Surface modification of titanium and its alloys for the enhancement of osseointegration in orthopaedics

Agnes Aruna John[1], Saravana Kumar Jaganathan[2,3,4,*], Eko Supriyanto[4] and A. Manikandan[5]

[1]Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia, Johor Bahru 81310, Malaysia
[2]Department for Management of Science and Technology Development, Ton Duc Thang University, Ho Chi Minh City 70000, Vietnam
[3]Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City 70000, Vietnam
[4]IJN-UTM Cardiovascular Engineering Centre, Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia, Johor Bahru 81310, Malaysia
[5]Department of Chemistry, Bharath Institute of Higher Education and Research, Bharath University, Chennai 600 073, India

Titanium (Ti) and Ti-based alloys are the best promising orthopaedic metal transplants. The Young's modulus of Ti and bone are nearer and so Ti implants are known as osseointegrated implants. However, the need for enhancing the osseointegration, corrosion resistance and biocompatibility cannot be ruled out in promoting the Ti as a golden standard. This review describes various surface modifications like acid etching, sand blasting, surface coating, alkali-heat treatment, plasma treatment and ion implantation of Ti-based implants which are the best solutions to promote biocompatibility, osseointegration and ultimately the longevity of implants. In addition, it gives an outline to accomplish the risky task in orthopaedics like recovering skeletal function by replacing the damaged bone for human being survival and it will assist the energetic collaboration of specialists in materials science, chemistry and biology for the quality enhancement.

Keywords: Corrosion resistance, osseointegration, orthopaedics, titanium and its alloys, surface modification.

BIOMATERIALS play a major role for the human beings born with some disabilities and disorders like congenital heart disease and for the aged people who require medical transplants to extend their life expectancy. Metals, polymers, ceramics and composites are the four main categories of biomaterials. Among these metallic implants gain enormous importance due to their high strength and strong resistance towards fatigue degradation. Metallic biomaterials represent around 70–80% of all implant materials and thus stand for a vital classification of biomaterials[1].

Titanium alloys, cobalt alloys and stainless steel are the most commonly preferred metallic biomaterials[2]. When the metallic implant is placed inside the living system, the electrochemical reaction degrades the metal surface called corrosion, due to the presence of corrosive environment that includes water, sodium, chlorine, proteins, plasma and amino acids[3]. The major problems related with metallic biomaterials are the compatibility with the blood and corrosion of metallic materials which are the main cause of rejection of metallic implants[4].

To tackle the above-mentioned limitations, a number of strategies were implemented to change the surface for the compatibility and corrosion resistance enhancement of the biomaterial known as surface modification[5]. The surface of the material has an important part in the response of biological system towards implant. After implantation, the communications between the implant surface and the biological system will not be affected, which means that the body should not treat the biomaterial as an external thing that will be achieved through effective selection of a material with desired properties. The required characteristics different from the naive material of the implant surface are attained through surface modification[6].

Ti-based alloys are one of the metallic implants mainly used in orthopaedics due to its outstanding tensile strength, better corrosion resistance, high specific strength, rigidity, fracture toughness, biocompatibility together making it as a replacement for hard tissue[4] (Figure 1). It is stated that Ti implants of about 1000 tonnes are inserted in patients every year worldwide. The chief physical characteristics of Ti in authority for biocompatibility include less electronic conductivity, elevated resistivity to corrosion and low ion-formation affinity in liquid environments[7].

Ti, Ti-based alloys are basically subjected to surface modification to enhance the resistivity towards corrosion, osseointegration also the biocompatibility for its applications in the medical field. To attain the characteristics required for biomedical application, surface modifications such as sandblasting, acid etching, surface coating, alkali-heat treatment, hydrothermal modification, plasma-spraying and ion implantation are employed. In this review, these surface modification techniques of Ti and Ti-based alloys for osseointegration enhancement are discussed.

*For correspondence. (e-mail: saravana@tdt.edu.vn)

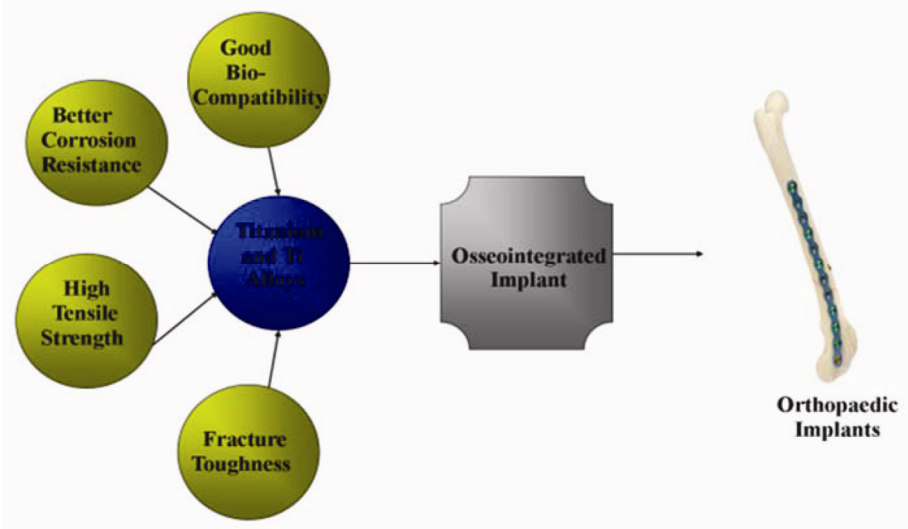

Figure 1. Osseointegrated implant characteristics.

Table 1. Chemical composition of mainly used titanium alloys in orthopaedics

Material	Chemical composition									
	N	C	H	Fe	O	Al	V	Zr	Nb	Ta
Ti–6Al–7Nb (ASTM F1295)	≦0.05	≦0.08	≦0.009	≦0.25	≦0.20	5.50~6.75	–	–	6.50~7.50	≦0.50
Ti–13Nb–13Zr (ASTM F1713)	≦0.009	≦0.05	≦0.005	≦0.08	≦0.1	–	–	≦13.0	≦13.30	–
Ti–6Al–4V (ASTM F136)	≦0.05	≦0.08	≦0.012	≦0.25	≦0.13	5.50~6.75	3.50~4.50	–	–	–

Titanium and its alloys in orthopaedics

Ti is less in weight and silver-gray has an atomic number 22 with low density and high strength, possesses better corrosion resistance. The non-toxic and biocompatibility of Ti makes its use in medical applications. Kroll process is employed for Ti production. The stepladder engaged in Ti production comprises extraction, purification, sponge production, alloy creation, forming and shaping. Pure Ti can be mixed with other chemical elements such as iron, aluminium, vanadium and molybdenum to create stronger and less weight alloys for various applications. Ti-based alloys such as Ti–6Al–7Nb, Ti–13Nb–13Zr, Ti–6Al–4V and Ti–12Mo–6Zr–2Fe are mainly used for the orthopaedic applications such as knee joints, bone plates, screws, elbow and hip prosthesis[8]. The chemical composition of Ti alloys is given in Table 1. It is found that nitrogen (N), carbon (C), hydrogen (H), iron (Fe) and oxygen (O) are some basic components present in Ti alloys.

Good mechanical strength is the basic need for orthopaedic implant. The implants for long-term use such as joint replacement prosthesis cannot tolerate mechanical failure. Ti has less elastic modulus, decent fatigue strength and corrosion resistance. The young's modulus of Ti (around 110 GPa) is almost closer to that of bone (10–30 GPa) compared to stainless steel (around 180 GPa) and Co–Cr alloys (around 210 GPa). So that the skeletal loads are equally shared between the implant and bones. This inbuilt titanium's capability and its ability of osseointegration formulate a promising hope for orthopaedic applications[9]. The mechanical properties such as fatigue strength, wear and corrosion resistance are improved through surface modification.

The factors affecting the osseointegration of metallic implants are the mechanical properties and the interaction of a metal surface with the biological system. If the body treats the metal implant as a foreign substance, the adherence of platelets activates the coagulation cascade which results in metal corrosion associated with inflammation. The corroded particles of the metallic implant may engulf by the macrophages or the implant weakens. These events might result in failure or rejection of the implant (Figure 2). The implant rejection can be reduced by improving the surface properties through the surface modification techniques. Hence, surface treatments of Ti and Ti-based alloy implants are required to enhance osseointegration, improve tissue adhesion and decrease bacterial adhesion that results in successful implantation[10]. After implantation, the

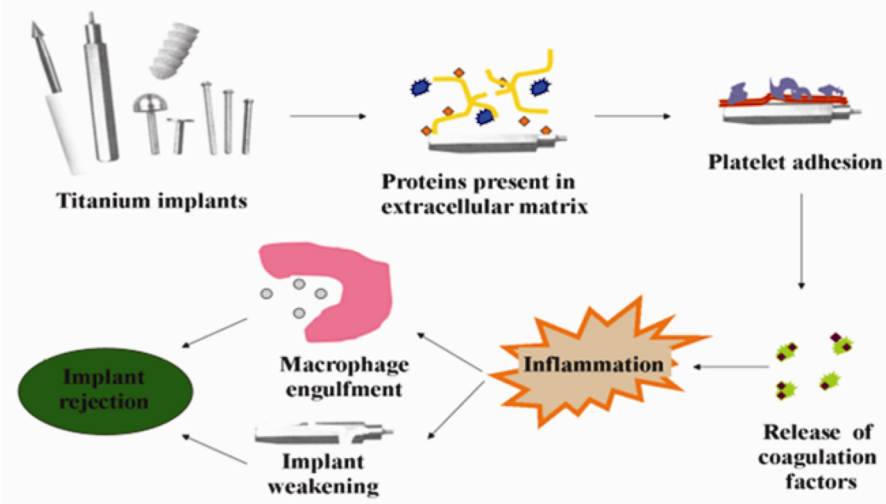

Figure 2. The sequential events lead to implant failure or rejection.

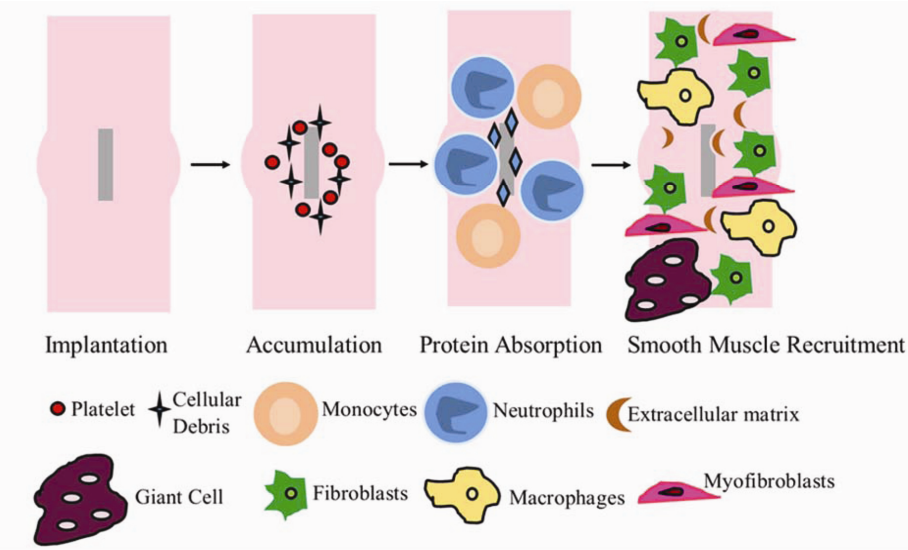

Figure 3. Biological responses to the implant.

biological responses to the implant include surrounding with cellular debris, platelets followed by protein absorption which lead to muscle recruitment (Figure 3). The surface modification-induced morphological changes found to advance the bioactivity, biocompatibility, resistivity to wear and corrosion and ultimately the biological performance of Ti and its alloys for the biomedical applications are summarized.

Osseointegration

An inflexible organ that establishes part of the vertebral skeleton is bone. It supports and safeguards the body parts, produces erythrocytes and leukocytes, stores minerals and facilitate movements. The tissue types seen in bones are marrow, endosteum, periosteum, nerves, blood vessels and cartilage. The cortical or compact bone is the hard outer layer and the cancellous or spongy bone tissue comprises the interior of the bone. The bone that holds the cancellous tissue is called as the bone marrow. The bone may fracture due to high impact force or injury and the repair of fractured bones is called as bone healing which will occur often. If it was failed, surgical methods are preferred. In complicated cases, low modulus metals like Ti and Ti-based alloys are utilized as bone plates, screws to avoid stress shielding[11].

Osseointegration denotes the formation of interface between the bone and implant surface. The implant which contains pores for the migration of osteoblast cells and connective tissue is known as osseointegrated implant. The surface properties of implant play a major role in osseointegration through which the molecular, cellular behaviours are affected. Osseointegration is the foremost

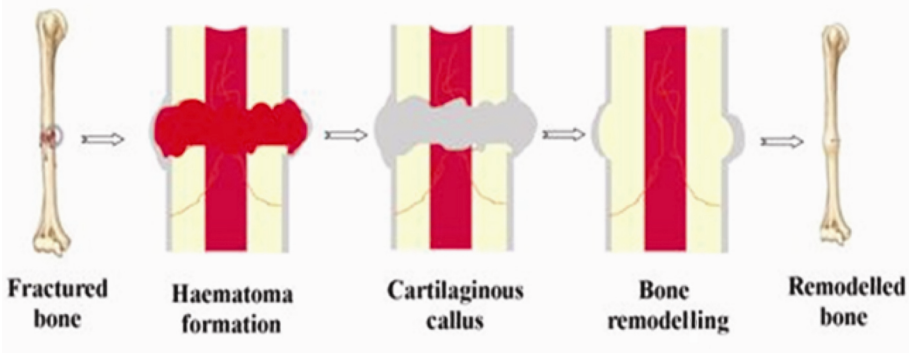

Figure 4. Mechanisms involved in bone healing.

consideration for the success of bone implants and it is frequently used to illustrate the bone tissue response to titanium material[12].

The mechanism of osseointegration is more similar to that involved in bone healing of fractured or damaged bone. The stages involved in fracture healing are granulation tissue formation, cartilage callus formation and remodelling of fractured bone into original bone contour[13] (Figure 4). In addition, osseointegration reduces the orthopaedic implant failure rate and plays a vital role in bone healing. To achieve better osseointegration, a physically powerful long-term relation between the implant surface and peri-implant bone should be established through surface modifications for the unwavering connection of the transplant at the location of implantation. The surface treatments such as acid etching, sand blasting, surface coating, alkali-heat treatment, plasma treatment and ion implantation techniques of Ti and its alloys for the osseointegration enhancement are reported in the following sections.

Acid etched and sand blasted Ti and its alloys

Alumina particles are propelled by force against the surface under high pressure. Acid treatment is performed to acquire a dirt-free material with even surface finishing. A combination of acids, which contains HNO_3 (10–30 vol%) and HF (1–3 vol%) in distilled water is taken as a standard solution for the acid pre-treatment of Ti and its alloys[14]. The selective removal of impurities from the metal surface is possible with the acid etching and the roughness depends on the exposure time and the acid used[15]. Takeuchi *et al.*[14] studied the decontamination efficiency of three acids, namely $Na_2S_2O_8$, H_2SO_4 and HCl to the surface of Ti. It was found that HCl is more efficient for Ti decontamination than the other two acids[14]. The acid treatment commonly results in skinny oxide layer formation over the surface of Ti which thickness is about less than 10 nm. This oxide layer is mainly composed of titanium oxide (TiO_2) and also the deposits from the solution are used for the treatment[16]. Korotin *et al.*[17] treated the Ti surface with hydrofluoric acid to improve its surface properties. The surface characterization was performed before and after the treatment. The results of X-ray photoelectron spectroscopy (XPS) measurement showed decreased hydrocarbon content with increased surface energy and bio-acceptability of Ti implant. In general, acid etching is mostly combined with other surface modifications to advance the characteristics of Ti and Ti alloys. Wen *et al.*[18] suggested that the bioactivity of Ti was increased by the acid treatment ($HCl + H_2SO_4$) followed by alkaline treatment. Celletti *et al.*[19] compared the bone response of acid etched and machined Ti surfaces by implanting it in a rabbit model. The bone-implant contact was higher in acid-treated surface than the machined Ti surface. Ti surface was treated with hydrofluoric acid to advance the bone-to-implant contact and strength of adhesion. XPS outcomes showed that the acid-treated Ti surface showed increased roughness, reduced cytotoxicity and enhanced biocompatibility than the control and the results suggested that the surface changes are time dependent (exposure or treatment time)[20]. Lan *et al.*[21] nanostructured the Ti surface through acid etching and the results demonstrated that the adhesion of bone mesenchymal stem cells (BMSCs) was increased with increase in cell proliferation.

Ti alloy (Ti13Zr13Nb) was sand blasted with alumina particles followed by H_2SO_4 etching. The surface morphology was studied using field emission scanning electron microscope (FE-SEM) and energy dispersive X-ray (EDX). The results indicated that the biocompatibility and osseointegration were improved for SLA surface than the raw Ti surface[22]. Li *et al.*[23] sand blasted the surface with Al_2O_3 particles for 30 s and subsequently etched with a solution mixture of acids (18% HCl and 49% H_2SO_4). The biocompatibility was examined using MG63 cells and was assessed by SEM, XPS and contact angle assay. The results showed that the surface roughness and biocompatibility were improved compared to the untreated Ti surface. Ti surface was sand blasted and then treated with oxalic acid to examine the topography

effects. SEM images of SLA-treated surface showed improved roughness as 4.54 μm. The cell studies revealed that the adhesion of human fetal osteoblast cells (CRL-11372) was increased after 24 h of incubation[24]. Kim *et al.*[25] etched the sand blasted Ti surface in hydrochloric acid to improve its osseointegration. The SLA modified Ti surface was subjected to surface classification, *in vitro* cell–surface interaction and *in vivo* animal tests. The results of this study suggested that the surface roughness was improved to 1.19 μm along with osteoblast cell growth. The *in vivo* animal study with rabbit tibia demonstrated a good bone to implant contact with a mean value of 29% (ref. 25). The SEM analysis of grit-blasted Ti surface showed better biocompatibility and the bone to implant contact area was quantified histomorphometrically. An Al_2O_3 free implant provides more bone to implant contact area with reduced shear resistance[26]. The sand blasted Ti was etched with concentrated H_2SO_4 and its effect on MC3T3-E1 cells was evaluated. SEM analysis showed that the surface roughness was improved in acid etched Ti surface than the sand blasted surface and there are no noteworthy differences in cell proliferation between acid etched and sand blasted Ti surface[27]. Ti surface was blasted with 25 μm and 250 μm aluminum oxide particles and a comparative study of bone response to the surfaces was made. After measuring the surface roughness of two blasted Ti surfaces, they were implanted into the rabbit tibiae. The bone to implant contact was higher in 25 μm particles blasted surface compared with the Ti surface blasted with 250 μm aluminum oxide particles. It was concluded that an extremely amplified surface roughness compared to an abstemiously improved one is a temporary drawback for bone tissue[28]. The roughened biocompatible Ti_6Al_4V surface was obtained by blasting the surface with biphasic calcium phosphate (BCP) particles. MTT assay of BCP blasted surface confirmed the viability of osteoblastic MC3T3-E1 cells. It indicated the non-toxicity and increased surface roughness of BCP blasted surface[29]. Ti was blasted with titanium oxide particles and the surface was analysed with an optical confocal laser profilometer. The blasted surfaces showed significantly increased roughness compared with untreated surface. Then the blasted and the untreated Ti implants were inserted in the maxilla or in the mandible for the histologic evaluation. The histomorphometrical analysis revealed that the contact of bone with metal was advanced in blasted surface placed in the maxilla and mandible[30]. Ti was subjected to sequential treatments including sandblasting and acid etching to produce hierarchically structured Ti surface with inherent antibacterial ability and outstanding osteo-conductivity[31]. The acid etched alumina blasted Ti_6AlV_4 surface was modified with immobilized oligonucleotides serving as anchor stands for rhBMP-2 and vascular endothelial growth factor A (rhVEGF-A) that enhances the osseointegration and biocompatibility of the implant[32].

Surface coating for osseointegration

The surface roughness, bone-implant bonding and ultimately the osseointegration of Ti implants were improved through the coating of implant material surface. Coatings comprising calcium phosphates, extracellular matrix (ECM) and immobilized growth factors aided osteointegration. The intention of osteogenic coatings must possess anti-infective property of orthopaedic devices[33]. For the orthopaedic implants, calcium phosphate has been in common use due to the resemblance with the mineral phase of the bone and the bioactive property[34]. The anchorage of implant with the bone and osseointegration was enhanced by the coating of implant with calcium phosphate, which acts as an intermediate layer between the implant surface and the surrounding tissue. This calcium phosphate layer encourages the formation of bone along the surface of the implant[35]. The implant surfaces were also coated with growth factors. For the orthopaedic implants, bone morphogenetic proteins (BMPs) are used to coat the implant surface that induces osteogenesis and supports implant integration. Orthopedic implants loaded with such growth factors showed improvement in the remodelling of bone ultimately, the osseointegration[36]. The utilization of growth factors in surface modification of orthopaedic devices was chosen based on their exposure time, release kinetics and the dosage was carefully considered to avoid harmful effects such as high initial burst rate, ectopic bone formation and short half-life[37]. The specific ossteogenic function was developed by coating of orthopaedic implants with peptide sequences. These peptides are the short segments of protein synthetically derived from original protein and have improved resistance to denaturizing effects. Peptide sequences generally used are RGD, YIGSR, IKVAV and KRSR to advance the cellular adhesion and bone matrix formation[38]. Zhao *et al.*[39] coated the NaOH-treated Ti_6Al_4V surface with TiO_2 powder and the surface morphology of the modified sample was studied by FE-SEM. The results showed that the bioactivity of the TiO_2-coated implant was induced by the formation of sodium titanate on the implant surface. The bio-apatite deposited on Ti implants showed better biocompatibility, increased bone growth with improved mechanical properties because of the chemical and biological resemblance of bio-apatite with the hard tissue. A bioactive hydroxyapatite (HA) coating can also be employed to assist osseointegration of orthopaedic implants[40]. Incorporating silver (Ag) into HA coatings is an active method to impart the coatings with antibacterial properties with good biocompatibility[41]. Ti–Nb–Hf alloy was coated with a number of small peptides like RGD (Arg–Gly–Asp)/PHSRN (Pro–His–Ser–Arg–Asn) and RGD (Arg–Gly–Asp)/FHRRIKA (Phe–His–Arg–Arg–Ile–Lys–Ala) and a comparative study was made with rat mesenchymal cells. Contact angle measurements and XPS were carried out to study the surface. The results

suggested that implant containing both RGD and PHSRN possesses improved cell adhesion for the mixtures of RGD and FHRRIKA[42].

The Ti surface was coated with inorganic titanium matrix in which various fractions of a biocompatible polymer, the poly-ε-caprolactone (PCL), have been included to enhance the surface properties. The surface morphology was analysed using SEM and it was shown that the presence of PCL allows crack-free coatings[43]. Ti–29Nb–13Ta–4.6Zr (TNTZ) surface is altered with HA coating followed by the calcium phosphate glass ceramic treatment to enhance the biocompatibility and biofunctionality[44]. Gelatin nano gold (GnG) composite was employed to modify the Ti and the modified surface was analysed via SEM. Western blot analysis was performed to assess the protein expression and the MC-3T3 E1 cell viability was assessed through trypan blue. The results showed that the MC-3T3 E1 cells are more compatible with modified surface than the pure Ti and GnG coating offered efficient communication between the implant surface and the osteoblast cells by regulating the cell signalling[45]. Zeng *et al.*[46] modified the Ti surface with calcium phosphate (CaP) coating and protein adsorption was evaluated by FTIR. The result showed that CaP modified Ti surface adsorbed enormous quantity of protein compared with the untreated surface. The protein adsorption capability of modified surface also changed the cellular behaviour on CaP surface. The HA-coated Ti implant was inserted into the cancellous bone of the intercondylar region of the distal femur of the dog. The outcome suggested that the biological fixation of Ti implant was improved by HA coating and showed better fixation after the fourth week of implantation[47]. HA-coated Ti implant was fixed into femoral canine cancellous bone of the dogs and the histomorphometric analysis was carried out after 12 weeks of implantation. The results demonstrated that the bone formation was enhanced with HA-coated surface than the untreated Ti surface[48]. The HA-coated and uncoated Ti plates were placed into the rabbit bones and the histologic examination confirmed more bone activity in 27 days and it was more prominent at 127 days with the coated implants compared with the uncoated Ti implants[49]. Sowa *et al.*[50] studied the bioactivity of anodic oxide coatings on Ti–13Nb–13Zr and human bone marrow derived mesenchymal stem cells (hBMSCs) were used to evaluate the biocompatibility. SEM and AFM analysis were carried out to illustrate the surface morphology. The results showed improved surface roughness, enhanced differentiation of stem cells into osteoblast cells that ultimately promoted the osseointegration of Ti implant. Ti implant surface was coated with nanocrystalline hydroxyapatite (NHA) and micron sized hydroxyapatite (MHA). The surfaces were analysed before and after coating using SEM and AFM. The results demonstrated that the osteoblast cells are adhered to the NHA-coated surface than the MHA-coated and uncoated surface[51]. Hydrogel was coated over the Ti surface and the modified surface was studied using SEM and EDX. The results revealed that the modified surfaces have three times higher roughness than the untouched Ti surface. The application of certain hydrogel coatings advances the implant biocompatibility, while improving the bond between the implant surface and the coating develops the longevity of the implant[52]. The biocompatibility of titanium carbonitride (TiCN) was improved by growing HA crystals over the TiCN-coated substrates. Formation of a coating with granular morphology was proved by SEM and X-ray diffraction. HA coating led to a reduction in thrombogenicity, resulting in controlled blood clot formation, therefore indicating an increased blood compatibility[53]. Ti_6Al_4V was coated with biodegradable chitosan (CS)-tripolyphosphate (TPP) nanoparticles directed to the visible film formation with thicknesses of ≈80 nm. The incorporation of BMP-2 into the nanoparticles results in an increased size. The biological activity of an incorporated BMP-2 was observed in an *in vivo* study in mice. The results showed that biodegradable CS-TPP coatings can be employed to present biologically active BMP-2 on usual implants[54]. A layer-by-layer technique has been established to formulate a stable BMP loaded or collagen/hyaluronic acid (Col/HA) polyelectrolyte multilayer (PEM) film on titanium coating to enhance osseointegration. Cell culture assay clarified that the functions of human mesenchymal stem cells, such as attachment, spreading, proliferation and differentiation were enriched by the covalently immobilized Col/HA PEM on Ti coatings than the absorbed Col/HA PEM[55,56]. Zinc-incorporated calcium silicate-based ceramic $Ca_2ZnSi_2O_7$ was coated on Ti–6Al–4V substrate. The *in vitro* response of MC3T3-E1 cells cultured on $Ca_2ZnSi_2O_7$ coating, $CaSiO_3$ coating and unmodified Ti–6Al–4V was studied. The results exhibited $Ca_2ZnSi_2O_7$ coating improved MC3T3-E1 cell attachment, proliferation and differentiation than the $CaSiO_3$ coating and control[57].

Alkali-heat treatment

Treating a material in the heat or at high temperature is a metal working procedure applied to modify the physical and chemical characteristics of a material[58]. The samples of Ti–6Al–4V, Ti–40Nb–1Hf and Ti–30Nb–1Fe–1Hf alloys were soaked in NaOH solution at 60ºC for a day, then the samples were rinsed with deionized water and dried for 24 h at 40ºC. Then the alkali-treated specimens were heated up to 600ºC for an hour. The morphological changes were analysed by SEM, FE-SEM and XPS. The biocompatibility of the treated surfaces was evaluated through cell studies. The results ensured that the biocompatibility and bone bonding were improved after the alkali-heat treatment compared with the untreated samples[59]. Lee *et al.*[60] inserted the alkali-heat treated and

untreated pure Ti, Ti–6Al–4V alloy implants in the mice. The surface and biocompatibility of the implants were characterized through XRD, SEM and XPS after three months of implantation. The results showed that the number of macrophages, which is responsible for inflammation, was adhered more on the untreated surfaces of pure Ti (40.3) and Ti_6Al_4V (43.3) than the treated surfaces which is about 8.7, 18.7 respectively. This indicated the biocompatibility improvement of alkali-heat treated surfaces. The NaOH-treated Ti was subjected to heat treatment to induce the apatite-forming ability of Ti metal by the generated sodium titanate bioactive layer over the surface. This bioactive Ti implant also showed improved bone-implant bonding thereby the enhanced osseointegration of the treated Ti surface[61,62]. Ti was placed at 45° in the mixture of solution containing $Ca(NO_3)_2$, Na_2HPO_4, calcium, phosphate and urea. Then it was heated at 150ºC for 3 h and the surface characterization was studied through SEM and FTIR. Alamar blue assay with the osteoblast cells of rats was made to evaluate the biocompatibility of heat-treated Ti surface. The results revealed that the hydrothermal modified surface induced the cell elongation and proliferation of osetoblast cells was more than the unmodified Ti surface[63]. The osteoblastic cell response to pure Ti and alkali-heat-treated $TiTa_8Ni_3$ was studied via cell proliferation and alkaline phosphatase (ALP) activity analysis of fetal rat calvarial cells. SEM and XPS were used to assess the surface characteristics of the metallic implant. The results demonstrated that the modified Ti alloy surface showed higher hydrophilicity and surface energy than the pure Ti. The modified Ti alloy surface also stimulates more osteoblast differentiation and the cell activity was 20% higher than the cells on naive Ti (ref. 64). Manganese-containing titanium oxide surface obtained by hydrothermal treatment was studied for its biological performance. The *in vitro* biocompatibility of modified surface was assessed in a mouse calvaria-derived osteoblastic cell (MC3T3-E1). The surface was analysed using SEM and inductively coupled plasma-atomic emission spectroscopy (ICP-AES). The results suggested that Mn containing Ti oxide surface has no significant positive effects on osteoblast cell function and proliferation[65]. The anodized and pre-calcified Ti_6Al_4V alloy surface was heat treated (APH) and their bioactivity before and after surface modification was evaluated. APH-treated surfaces displayed increased roughness, biocompatibility and bone-implant contact than the untreated surface. The treated Ti_6Al_4V alloy showed more bioactivity and bone regenerative capability compared with anodized and heat-treated (AH) surfaces[66]. Ti–35Nb–5.7Ta–7.2Zr (TNZT) and Ti–35Nb–5.7Ta–7.2Zr–0.5B (TNZTB) alloys were heat treated and the effects on their mechanical properties were evaluated. The TNZTB showed elevated hardness and tensile strength compared to TNZT alloy due to the formation of hard TiB precipitates. The cell adhesion and cell spreading were better in both heat treated alloys than the unmodified alloy, but the boron free Ti-alloy (TNTZ) enhanced cell attachment compared with boron containing Ti-alloy (TNZTB)[67].

Silva *et al.*[68] examined the effect of heat treatment on Ti–Nb alloy by conducting some examinations such as density measurements, X-ray diffraction, optical microscopy, Vickers micro-hardness on both untreated and heat treated Ti–Nb alloy. The results indicated that the surface roughness was increased due to the heat treatment of Ti–Nb alloy that ultimately resulted in enhanced biocompatibility of heat treated Ti–Nb alloy than the untreated Ti–Nb (ref. 68). Ti–10Zr–5Ta–5Nb alloy was modified by hot rolling accompanied with heat treatment and the biocompatibility of modified surface was examined. The results suggested that there is no effect on cell viability of human fetal osteoblast cell line hFOB 1.19 and the corrosion resistance was improved with increased biocompatibility[69]. The surface morphology of alkali-heat treated Ti–27Nb alloy was characterized via SEM, XRD. The results demonstrated that the surface roughness of treated samples was improved by the formation of sodium-titanate layer over the modified surface and it was useful for the enhancement of biocompatibility of alkali-heat treated Ti alloy[70]. Cui *et al.*[71] transformed the amorphous titania layer into a crystalline structure by treating with hot water and heat. The capability of the formation of apatite layer was studied through SEM and XRD analysis. The results revealed that the hot water and heat treatment are the powerful techniques to modify the amorphous structure into anatase. The hot water formed the bond between OH and Ti and the heat treatment enhanced the strength of bond between apatite layer and Ti substrates that makes Ti suitable for load-bearing applications (orthopaedics). Ti6Al4V alloy was allowed to oxidize by treating at 500ºC for an hour in air that formed an outer ceramic layer to improve the behaviour of osteoblast cells. The attachment, spreading, proliferation and viability of osteoblast cells and procollagen I peptide secretion of human primary osteoblasts were significantly increased. The *in vitro* studies proposed that the thermal treatment enhances the osseointegration of the orthopaedic implant through the efficient bonding of thermally modified surface and osteoblast cells[72]. Ti surface was modified by hydrothermal treatment at 280°C and the pressure was maintained at 6.3 MPa. The bioactivity of modified surface was evaluated using simulated body fluid (SBF). The result showed that the bioactivity and biocompatibility were improved after the high-pressure, hydrothermal treatment[73]. Shi *et al.* improved the surface hardness of Ti through gas nitriding and then subjected to hydrothermal treatment in the solution of calcium chloride. NIH3T3 fibroblast cells were used to investigate biocompatibility of modified Ti surface. The results indicated that the cell spreading and proliferation were enhanced with outstanding abrasion resistance[74]. Ti

surface was sequentially treated with NaOH, strontium acetate, heat and water to produce strontium (Sr) modified titanium. The hydrophilicity of modified titanium was improved through the insertion of Sr ions and water treatment. The cell studies showed stimulated cellular adhesion, spreading and growth than the control Ti surfaces. The results indicate that Sr-modified Ti may increase the bioactivity *in vitro*. Treating with water promoted the response of osteoblasts through upregulating the expression of osteogenesis-related genes. Ti plates heat treated at 700°C displayed improved bioactivity than those treated at 600°C (ref. 75). A calcium titanate layer was formed on Ti metal, when it was heat-treated after exposure to NaOH followed by $CaCl_2$ solutions and finally soaked in hot water. Bone growth-promoting ions like Mg, Sr and Zn, and also antibacterial ions like Ag were integrated into the calcium titanate surface layers, to be gradually released in the living body. Porous Ti metal grown with titanium oxide on its surface presented both osteoconduction, osteoinduction and plays an essential role in repairing injured bone tissues[76]. Radiofrequency glow discharge (RFGD) does not change the roughness, topography, elemental composition or thickness of the alloy's surface oxide layer. In other case, heat treatment transformed the oxide topography via creating a oxide elevations of about 50–100 nm in diameter. These nanostructures exhibited a three-fold increase in roughness in comparison with the control surfaces[77].

Osseointegration enrichment by plasma treatment and ion implantation

Plasma surface modification (PSM) is an efficient and cost effective method for the biomaterials to enhance their biocompatibility. Ran *et al.*[78] treated the Ti implant with plasma micro-arc oxidation (MAO) and the osteogenic capability of MAO modified implant was examined by implanting in dogs. The histological analysis and SEM were carried out in the twelfth week of implantation. The rapid and efficient osseointegration occurred with MAO-treated Ti implant than the control. The formation of new bone with more load-bearing capacity was promoted by MAO treatment of the metal implants. The plasma treated Ti_6Al_4V and Ti_6Al_7Nb alloys were analysed for the surface changes using electron microscopy. The plasma induced surface energy was evaluated by drop shape analysis. The results suggested that the low pressure plasma treatment induced more surface free energy on the implant surface ultimately resulting in improved biocompatibility of modified surfaces than the unmodified surfaces[79]. The plasma modified Ni–Ti surface showed enhanced cell adhesion and growth compared with the alkali-treated, thermally oxidized and control groups. Among these three treatments (alkali treatment, thermal oxidation and plasma treatment), the plasma treatment offers the most favourable environment for the cells to grow even though there was no notable chemical changes with the plasma treated Ni–Ti surface compared with the untreated surface[80]. Ion implantation is the process involved in material engineering in which the electric field is used to accelerate the ions of a material and impacted into a solid to modify its physical characteristics. Silver plasma immersion ion-implantation (Ag-PIII) technique was implemented on Ti implant to enrich the osseointegration of sandblasted and acid-etched medical implants. The SLA treated and the Ag-PIII modified Ti implants were implanted into the Labrador dogs to evaluate the biocompatibility of the implants. SEM, XPS, histologic analysis and histomorphometric examination were conducted to assess the *in vivo* biocompatibility. The results revealed that the Ag-PIII treated implants have more stability, enhanced new bone formation and increased bone mineral density than the SLA-treated implants[81]. Liang *et al.*[82] implanted the zinc ions (Zn) into the pure Ti surface and the consequence of surface modification on biocompatibility was evaluated. SEM and XPS were implemented to analyse the structure and chemical composition of modified surface. MG-63 bone cell line was used to find the cellular responses to the treated surface. The results demonstrated that the cell growth was predominantly increased for Zn altered Ti surface than the unalloyed commercial Ti implant surface. The proliferation of MG-63 cells was promoted and the cell death was reduced by the Zn ion implantation. These results suggested that the osteoblast cell compatibility was advanced via plasma immersion ion implantation and deposition (PIIID) technique.

Some recent trends in osseointegration improvement

The technical demand for orthopaedic materials has led to noteworthy developments in the field of nanomedicine, which includes designing and surface modification of nanomaterials for orthopaedics[83]. Application of nanotechnology to modify the titanium implant surfaces can significantly advance cellular and tissue responses, cell proliferation that may benefit osseointegration[84]. Nano modification introduces an innovative bioactive capacity into the field of metallic materials, but still remains to be tested *in vivo*[85]. In addition to the above discussed techniques, laser treatment, electropolishing and pulsing technique, micro-arc oxidation are also performed recently. Ti_6Al_4V was treated with CO_2 laser and the influence of laser treatment on the biocompatibility of Ti_6Al_4V implant was studied. The results showed increased surface hardness of 67% with reduced cytotoxicity. The biocompatibility enhancement was improved through laser treatment[86]. Ti was altered through micro-arc oxidation (MAO) in calcium acetate (CA; $C_4H_6CaO_4$)

Table 2. Advantages and disadvantages of surface modification techniques of titanium and its alloys

Surface treatment	Advantages	Disadvantages
Acid etching and sand blasting	Removes contaminants from the surface Selective elimination of impurities Comparatively long lasting	Surface damages Mechanical properties may be affected Mostly combined with other surface treatments
Surface coating	Acts as an intermediate between the bone and implant surface Strong bone-implant bonding	Breakage of oxide layer may occur Less life span Possibility of ectopic bone formation
Alkali heat treatment	Formation of highly bioactive layer Induces the apatite forming ability of bone Better biocompatibility Increased longevity	Effects based on chemical reagents used for modification Very high operating temperature
Plasma treatment	Enhanced osteoblast cell adhesion, growth Cost effective Longer Life	No significant chemical changes
Ion implantation	Improved antibacterial activity Increased wear resistance	Very deep profiles are complicated Expensive Presence of high impurity content Temporary

Table 3. Patents on surface modification of Ti and its alloys for orthopaedic applications

Title	Inventors	Metal	Key findings	Reference
Nano surface modified metallic titanium implants for orthopaedic or dental applications and method of manufacturing thereof	Menon Shantikumar V Nair Manzoor Koyakutty Divya Rani VV Vinothkumar Lakshmanan Deepthy	Ti	The nano structured titanium implants were tested both *in vitro* and *in vivo*, providing confirmed osteoblast cell response through enhanced cellular adhesion, proliferation as well as differentiation. Enhanced osteointegration was proven *in vivo*	89
Method of surface hardening orthopaedic implant devices	H. Ravindranath Shetty, Walter H. Ottersberg, Jack E. Parr, Roy D. Crowninshield	Ti–6Al–4V	Surface hardening enhances the surface hardness, wear properties of the titanium or titanium alloy by thermal reaction of nitrogen gas at low temperatures with minimal loss in fatigue strength.	90
Method for ceramic peening of orthopaedic titanium alloy implants	Neil B. Beals, Willard L. Sauer	Ti–6Al–4V	A controlled ceramic shot peening process is used to induce a controlled surface roughness and uniform compressive stress on and into the surface of the titanium orthopaedic implant. These factors enhance the overall structural integrity of the implant and reduce the potential for metallic debris to be liberated from the implant.	91
Titanium based biocomposite material useful for orthopaedic and other implants and a process for its preparation	Bhagwati Prasad Kashyap, Tallapragada Raja Rama Mohan, Ranganathan Sundaresan, Malobika Karanjai	Ti	Immersion of the biocomposite in simulated body fluids, led to precipitation of bioactive phases like calcium hydroxyapatite, tricalcium phosphate, sodium calcium phosphate and calcium hydrogen phosphates on the surface, indicating biocompatibility of the implantable material having required interconnected porosity for facilitating tissue growth.	92
Plasma nitrided titanium and titanium alloy products	Efstathius Meletis	Ti and Ti–6Al–4V	Nitrided specimens showed a three-fold increase in surface hardness. Surface roughness was found to be a function of the degree of plasma intensification. Processing of Ti–6Al–4V resulted in a higher wear, corrosion and wear-corrosion resistance.	93

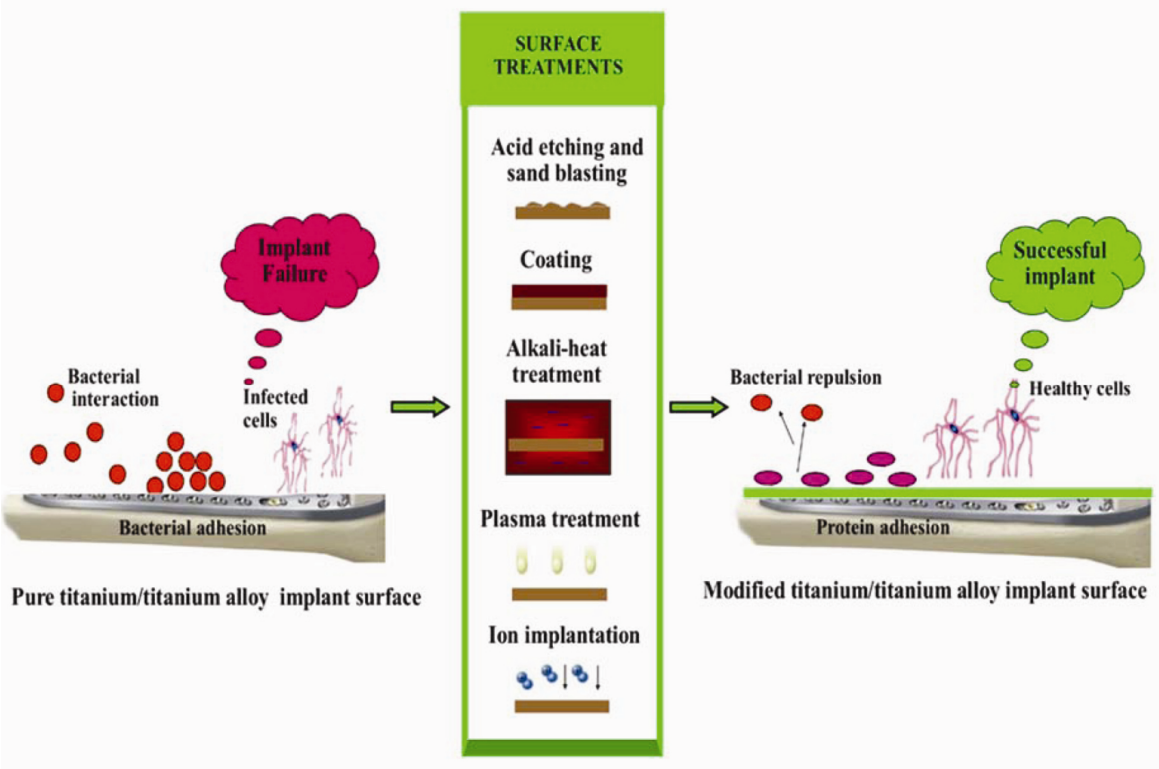

Figure 5. Effect of surface modification on biological aspects of titanium and titanium based alloy implants.

electrolytic solution and morphological changes were analysed through SEM and EDX. The cytocompatibility studies were performed using MC3T3-E1 preosteoblasts to observe the cell adhesion and proliferation. The results illustrated an enhanced cell adhesion, proliferation, cytocompatibility and osseointegration of MAO modified Ti than the raw Ti surface[87]. The microstructure and mechanical characteristics of Ti_6Al_4V were modified by electropulsing treatment (EPT) for biocompatibility enhancement. The high energy electric pulses formed the selective TiO_2 microwave structure on the treated surface that supports new bone formation. The EPT of Ti_6Al_4V resulted in increased cell viability, surface energy and wholly the biocompatibility[88].

Conclusion

The need of orthopaedic implants and modernization of biomaterials has been in ever growing demand because of musculoskeletal diseases linked with osteolysis. With the constantly increasing number of patients, necessitating orthopaedic reconstructions and improvement of Ti and its alloys with structural and biological prospective to direct bone injury would be attractive. In this review, various surface modification techniques performed to enhance the surface properties and biological performance of Ti and its alloys have been discussed and summarized. The merits and demerits of each technique are given in Table 2 and the related patents are given in Table 3.

From the reported literatures, surface coating and acid etching are employed most commonly due to cost effectiveness and still there is problem with longevity of implants. Plasma treatment can be performed to attain a reasonable longevity of an implant. In some cases, the treatments may be sequentially employed to achieve the desired characteristics. The ion implantation technique is preferred to attain better osseointegration but it is very expensive. To reduce this cost, a number of novel surface treatment technologies such as laser treatment, microarc oxidation and electropulsing techniques should be studied to promote the properties of Ti and its alloys for attaining the medical requirements particularly for the orthopaedic applications.

It has been inferred that surface modifications of Ti-based alloys depend not only on material properties but also its biological viewpoints such as protein adhesion, cell growth, cell proliferation and antibacterial activity (Figure 5). However, with the advent of new surface modification techniques, the future will give simultaneous solutions for the existing tribiological and clinical problems.

In the current fashion of surface modification, the antibacterial effect and the wear resistance are the twin properties achieved at the same time through an ion implantation technique than the other thermal modification

treatments. On the other hand, complete understanding of the biological response at the bone-implant interface is still deficient and additional studies are necessary to comprehend the biological practices taking place at the bone-implant boundary. Further, how these biological activities are influenced and controlled by specific surface properties of the implant material is still a matter of growing interest.

The well-organized functionalization methods of Ti and its alloy will unquestionably assist to achieve one of the unfathomable tasks in orthopaedics, which is substituting the damaged bone and renovating the skeletal functions to recover human health. The present review gives an idea about accomplishing this objective and it will assist the energetic collaboration of specialists in materials science, chemistry and biology to promote the quality of the implant material.

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