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Review article

Biofunctionalization of titanium for dental implant

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Summary Surface modification is an important and predominant technique for obtaining biofunction in metals for biomedical use including dentistry. One 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 to improve the hard tissue compatibility of titanium have been developed. Hydroxyapatite layer, titanium oxide layer, and calcium titanate layer with various morphologies are deposited using electrochemical treatment including micro-arc oxidation. Also, surface modification layers without hydroxyapatite and calcium phosphate are chemically formed that accelerate bone formation. Other approach is the immobilization of biofunctional molecules such as poly(ethylene glycol) to the metal surface to control the adsorption of proteins and adhesion of cells, platelets, and bacteria. In the case of immobilization of biomolecules such as collagen and peptide, bone formation and soft tissue adhesion are improved.

Contents

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1. Introduction

Metals have a long history in the treatments of dentistry. However, metals are typically artificial materials and have no biofunction that leads to low attraction of metals as biomaterials. In this review, ''biofunction'' is defined not only as ''inhibition of the non-specific adsorption of protein and adhesion of cells'', but also as ''enhancement of them''. In addition, ''metal-free'' or ''de-metallic'' treatment is a trend in dentistry from the esthetic viewpoint. On the other hand, abrupt technological evolution on ceramics and polymers make it possible to apply these materials to medical devices the last three decades. In particular, excellent biofunctions of ceramics and polymers are expected to show excellent properties as biomaterials; in fact many devices consisting of metals have been substituted by those consisting of ceramics and polymers. In spite of this event, over 70% of implant devices in medical field including dentistry still consist of metals and this share is currently maintained, because of their high strength, toughness, and durability. Metallic biomaterials cannot be replaced with ceramics or polymers at present.

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. In addition, metals with biofunctions have been required in the recent past. In dentistry, dental implants require hard tissue compatibility for osseointegration and bone formation, soft tissue compatibility for adhesion of gingival epithelium, and antibacterial property for the inhibition of biofilm formation. These biofunctional properties consist of two conflicting properties: the inhibition and enhancement of protein adsorption or cell adhesion.

When a metallic material is implanted into a human body, immediate reaction occurs between its surface and the living tissues. In other words, immediate reaction at this initial stage straightaway determines and defines a metallic material's biofunction. With surface modification, biofunction of surface layer could be improved. For these purposes, many techniques for surface modification of metals are attempted on a research stage and some of them are commercialized. Reviews on surface modification of titanium (Ti) have already been published on sputter deposition [1] and electrochemical treatments [2]. In this review, surface modification techniques of Ti for dental implants are categorized and explained.

2. Surface modification techniques

In Table 1, surface modification techniques are categorized according to their processes and purposes. Major purpose of surface modification is to improve hard tissue compatibility or accelerate bone formation. Research to improve hard tissue compatibility involves two approaches based on the resultant surface layer: a calcium phosphate and titanium oxide layer with the thickness measured in micrometers and a surface-modified layer with the thickness measured in nanometers. Most of these processes have been developed since the 1990s. Fig. 1 shows the history of the surface treatment technique to improve hard tissue compatibility.

Surface property is particularly significant for biomaterials, and thus surface modification techniques are particularly useful to biomaterials. Dry process (using ion beam) and wet process (which is performed in aqueous solutions) are predominant surface modification techniques. In particular, electrochemical technique in the wet process is important near recently. Immobilization of bone formation factors such as bone morphological protein, BMP, or biomolecules such as collagen and peptide to metal surface is another technique to improve hard tissue compatibility. On the other hand, the immobilization of biofunctional molecules such as poly(ethylene glycol), PEG, to the metal surface to control the adsorp-

Table 1 Categorization of surface treatment techniques of metals for medical devices according to the process and purpose.

	Dry process	Electrochemical process Micro-arc oxidation	Chemical and hydrothermal process
Hydroxyapatite or calcium phosphate coating	Commercialized	Commercialized	Studied
TiO_2 or $CaTiO_3$ coating	Commercialized	Commercialized	—
Surface-modified layer formation[a]	—	—	Commercialized
Immobilization of functional molecules and biomolecules[b]	—	Studied	Studied

[a] Techniques forming a surface layer that enhances hard tissue compatibility, while the layer does not contain HA and calcium phosphate. See Section 5.
[b] Techniques immobilizing organic molecules including biomolecules to inhibit the adsorption of proteins or the adhesion of cells and to enhance them. See Section 7.

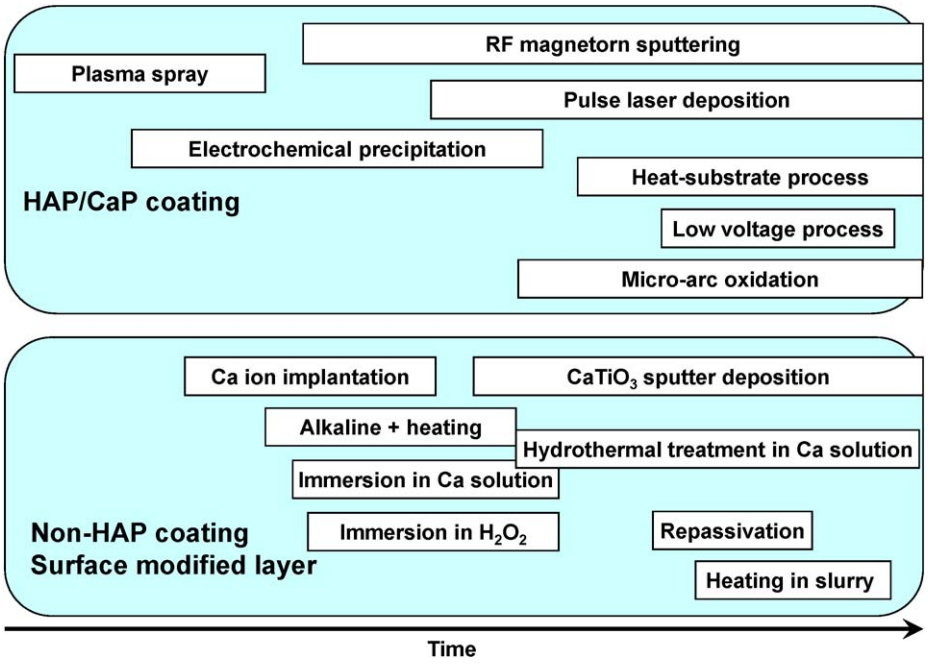

Figure 1 History of surface treatment technique to improve hard tissue compatibility. Approaches to improving hard tissue compatibility are categorized based on the resultant surface layer: calcium phosphate layer formation with thickness measured in micrometers and surface-modified layer formation with thickness measured in nanometers.

tion of proteins and adhesion of cells, platelets, and bacteria is attempted.

3. Dry process coating

3.1. Dry process

Most dry processes are performed using the ion beam. Ion beam technology is particularly useful in the engineering field, especially in silicon technology. Ion beam technology permits the formation of thin films at atomic and molecular levels, as well as low temperature syntheses utilizing ionic effects. The process is thermal-unequilibrium, thus making it possible to synthesize unnatural substances. Ion beam technology has contributed significantly to the modification of biomaterial surfaces. It can be classified according to the effects on solid surface: film formation, sputtering and ion implantation. When ion impacts a material surface, attaching, sputtering and implantation effects occur according to the ion's energy (Fig. 2). By utilizing these effects, thin film formation, graded-composition layer and unequilibrium layer are obtained.

3.2. Hydroxyapatite (HA) coating

Currently, plasma spraying of HA on metallic materials is widely used to form the HA layer—which is the nucleus for active bone formation and conductivity. In the case of plasma-sprayed HA, however, the HA—Ti interface or HA itself may fracture under relatively low stress because of low interface bonding strength and low toughness of the sprayed layer itself [3]. Solubility of ceramics increases as its crystallinity decreases. The crystallinity of a thin film formed with ion beam is low and the solubility is large. The crystallinity of coated HA is an important factor because crystallinity governs solubility in the human body. Low crystalline film on Ti dissolves rapidly when the Ti is implanted into a human body. Thus heat treatment of HA film is necessary to increase its crystallinity and reduce its solubility [3,4]. Calcium ions are implanted during the mixing process to induce strong bonding between the HA film and the Ti substrate, with implanted calcium ions serving as binders [5]. HA and calcium phosphate coatings with RF magnetron sputtering have been applied [6,7].

3.3. TiO_2 and $CaTiO_3$ coating

Nanoscale TiO_2 coating on Ti using RF magnetron sputtering does not show better osseointegration than untreated Ti

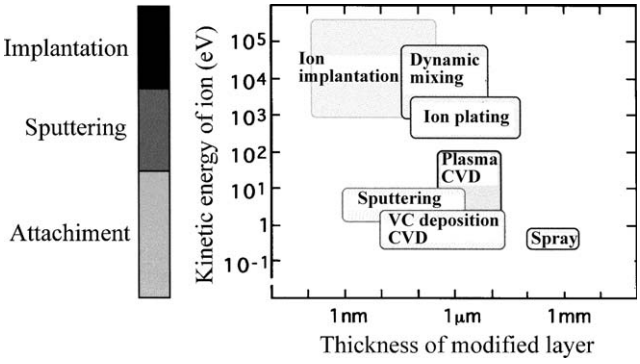

Figure 2 Effects of ion irradiation on solid surfaces due to ion energy, the ion beam technique according to the kinetic ion energy, and the resultant thickness of modified layer.

surface [8]. A $CaTiO_3$ coating controlling thickness and crystallinity is also effective for bone formation [9].

4. Electrochemical and chemical coating

4.1. HA coating

Electrochemical treatment is used commonly to form an HA layer on Ti [10,11]. Through an electrochemical process, carbonate-containing HA 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 [12,13]. Beta-TCP is cathodically coated on Ti for immobilization of collagen [14]. Low-voltage alternating current is also effective to precipitate calcium phosphate on Ti [15], as shown in Fig. 3. This technique is useful for the treatment of thin wire and fiber without the dissolution of Ti. HA is electrodeposited with pulse current [16].

Nano-grained calcium phosphate is electrochemically deposited on Ti using acidic electrolytes [17]. The coating layer contains dicalcium phosphate dihydrate (55—85 nm in grain size) with a small amount of HA (20—25 nm); the content of HA increases with the increase of the current density [18]. An electrochemical method of producing nanocrystalline HA coatings on Ti surface is reported [19,20]. Also, HA is coated by dynamic voltage during electrophoretic deposition [21].

4.2. TiO_2 coating

Anodic oxidation and oxide films of Ti in electrolytes of calcium glycerophosphate and calcium acetate is attempted [22]. Self-organized porous nanotubular TiO_2 is anodically formed on Ti in Na_2SO_4 electrolyte containing NaF. The oxidation carried out at 20 V with the baths stirred using magnetic pellet and ultrasonic vibration. (1 0 1) and (2 0 0) poles were randomly oriented [23]. TiO_2 nanotube-type oxide film on Ti substrate has been fabricated using an electrochemical method. The formation and growth of a self-organized nanotube layer can be achieved directly by anodization in NH_4-containing electrolytes. The diameter, length, and wall thickness of the nanotube are significantly influenced by anodizing condition such as voltage, current density, and anodizing time [24].

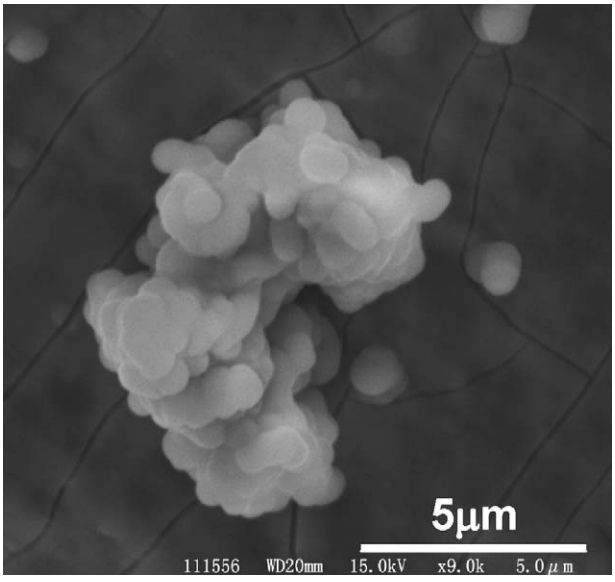

Figure 3 Scanning electron micrograph of calcium phosphate precipitated on a titanium woven texture after a low-voltage alternating current treatment and immersion in Hanks' solution: alternative current between −1 and 1 V with 1 Hz frequency, followed by immersion in Hanks' solution for 1.0 M.

4.3. Micro-arc oxidation

Micro-arc oxidation (MAO, also named as plasma electrolytic oxidation or anodic spark oxidation) is a relatively convenient technique for forming oxide layer on metals. MAO is effective for the formation of porous or irregular-shaped TiO_2 layer on Ti substrate and ZrO_2 layer on Zr substrate (Fig. 4). The advantage of MAO is that coating layer is not only porous but also uniformly coated on metal surfaces with complex geometry. Anodically electrochemical deposition and micro-arc oxidation are not clearly distinguished. In the case of the formation of an oxide layer with connecting pore to the substrate metal by high voltage, this technique is usually categorized as micro-arc oxidation. In this sense, some of the electrochemical techniques explained above belong to MAO. MAO is currently used to obtain thick and porous oxide or HA layer [25—28]. Ultraviolet irradiation of micro-arc oxidation titania coating in distilled water enhances bioactivity [29]. This technique is also applied to zirconium (Zr) [30,31].

5. Surface-modified layer formation by chemical treatment

Hard tissue compatibility can be improved by modifying the Ti surface instead of the HA coating. Many surface modification techniques with neither a HA 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 [32,33]. This superiority of

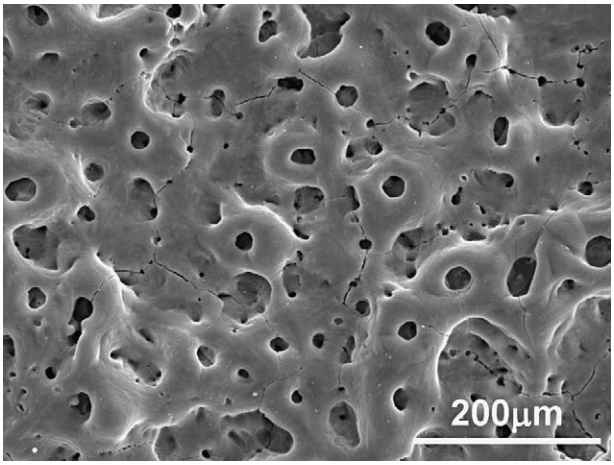

Figure 4 Scanning electron micrograph of porous ZrO_2 layer formed on Zr substrate by micro-arc oxidation.

calcium-ion-implanted Ti was due to a modified surface with calcium-ion implantation. The modified surface of calcium-ion-implanted Ti contained TiO_2 containing calcium in the chemical state of $CaTiO_3$ [34].

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 [35]. 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 titania-hydrogel, which increases the magnitude of the supersaturation of HA in the solution near the surface. The gel induces HA nucleation, and the HA layer is rapidly formed. This process is already commercialized for the stem of an artificial hip joint. Immersion of Ti in $TaCl_5$-containing H_2O_2 accelerates HA formation in simulated body fluid [36]. A pullout test of specimens from rat tibia revealed increased bonding strength of Ti to the bone. Acid etching is also effective to cell activity of Ti [37,38]. Also, the combination of acid etching and alkaline treatment is effective to accelerate HA coating [39].

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 [40]. The most effective means to precipitate HA is immersion in an alkaline solution. While in identical calcium-containing solutions, hydrothermal modification of Ti was performed using an autoclave [41] and hydrothermal treatment with high pressure [42]. Ti is chemically treated with H_2O_2/HNO_3 solutions at 353 K for 50–60 min to introduce TiO_2 and $CaTiO_3$ layer and hydrothermally treated in an autoclave at 453 K for 12 h [43]. This modification promotes the precipitation of HA. Ti is mounted in calcium-hydroxide slurry and heated and this modification is effective to form $CaTiO_3$ layer [44,45].

6. Inhibition of bone formation

When Ti alloys are used for bone fixators such as bone screws and bone nails implanted in bone marrow in orthopedics, Ti alloys form callus 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 [46,47]. Stainless steel is used for complete retrieval after healing. Therefore, surface treatments that do not cause callus formation are necessary for the safe utilization of Ti alloy devices. It has been reported that zirconium (Zr) forms zirconium phosphate but not calcium phosphate [48].

The coating of Zr inhibits the formation of calcium phosphate on Ti [49]. Calcium was not detected from Zr-coated Ti and Zr. Therefore, Zr coating is a useful technique to inhibit the assimilation of Ti alloys with bone. Inhibition of calcium phosphate formation on zirconium is confirmed by another study [50]. Neither calcium nor phosphate stably exists alone on titanium, and calcium phosphate is naturally formed on it; calcium phosphate formed on titanium is stable and protective. On the other hand, calcium is never incorporated on zirconium, while zirconium phosphate, which is easily formed on zirconium, is highly stable and protective.

7. Immobilization of functional molecules

7.1. Immobilization of PEG

The immobilization of biofunctional polymers on a noble metals such as gold is usually conducted by using the bonding —SH or —SS— group; however, this technique can only be used for noble metals. The adhesion of platelets and adsorption of proteins, peptides, antibodies, and DNA is controlled by modifications of the above technique. On the other hand, PEG is a biofunctional molecule on which adsorption of proteins is inhibited. Therefore, immobilization of PEG to metal surface is an important event to biofunctionalize the metal surface. A class of copolymers based on poly(L-lysine)-g-poly(ethylene glycol), PLL-g-PEG, has been found to spontaneously adsorb from aqueous solutions onto TiO_2, $Si_{0.4}Ti_{0.6}O_2$, and Nb_2O_5 to develop blood-contacting materials and biosensors [51,52]. In another case, TiO_2 and Au surfaces are functionalized by the attachment of poly(ethylene glycol)—poly(DL-lactic acid), PEG—PLA, copolymeric micelles. The micelle layer can enhance the resistance to protein adsorption to the surfaces up to 70% [53]. A surface of stainless steel was firstly modified by a silane-coupling agent, SCA, (3-mercaptopropyl)trimethoxysilane. The silanized stainless steel, SCA-SS, surface was subsequently activated by argon plasma and then subjected to UV-induced graft polymerization of poly(ethylene glycol)methacrylate, PEGMA. The PEGMA graft-polymerized stainless-steel coupon, PEGMA-g-SCA-SS, with a high graft concentration and, thus, a high PEG content was found to be very effective to prevent the absorption of bovine serum albumin and γ-globulin [54]. These processes require several steps but are effective for immobilization; however, no promising technique for the immobilization of PEG to a metal surface has been so far developed. Photoreactive PEG is photoimmobilized on Ti [55].

7.2. Electrodeposition of PEG

Both terminals of PEG (MW: 1000) were terminated with $-NH_2$(NH_2—PEG—NH_2), but only one terminal was terminated

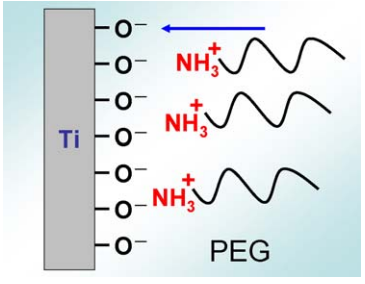

Figure 5 Both terminals of PEG were terminated with $-NH_2$, only one terminal was terminated with $-NH_2$. The amine bases dissociated and were positively charged in aqueous solution. The cathodic potential was charged to Ti from the open circuit potential to −0.5 V vs. a saturated calomel electrode and was maintained at this potential for 300 s. During charging, they were electrically attracted to the Ti surface with a cathodic charge (blue arrow). PEG molecules were eventually immobilized. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

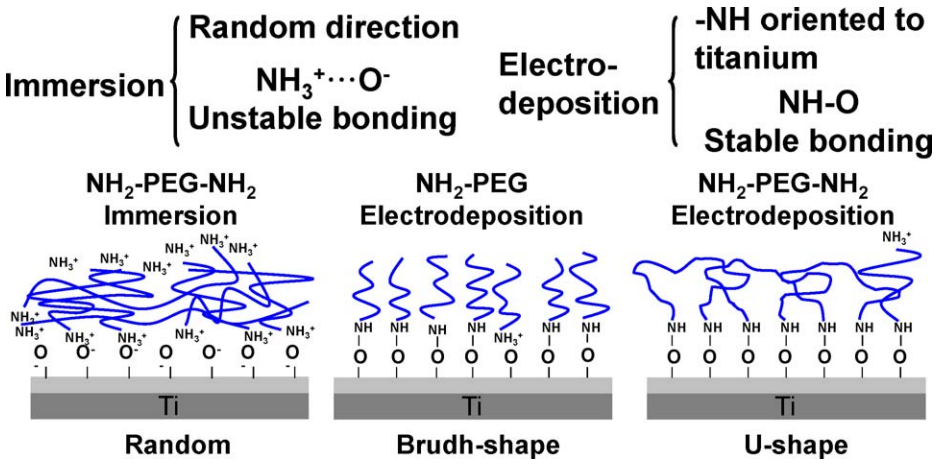

Figure 6 Schematic model of immobilized PEG to a Ti surface with immersion and electrodeposition. More terminated amines combined with Ti oxide as an ionic NH—O by electrodeposition, while more amines randomly existed as NH_3^+ in the PEG molecule by immersion. The difference in amine termination led to different bonding manners: U-shaped in NH_2—PEG—NH_2 and brushed in NH_2—PEG.

with —NH_2(NH_2—PEG) [56]. The cathodic potential was charged to Ti from the open circuit potential to —0.5 V vs. 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 Fig. 5. 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 an NH—O bond by electrodeposition, while more amines randomly existed as NH_3^+ in the PEG molecule by immersion

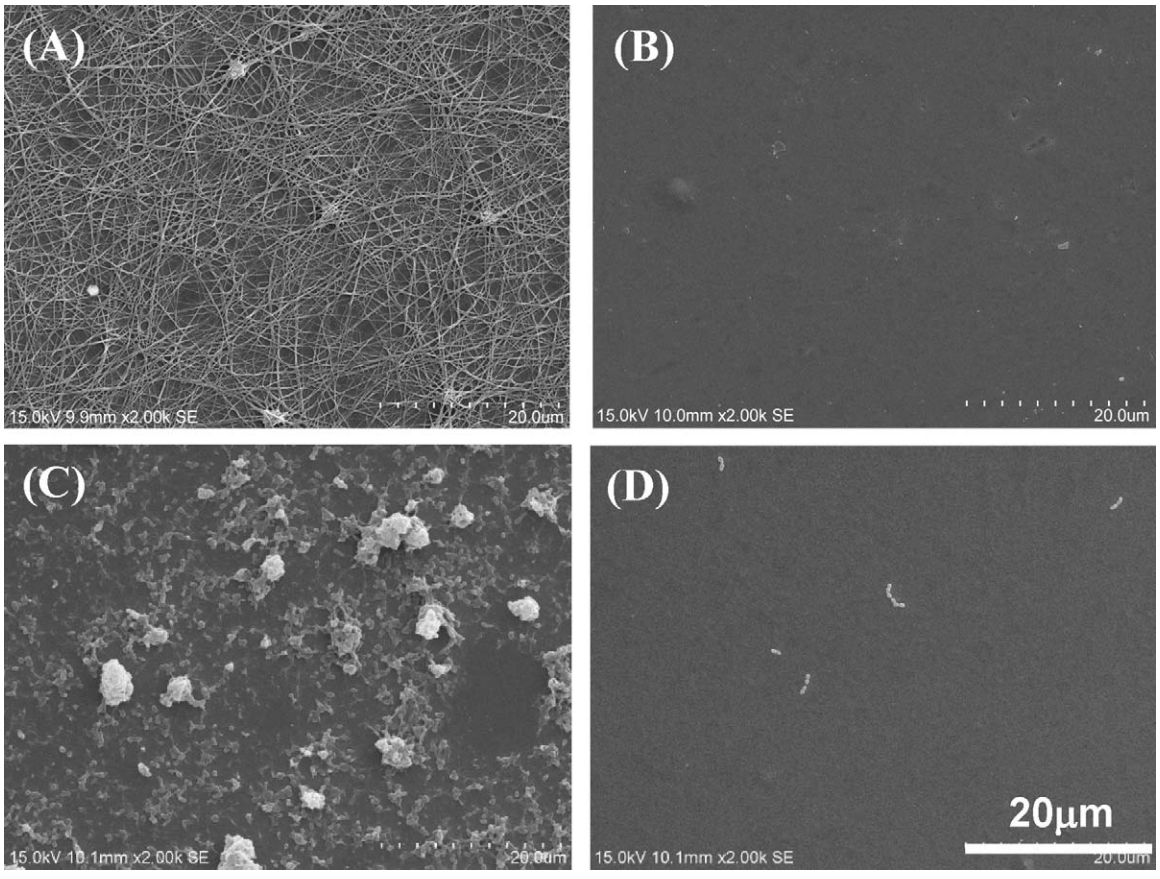

Figure 7 Platelets adhered to the untreated Ti surface, and a fibrin network was formed on it (A). Platelet adhesion was inhibited on the PEG-electrodeposited Ti surface (B). Bacteria (*S. mutans* MT8148) adhered to an untreated Ti surface (C), while bacterial adhesion was inhibited on a PEG-electrodeposited Ti surface (D).

(Fig. 6) [57]. 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 [58]. The PEG-immobilized surface inhibited the adsorption of proteins and cells, as well as the adhesion of platelets and bacteria (Fig. 7), indicating that this electrodeposition technique is useful for the biofunctionalization of metal surfaces. It is also useful for all electroconductive and morphological materials.

7.3. Immobilization of biomolecules

Biomolecules are also used to accelerate bone formation and soft tissue adhesion on a material. Type I collagen is immobilized by immersion in the collagen solution [59]. Type I collagen production increases with modification by ethane-1,1,2-triphosphonic acid and methylenediphosphonic acid grafted onto Ti [60]. Type I collagen is grafted through glutaraldehyde as a crosslinking agent [61]. Bone morphogenetic protein-4 (BMP-4) is immobilized on a Ti—6Al—4V alloy through lysozyme to improve the hard tissue response [62]. Proteins are silane-coupled to the oxidized surfaces of the Co—Cr—Mo alloy, the Ti—6Al—4V alloy, Ti, and the Ni—Ti alloy to improve tissue compatibility [63]. Fibronectin is immobilized directly on Ti using tresyl chloride activation technique [64]. L-Threonine and O-phospho-L-threonine are immobilized acid-etched Ti surface [65].

Peptides consisting of arginine (R)-glycine (G)-asparaginic acid (D) sequence, RGD peptide, accelerate cell attachment and extension of bone cells on Ti [66]. RGD is a peptide known to involve cell adhesion, which is involved in many extracellular matrix proteins [67]. Bone formation is accelerated by immobilizing RGD on a Ti surface [68]. Peptides with terminal cysteine residues were immobilized on maleimide-activated oxides [69—71].

To immobilize RGD to the electrodeposited PEG on Ti, PEG with an $-NH_2$ group and a —COOH group (NH_2—PEG—COOH) must be employed. One terminal group, $-NH_2$, is required to bind stably with a surface oxide on a metal. On the other hand, the other terminal group, —COOH, is useful to bond biofunctional molecules such as RGD [72]. This RGD/PEG/Ti surface accelerated calcification by MC3T3-E1 cell [73].

Glycine (G)-arginine (R)-glycine (G)-asparaginic acid (D)-seine (S) sequence peptide, GRGDS peptide, is coated with chloride activation technique to enhance adhesion and migration of osteoblastic cells [74]. The expression levels of many genes in MC3T3-E1 cells are altered.

8. Conclusions

Titanium is widely used in dental implants and their surface may be biofunctionalized by various techniques such as dry and wet processes, the immobilization of biofunctional molecules. Major purpose of surface modification is to improve hard tissue compatibility or accelerate bone formation. On the other hand, the electrodeposition technique is useful for all electroconductive and morphological materials not only to inhibit the adhesion of platelet and bacteria but also to enhance bone formation. These techniques make it possible to apply metals to a scaffold in tissue engineering.

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