity of sulphur in the γ-MPS layer increased with the number of γ-MPS molecular units and the thickness of the γ-MPS layer. The shear bonding stress of the Ti/γ-MPS/SPU interface increased with the increase in the concentration of the γ-MPS solution only in the case of 1 min immersion. On the other hand, the shear bonding stress of the Ti/γ-MPS/SPU interface formed from 1.0 to 2.0 per cent γ-MPS solutions significantly increased with immersion times. The Ti-SPU composite was fractured, leaving the SPU component elements on the fractured surface, as determined by XPS. However, more residual SPU existed on the fractured surface of the Ti-SPU composite with a γ-MPS layer than on that without a γ-MPS layer. The SPU elements remained on the fractured surface as a result of the presence of the γ-MPS layer. The thicker the γ-MPS layer the larger the SPU area fraction on the fractured surface (figure 11).

On the other hand, the shear bond strength of the Ti/SPU interface increased with ultraviolet (UV) irradiation according to the increase of the cross-linkage in SPU. UV irradiation of a Ti–SPU composite is clearly a factor governing the shear bond strength (Sakamoto et al. 2008a). In addition, active hydroxyl groups on the surface oxide film are clearly factors governing the shear bond strength (Sakamoto et al. 2008b).

Platelet adhesion to Ti is inhibited by SPU, as shown in figure 12. This technique is used for the creation of a new metal-based material having high strength, high toughness and biofunction. Whenever good bonding is produced between metal and polymer, we could apply biofunctionalization techniques developed in the field of polymers to the composite materials.
5. CONCLUSIONS

Metallic materials are widely used in medicine not only for orthopaedic implants but also for cardiovascular devices and other purposes. The metal surface may be biofunctionalized by various techniques, such as dry and wet processes, the immobilization of biofunctional molecules and the creation of metal–polymer composites. In particular, the electrodeposition technique is useful for all electroconductive and morphological materials. These techniques make it possible to apply metals to scaffolds in tissue engineering.

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REFERENCES


Pierschbacher, M. D. & Ruoslahti, E. 1984 Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. Nature 309, 30–33. (doi:10.1038/309030a0)


