Bio-Functionalization of Titanium Surfaces for Dental Implants

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Since dental implants are used in contact with many different tissues, it is necessary to have optimum surface compatibility with the host bone tissues and soft tissues. Furthermore, dental implant surfaces exposed to the oral cavity must remain plaque-free. Such materials can be created under well-controlled conditions by modifying the surfaces of metals that contact those tissues. “Tissue-compatible implants,” which are compatible with all host tissues, must integrate with bone tissue, easily form hemidesmosomes, and prevent bacterial adhesion. This research was aimed at developing such tissue-compatible implants by modifying titanium surfaces using a dry process for closely adhering to the titanium substrate and ensuring good wear resistance. The process includes ion beam dynamic mixing (thin calcium phosphates), ion implantation, titania spraying, ion plating and ion beam mixing. At the bone tissue/implant interface, a thin calcium phosphate coating and rapid heating with infrared radiation was effective in controlling the dissolution without cracking the coating. This thin calcium phosphate coating may directly promote osteogenisis, but also enable immobilization of functional proteins or drugs such as bisphosphonate for drug delivery system. At the oral fluid/implant interface, an alumina coating and F⁺-implantation were responsible for inhibiting the adhesion of microbial plaque. In conclusion, dry-process surface modification is useful in controlling the physicochemical nature of surfaces, including the surface energy and the surface electrical charge, and in developing tissue-compatible implants.

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1. Introduction

Dental implants have no periodontal ligament and junctional epithelium, in contrast to the natural teeth. In addition, dental implants are partially exposed to the oral cavity (Fig. 1). Therefore, the tissues surrounding dental implants are prone to bacterial infection and invasion of the epithelia, thus, dental implants run the risk of being expelled from the host body. In addition, dental implants undergo occlusal stresses and other forms of mechanical stress such as wear. This review will focus on the surface modification of titanium for developing “tissue-compatible implants,” which are compatible with all the surrounding host tissues including in host bone tissues, soft tissues and surfaces exposed to the oral cavity.

2. Control of Surface Topography and Surface Chemistry

The vital reaction of biomaterials is affected by the surface topography and surface chemistry of the materials. Surface topography has marked effects on cell behavior. Generally, cell adhesion is greater on rough surfaces than on smooth surfaces, but the actual rate of adhesion depends on the type of cell. Contact guidance, the phenomenon in which cells align along grooves of the substrate, is an example of surface topography controlling cell behavior.¹,² Surface roughness alters osteoblast proliferation, differentiation, and matrix production in vitro, and plays a role in determining the phenotypic expression of cells in vivo.³,⁴ Surface chemistry involves the adsorption of proteins, bacteria, and cells on biomaterials. This adsorption reflects the affinity between two substances, and the strength of adsorption follows the order: chemical adsorption including covalent bonds and ionic bonds > electrostatic force found in electrokinetic potential, or zeta potential > hydrogen bonds involved in hydrophilic groups such as −OH, −COOH, and −NH₂ > hydrophobic interaction (i.e., adsorption of hydrophobic substances in water) > van der Waals forces. Adsorption characteristics are primarily evaluated by hydrophobicity (wettability), which can be determined by measuring the surface energy (contact angle), and electrokinetic potential (zeta potential, isoelectric point), which reflects surface electric charges (Fig. 2).

3. Surface Modification of Titanium for Developing “Tissue-compatible Implants”

Tissue-compatible implants must integrate with bone tissues (bone tissue/implant interface), strongly adhere to subepithelial connective tissues, easily form hemidesmosomes (soft tissue/implant interface), and remain plaque-free on the surfaces exposed to the oral cavity (oral fluid/implant interface) (Fig. 1). This modified layer should closely adhere to the metal and have good wear resistance. The methods of surface modification of titanium implant materials related to the surface topography and chemistry of different host tissues are described as follows.

3.1 Bone tissue/Implant interface

The integration of bone tissue with an implant requires a rough surface topography that maximizes the area in contact with the bone and allows the contact guidance for the development of osteoblasts. As for surface chemistry, surface modification with a thin calcium phosphate coating is useful in pro-
Fig. 1 Structural differences between (a) natural teeth and (b) dental implants, and (c) concept of tissue-compatible dental implants.

Fig. 2 Electrokinetic potential (zeta potential).

Bio-functionalization of titanium surfaces for dental implants is a strategy to enhance osseointegration and avoid tissue rejection and infection. 

Titanium is known to have a greater ability than other metals to facilitate osseointegration, which is defined as a close contact between bone tissue and implant material such that there is no progressive relative motion of living bone and implant under functional levels and loading for the life of the patient.

**3.1.1 Osseointegration of titanium**

Titanium is known to have a greater ability than other metals to facilitate osseointegration, which is defined as a close contact between bone tissue and implant material such that there is no progressive relative motion of living bone and implant under functional levels and loading for the life of the patient.

Generation of the titanium oxide film on the surface of titanium is one reason for this ability and its high level of corrosion resistance. In addition, the degree of the deposition of calcium phosphates in body fluid is greater on titanium than on other metals. Presently, adsorption of osteogenic proteins such as osteocalcin (Oc) and osteopontin (Op) to the titanium surface is a main function of the osseointegration of titanium.

There are two mechanisms involved in the adsorption of osteogenic proteins, as shown in Fig. 3. Titanium oxide has a similar number of isoelectric points (pl) at approximately pH = 5 as those of pH = 4.7–4.9 on osteogenic proteins. Accordingly, at around pH 7, both titanium oxide and osteogenic proteins are negatively charged. The figure on the left shows the positively charged divalent ion (such as Ca^{2+} ion and/or Mg^{2+} ion) mediated mechanism. The hydration effect of terminal OH radicals (dissociation constant, pK = 12.7, basic), which are positively charged, is also considered as playing a role in protein adsorption, as shown in the figure on the right.

Even when light microscopy confirms osseointegration of titanium implants, examination by electron microscope reveals that the bone and the implant are not crystallographically continuous. Albrektsson and Jacobsson observed thin amorphous structures between the bone and the titanium implant. Recently, in an ultrastructural study, Ayukawa et al. found a thin amorphous zone at the osseointegrated layer between the titanium and bone interface. Based on this investigation, it can be speculated on the process occurring in the implant hosts (Fig. 4). In the early stage of implantation, at first, osteogenic proteins, Oc and Op, are adsorbed on the titanium surface through Ca^{2+} or OH radicals as mentioned above. These osteogenic proteins cause the osteoblasts to migrate to the titanium. There, the osteoblasts secrete Oc and Op to the opposite side while simultaneously collecting collagen and hydroxyapatite, and stimulating bone formation. At the osseointegration stage, these proteins turn into noncollagenous protein such as proteoglycan. Thus, amorphous structures are formed at the interface between the titanium and the bone, that is, there is no direct contact between titanium and bone.

Dissolution of different metal ions from Ti and Ti alloy (Ti–6Al–4V) implants has been reported. The presence of the amorphous structures, as described above, introduces the risk of large gap formations and a loss of osseointegration under unfavorable conditions such as infection and overloading of
the implants. In addition, there have been a number of reports showing that the rate of osteogenesis in Ti and Ti alloy implants is lower than that in calcium phosphate implant materials. These phenomena all suggest the surface modification of titanium.

3.1.2 Surface modifications of titanium with a thin calcium phosphate (Ca–P) coating

(1) Calcium phosphate (Ca–P) coating by plasma spraying

Ca–P implants, including hydroxyapatite (HA), are well known for good osteoconductivity (the early stage of osteogenesis) as well as for direct binding to bone tissue in vivo. Alkaline phosphatase expression and parathyroid hormone response were higher in cultures grown in HA than in cultures grown in titanium, and the in vitro formation of extracellular matrices was greater on Ca–P coatings than on titanium. Several mechanisms of the principal factors involved in osteogenesis on Ca–P ceramics have been considered. First, implanted Ca–P (HA) acts as a nucleation site and exhibits crystallographic properties in an epitaxial process of the newly developed structure. The calcium ions dissolve from the Ca–P surface, resulting in the deposition of a mineralized layer. This stimulates the bone cells to continue extracellular matrix (bonding zone) synthesis and calcification. Ca–P ceramics adsorb many osteo-conductive and/or osteo-inductive proteins, which have an important role in the mineralization of bone tissues.

In spite of their rapid and strong bonds to living bone tissues and favorable osteogenic ability, Ca–P ceramics alone cannot be used for implants because of their lack of strength. Accordingly, Ca–P coatings on Ti implants produced by the plasma spraying have frequently been used. These Ca–P coated implants, however, often develop fractures in their coatings as well as at the titanium interface after implantation. The reason for this is thought to originate in the comparatively thick, porous, nonuniform (crystalline surrounded by an amorphous mass), and poorly adherent Ca–P layer produced by plasma spraying. These fragments of a certain size cause phagocytosis by macrophages, leading to inflammation. It is therefore desirable for the materials to be rapidly and completely absorbed in the host tissues and to be entirely replaced with bone tissue. When osteogenesis occurs at the site where old bones are absorbed (remodeling of bones), the Ca–P coatings should be no thicker than necessary.

(2) Employing a dry process to produce thin Ca–P coatings

For composite materials that combine brittle ceramics and metals, it is of cardinal importance to minimize the thickness of the ceramic layer. In bending tests, the radius of maximal flexion without breakage of the ceramics can be obtained with a thin ceramic layer of not more than 10 μm (Table 1). The beneficial properties of the metals may be optimized by reducing the thickness the ceramic layer as long as there are few defects in the ceramics and superior adhesion between
Table 1  Radius of maximal flexion without breakage of ceramics at three point bending test.

<table>
<thead>
<tr>
<th></th>
<th>Proof stress (MPa)</th>
<th>Elastic modulus (GP)</th>
<th>Radius of maximal flexion (mm)</th>
<th>Thickness of film (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti (grade 2)</td>
<td>350</td>
<td>100</td>
<td>0.53</td>
<td>—</td>
</tr>
<tr>
<td>Alumina</td>
<td>500–1000</td>
<td>350–400</td>
<td>0.19–0.38</td>
<td>19–38</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>100–200</td>
<td>35–120</td>
<td>0.13–0.25</td>
<td>13–25</td>
</tr>
</tbody>
</table>

Fig. 5 Apparatus of ion beam dynamic mixing (IBDM), and cross-section of Ca–P coatings produced by IBDM (lower: enlargement of upper picture).

Accordingly, attempts have recently been made to solve problems in the plasma spraying technique using the dry process. In the dry process, ion-plating and the ion sputtering, which are a kind of physical vapor deposition (PVD), are used to produce implant materials consisting of a thin, homogeneous, and adherent Ca–P coating. Ion beam dynamic mixing (IBDM) was also introduced as a suitable technique for fabricating a thin and adherent ceramic layer (Fig. 5). This method is a combination of ion implantation and PVD and has the advantages of a high deposition rate, producing defect-free transparent thin films, and excellent adhesion compared to conventional thin-film deposition techniques. Figure 6 shows a commercially available Brånemark implant and a Ca–P coated implant. The slight color change is recognized in the coated implant caused by the thin (1 µm) and defect-free coating. Good degrees of osteogenesis and bond strength with bone are obtained in the thin Ca–P coated implants.

(3) Heat treatment of thin Ca–P coatings

The deposited coatings produced by the dry process are amorphous, resulting in films that easily dissolve in simulated body fluids and that crystallize during heat treatment. However, the film coatings tend to crack easily with conventional heat treatment in an electric furnace because of the debonding of the coatings. Rapid, homogeneous, and comparatively low-temperature heating at around 600°C, such as defocused infrared radiation, controls Ca–P solubility and ensures the adherence of the coatings for both ion-sputtered coatings and IBDM coatings (Fig. 7).
uniform temp. area: $\phi \: 30 \text{mm}$

Fig. 7 Rapid heating with infrared radiation (left), and SEM of coatings after immersion in simulated body fluid for 35 d (right 1: rapid heated at $600 ^\circ \text{C}$, time until $600 ^\circ \text{C}$ is 13 s, right 2: furnace heated at $500 ^\circ \text{C}$ for 1 h).

Uniform temp. area: $\phi \: 30 \text{mm}$

Fig. 8 XPS depth profiles of Ti substrates, in which Ca–P coatings were removed by epoxy glue.

XPS depth profiles of Ti substrates, in which Ca–P coatings were removed by epoxy glue revealed that the intensities of $P^{3-}$ state of the furnace heated and rapid heated at $800 ^\circ \text{C}$ specimens were larger than those of the as coated and rapid heated at $600 ^\circ \text{C}$, the intensities of Ca2p spectrum were decreased in the furnace heated and rapid heated at 800°C specimens. In addition, there was no remarkable difference in the Ti-oxidation state among the specimens (Fig. 8). From these results, too much growth of the Ti–P compounds and the decrease in the thickness of Ca-implanted layers were considered to be a major reason for causing the cracks. Titanium oxide is considered to have no dominant role in the bonding between thin Ca–P coatings and Ti substrates. (Fig. 9).

(4) Immobilization of bisphosphonates

Bisphosphonates are a new class of drugs that have been developed for the treatment of osteoporosis by inhibiting the activity of osteoclasts. For dental implants, the use of bisphosphonates is expected to promote osteogenesis at the bone-implant interface. We found that the bisphosphonates were able to immobilize to titanium through the thin calcium phosphate coatings (Fig. 10). The reason for this was considered due to the marked affinity of the bisphosphonates to the calcium phosphates. The alkaline phosphatase expression activity of osteoblastic cells cultured on plates immo-
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Fig. 11 Light and a fluorescent micrograph of bisphosphonate-coated Ti implant/bone interface 12 weeks after implantation in beagle dog.

Fig. 12 Multi-grooves, a combination of micro-grooves and macro-grooves, to control the orientation of both cells and extracellular matrix (ECM) such as collagen.

Fig. 13 X-ray photograph around peri-implantitis (black arrows).

Fig. 12 Multi-grooves, a combination of micro-grooves and macro-grooves, to control the orientation of both cells and extracellular matrix (ECM) such as collagen.

3.2 Soft tissue/Implant interface

Dental implants lack the structures that maintain the continuity between the epithelium and connective tissues that are normally formed by hemidesmosomes and the basal lamina, which connect dental enamel and adhesive epithelium. Peri-implant epithelium has a lower capacity to act as a proliferative defense mechanism than does junctional epithelium. Therefore, to prevent the invasion of the bacteria and epithelium, a system of biological sealing is required. We found that the extension and spread of fibroblasts and epithelial cells were critically influenced by the pore diameter of 1.2–3.0 µm in Millipore filters. Our observation of in vitro experiments also suggests that a range in hole size of 50 to 100 µm is most critical for the connective tissue cells to migrate and orient at right angles to the implant surface, similar to Sharpey’s fibers. Multi-grooves, a combination of microgrooves and macro-grooves, are also expected to be able to control the orientation of both cells and extracellular matrix (ECM) such as collagen (Fig. 12). These surface topographies may help in providing a biological seal around the implant.

As for the surface chemistry, methods of modifying the titanium surface using adhesive proteins such as osteonectin, fibronectin or laminin compatible with the soft tissue/implant interface have been proposed. For the implant surface in contact with subepithelial connective tissues, glutaraldehyde is used to fix the selected proteins to the amino residues brought to the surface of the alloys during silane-coupling treatment or carbodiimide treatment. The gingival epithelium attached to dental implants through the formation of hemidesmosomes using laminin. Tresyl chloride (2,2,2-trifluoroethanesulfonyl chloride) treatment on titanium surface is also useful for the development of tight connection of dental implant to subepithelial connective tissues and/or peri-implant epithelium through the basic terminal OH groups. A stable coating and prevention of protein denaturation at the time of implantation are necessary.

3.3 Oral fluid/Implant interface

Microbial plaque accumulation surrounding dental implants may develop into peri-implantitis, which is defined as inflammation or infection around an implant, with accompanying bone loss (Fig. 13). Plaque accumulations are observed surrounding titanium implants, and many kinds of bacteria, which were confirmed to be the same as periodontopathic bacteria, are recognized in the plaque formation (Fig. 14).

It is therefore important to maintain the surface of dental implants exposed to the oral cavity (Oral fluid/Implant interface) free of plaque to prevent peri-implantitis. There are at least two methods of inhibiting the formation of microbial
Table 2 Surface modification of titanium with dry process

<table>
<thead>
<tr>
<th>Modification/Treatment</th>
<th>Characterization</th>
<th>Knoop hardness</th>
<th>Thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti-polished</td>
<td>TiO₂</td>
<td>148 (5)</td>
<td>&lt;30 nm</td>
</tr>
<tr>
<td>HA-coated</td>
<td>Ion beam dynamic mixing</td>
<td>Hydroxyapatite</td>
<td>—</td>
</tr>
<tr>
<td>Ca²⁺-implanted</td>
<td>Ion implantation</td>
<td>CaTiO₃, TiO₂, TiO</td>
<td>264 (17)</td>
</tr>
<tr>
<td>N⁺-implanted</td>
<td>Ion implantation</td>
<td>TiN, Ti₂N, TiO₂</td>
<td>232 (14)</td>
</tr>
<tr>
<td>F⁺-implanted</td>
<td>Ion implantation</td>
<td>TiF₃, TiOF, TiOₓ</td>
<td>230 (19)</td>
</tr>
<tr>
<td>Titania-coated</td>
<td>Flame spraying</td>
<td>TiO₂ (rutile, anatase)</td>
<td>227 (25)</td>
</tr>
<tr>
<td>TiN-coated</td>
<td>Ion plating</td>
<td>TiN</td>
<td>1837 (227)</td>
</tr>
<tr>
<td>Alumina-coated</td>
<td>Ion plating</td>
<td>Al₂O₃ (corundum)</td>
<td>1355 (66)</td>
</tr>
<tr>
<td>Ag-IBM</td>
<td>Ion beam mixing</td>
<td>Ag, TiO₂, TiOₓ</td>
<td>250 (8)</td>
</tr>
<tr>
<td>Sn-IBM</td>
<td>Ion beam mixing</td>
<td>Sn, TiO₂ TiOₓ</td>
<td>212 (6)</td>
</tr>
<tr>
<td>Zn-IBM</td>
<td>Ion beam mixing</td>
<td>Zn, TiO₂ TiOₓ</td>
<td>213 (13)</td>
</tr>
<tr>
<td>Pt-IBM</td>
<td>Ion beam mixing</td>
<td>Pt, TiO₂</td>
<td>225 (19)</td>
</tr>
</tbody>
</table>

Fig. 14 Microbial plaque accumulation surrounding the abutment of dental implants.

plaque. The first is to inhibit the initial adhesion of oral bacteria. The second is to inhibit the colonization of oral bacteria, which involves surface antibacterial activity. The adhesion of bacteria is greatly influenced by electric charges on the implant surface because bacteria have a large specific surface area. Antibacterial modification can be effective for the implant surface. Another requirement for the modified surfaces is their resistance to wear when the teeth are brushed.

3.3.1 Initial adhesion of oral bacteria

The initial adherence of oral bacteria on cp-titanium and titanium surfaces modified with a dry process was investigated.²⁹ Surface modifications were conducted with dry processes that included ion implantation (Ca⁺, N⁺, F⁺), oxidation (titania spraying), ion plating (TiN, alumina), and ion beam mixing (Ag, Sn, Zn, Pt) with Ar⁺ on polished pure titanium plates. (Table 2).

The results showed that comparatively large amounts of P. gingivalis and A. actinomycetemcomitans, which are major periodontopathic bacteria, adhered to polished cp-titanium (Fig. 15). These findings indicate that there is a probable risk of bacterial adhesion to titanium surfaces at the supra- and sub-gingival portions of implants, and surface modification to inhibit the adherence of oral bacteria is required. The degree of P. gingivalis adhesion showed a positive correlation with surface energy and the amount of calcium-ion adsorption (Fig. 16).

The level of bacterial adhesion on calcium-implanted surfaces was greater than on polished titanium, despite similar degrees of surface roughness. The reason for this is believed to be that the Ca-rich surfaces on the calcium-implanted specimens promoted protein adsorption in saliva and, ultimately, bacterial adhesion. Accordingly, even though calcium-ion implantation is beneficial in bonding implants to bone tissue, this treatment carries with it the risk of promoting the adhesion of plaque on surfaces exposed to the oral cavity. In contrast, the level of initial adhesion of bacteria decreased on the
alumina-coated specimen (Fig. 15). This is related to the non-adsorption of calcium ions on alumina-coated specimens. In contrast to titanium oxide, the isoelectric point of $\alpha$-Al$_2$O$_3$ is 9.2 and that of $\gamma$-Al$_2$O$_3$ is 8.0, respectively. Therefore, the surface of the alumina-coated specimen is considered to be positively charged, and calcium ions were not adsorbed on the surface, resulting in a decrease in the initial levels of adhered $P. gingivalis$.

### 3.3.2 Antibacterial activity

Antibacterial activity was also investigated on the same specimens as the initial adhesion assay. $^{30}$F$^+$-implanted specimens significantly inhibited the growth of both $P. gingivalis$ and $A. actinomycetemcomitans$ (Fig. 17). Fluoride is widely used as a highly effective anticaries agent. The principal antibacterial mechanism considered was that a metal fluoride complex affects bacterial metabolism as an enzyme inhibitor. Incidentally, it was confirmed that F$^+$-implanted surfaces did not influence the proliferation of mouse-fibroblast cells. Titania-sprayed specimens generated no antimicrobial activity despite the anatase that formed on the surfaces. This may be because no UV light was used, and no coupling metals were used for stimulating photocatalytic reactions.

In conclusion, dry-process surface modification is useful in controlling the physicochemical nature of surfaces, including the surface energy and the surface electrical charge, and in developing tissue-compatible implants. Bio-functional implants that maintain homeostasis can be created under highly controlled conditions by modifying surfaces using dry process.

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### REFERENCES