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Impact of surface topography and coating on osteogenesis and bacterial attachment on titanium implants

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Abstract

Titanium (Ti) plays a predominant role as the material of choice in orthopaedic and dental implants. Despite the majority of Ti implants having long-term success, premature failure due to unsuccessful osseointegration leading to aseptic loosening is still too common. Recently, surface topography modification and biological/non-biological coatings have been integrated into orthopaedic/dental implants in order to mimic the surrounding biological environment as well as reduce the inflammation/infection that may occur. In this review, we summarize the impact of various Ti coatings on cell behaviour both in vivo and in vitro. First, we focus on the Ti surface properties and their effects on osteogenesis and then on bacterial adhesion and viability. We conclude from the current literature that surface modification of Ti implants can be generated that offer both osteoinductive and antimicrobial properties.

Keywords

Titanium implant, topography, osteogenesis, bacterial adhesion, surface coating

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Introduction

Titanium (Ti) and its alloys are commonly used materials in orthopaedic and dental implants due to their mechanical and chemical properties; these include high strength to weight ratio and high yield and fatigue strength along with a relatively low Young's modulus counteracting the effects of stress shielding. An instantaneously forming passive oxide layer leads to corrosion resistance and biocompatibility.[1–5] Moreover, Ti is amenable to alterations in physical and chemical properties, including changing the surface oxide composition, thickness and topography, together making Ti a suitable material for enhancement via surface modification.[6] The biocompatibility of Ti and its alloys are related to the capacity of the Ti oxide layer to react with water ions and serum proteins as well as the resistance to corrosion that provided by the oxide layer.[7–10]

Scaffold surface features need to be biocompatible, bioactive and perhaps biodegradable as they are replaced by natural tissue during the regenerative process. Replicating the key structures of the extracellular matrix (ECM) and providing stem cell environments are powerful bioactive strategies that material scientists can copy and exploit.[11] Although Ti materials have many favourable properties, there are known potential shortcomings. For example, aluminium in Ti alloys may be associated with neurological disorders.[12] In addition, intra-articular injection of Ti dioxide (TiO_2) nanoparticles in rats has been noted to cause

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toxicological effects in lungs with follicular lymphoid hyperplasia and inflammatory cells aggregated around the bronchia.[13] Moreover, ionic Ti may have a mutagenic effect on cells either directly by damaging DNA via free radicals or indirectly by inhibiting the DNA repair[14] and may also induce some allergic reactions.[4,15] The biological response to orthopaedic and dental implants is determined by the physical and chemical features of the implant surface. These include surface topography, surface free energy, oxide thickness and oxide composition. The interaction between cells and the interface will be affected by one or more of these factors and any change in one will affect the other parameters.[16–19] Surface topography has the ability to regulate the cell behaviour in a reproducible manner.[20] Furthermore, advances in topographical fabrication are making nanoscale topographical features achievable in a large scale on more complex materials (traditionally only flat surfaces and small surface areas have been able to be patterned at the nanoscale).[21] The use of topography to guide mesenchymal stem cells (MSCs) may, in fact, play a key role in bone tissue engineering as, unlike chemical and mechanical alterations, topographical modifications do not affect the bulk properties of materials and orthopaedic materials need to be able to support load. The stem cells' ability to adhere and spread into specific surfaces has shown a dramatic effect in cellular development.[22] Osseointegration is the direct contact between bone and the implant, with histological evidence suggesting that new bone is forming around the inert object. The quality and amount of osseointegrated bone around the implant, in addition to other factors such as the degree of inflammation, an excessive force, may affect their stability and consequently their failure rates.[23] Osseointegration and subsequent mineralization is dependent on the initial adhesion of fibrin in blood-mediated osseointegration of osteoblasts or MSCs onto the implant surface.[12,24,25] Failure to achieve osseointegration will lead to premature implant failure and this integration is required to be maintained throughout the implant's lifespan to ensure longevity[26] although patient and surgical related technical/environment factors may also contribute to failure.[27] For instance, among patient factors, male gender, smoking, autoimmune disease and penicillin allergy showed a trend towards greater failure rates.[28–30] Late-stage failure tends to occur as a result of implant overloading, wear and peri-implantitis.[31]

Moreover, implant infection is the most serious issue after surgery. Biomaterial centred infections (BCI) and prosthetic implant infections (PIIs) have a significant contribution in prosthetic implant failure and aseptic loosening[32,33] with the average rate 2%–5%.[34] Host defence mechanisms and current antibiotic treatments become ineffective when bacterial biofilms build up.[35,36] However, Ti is generally considered a very safe and highly biocompatible material that has had extensive clinical use for many decades.

Surface properties

Albrektsson and Wennerberg[37] subdivided the implant surface quality into three categories: mechanical properties, topographical properties and physicochemical properties. They conclude that these characteristics are related and by altering any of these groups, the others will also be affected. With Ti, altering the mechanical properties within the physiological range is hard to achieve and so chemistry and topography are the main focus.[37]

Biological (in the bone forming sense) materials can be roughly classified into three categories: (1) biotolerant materials where a thin fibrous tissue interface is formed; (2) bioinert materials, like Ti, that can have direct bone contact under osteopermissive conditions; and (3) bioactive materials like calcium phosphate ceramics which can have high degree of direct contact bond with the surrounding bone which is believed to be due to the presence of free calcium and phosphate at the implant interface.[38] More recently, these have been re-categorized as first generation (structural, biocompatible), for example, Ti, second generation (bioactive), for example, hydroxyapatite (HA), bioglass and third generation (reproducible molecular control), for example, nanotopography.[39]

Biocompatibility is important to prevent an immune response and foreign body reaction when the material is introduced into the human body.[40] The primary interaction between material and host starts with a thin interface zone, which includes rapid protein adsorption and interaction with the connective tissues. This first interaction is controlled by physical and chemical properties such as roughness, structure, defects and oxide thickness and is critical for long-term implant success.[6,41]

In this review, we will discuss the importance of Ti surface properties on the bioactivity of implants.

Surface wettability

Wettability is measured by contact angle measurement, usually of water, at the solid/liquid interface while surrounded by a gas phase or another liquid phase and provides gross surface characterization. A low contact angle of less than 90° indicates a hydrophilic surface; the liquid will subsequently spread over the surface. A large contact angle of more than 90° signifies that the surface is hydrophobic leading to droplet of liquid forming on the interface. However, this reaction is controlled by the molecular interaction between the different phases.[42,43] Other factors such as surface tension and surface energy are also determined by surface wettability.[44]

Liquids can interact with two different types of solid surfaces: high and low energy solid surfaces. Metals, glass and ceramics are examples of solid surfaces with high energy (hard solids) where molecular liquids achieve complete wetting on these solids. The weak solids like fluorocarbons and hydrocarbons have a low energy, where liquid

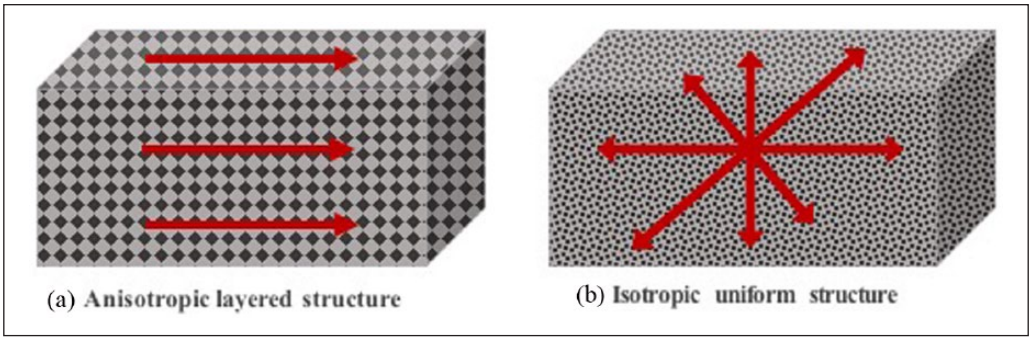

Figure 1. The difference between anisotropic and isotropic surfaces. (a) Anisotropic surfaces have clear directionality, differ considerably in roughness and the materials properties are not the same at all points or directions. (b) Isotropic surfaces have the same topography independent of measuring direction and the physical property is the same at any point/direction through the material.

molecules would take a very low energy to break them providing a complete or partial wetting depending on the liquid chosen.[42,45]

However, increasing the surface wettability may enhance the fibrin adhesion and provide contact guidance for osteoblast migration along the surface.[46] Moreover, any change in surface wettability will affect protein adsorption which consequently changes cell adhesion through integrins and non-integrin receptors.[47]

Surface chemistry

The surface chemistry is an important factor in improving the osseointegration. The chemistry of the surface will dictate the interaction of cells with surface proteins in a number of ways: (1) chemical adsorption including covalent bonds and ionic bonds, (2) electrostatic forces found in electrokinetic potential or zeta potential, (3) hydrogen bonds involved in hydrophilic groups, (4) hydrophobic interaction and (5) van der Waals forces.[48] It is known, for example, that osteoblasts are sensitive to subtle differences in surface chemistry.[49]

For instance, the fluorine-modified implant surface accelerated osseointegration in the early stage of healing which improved the growth of peri-implant tissue, enhanced the adhesion strength and influenced the osteogenesis gene level.[50,51] Also, through control of the oxide chemistry and surface charge, charged antimicrobials may be applied to help fight potential infections.[52] Shibata et al.[53] showed that the TiO formed on the Ti–Cl surface enhanced cell extension and cell growth through a larger adsorption of fibronectin (FN) compared with control, while the $TiCl_3$ contributed to the antibacterial activity of Ti–Cl.

Oxide thickness

Ti, in the presence of air or water, reacts with oxygen to form a protective, chemically stable oxide layer that has the capability to reform immediately after any disturbance. This oxide layer gives the implant increased corrosion resistance, a low rate of ion release and good biocompatibility via plasma protein interactions (e.g. fibrin, fibronectin, vitronectin).[6,54,55] An interesting characteristic of the oxide layer is that it can be induced to provide antibacterial behaviour through light excitation without affecting mammalian cell cytocompatibility.[56] For example, light irradiation of amoxicillin gold nanoparticle composites (amoxi@AuNPs) showed a photo-antimicrobial effect on *Staphylococcus aureus*.[57] Furthermore, studies have shown the importance of the oxide layer thickness in bone formation on implants where bone contact may improve via increasing the oxide layer thickness.[58]

Surface roughness and nanostructure

Surface roughness has a vital role in bone healing and enhancing the biomechanical properties by increasing the mechanical retention (interdigitation) and providing good stress distribution. Surface roughness can be divided into three levels: macro-roughness (*Ra* scale around 10 µm), micro-roughness (*Ra* scale around 1 µm) and nano-roughness (*Ra* scale < 200 nm). *Ra* is an arithmetic average of the absolute values of vertical deviations from a mean plane.[46,59]

Implant roughness can also be classified depending on feature morphology such as concave textures, for example, HA coating/titanium plasma spraying and convex textures, for example, etching and blasting treatments.[60] Another classification of implant roughness is the orientation of surface irregularities such as isotropic surfaces where topographies are independent of direction and anisotropic surfaces that have a clear direction[4,61] (Figure 1).

In cases of poor bone quality and reduced bone volumes, surface roughness is often used in clinical situations to help accelerate and enhance osseointegration and bone interlocking.[46] Previous studies have shown that the optimal *Ra* needs to be around 1–1.5 µm; otherwise, the

implant fixation would be weakened.[62] Increasing surface roughness can, however, via increased surface area, increase the potential of microbial colonization and provide a shelter to bacteria, hence avoiding removal by antibiotics.[26,63] However, previous studies have shown that surface roughness below 0.2 μm was less likely to promote bacterial adhesion as most bacteria are larger in size.[64,65] The above studies, however, really only deal with topography for mechanical integration rather than cellular integration (osseoinduction). Surface topography can indeed influence the rate at which bone is formed next to the surface, and perhaps more so than the surface oxide thickness or microstructure[66] as will be further discussed.

There has been some success with improving secondary cellular fixation, using topographical modifications. Most techniques that are used to produce nanotopography on Ti such as sand-blasting,[67] acid etching,[68,69] cluster deposition,[70] layer-by-layer assembly[71] and anodization[72] generate less defined features, lacking precise control and tunability of the topographies.[73] While such nanoscale features may lead to changes in the cell number, size, focal adhesion arrangements, cytoskeletal and nucleoskeletal organization, reproducible changes may be hard to achieve because of batch to batch variations. More precise control over nanofeatures traditionally requires lithographical techniques that are hard to use with materials such as Ti.[74] However, techniques such as through mask anodization have allowed reproducible features to be created.[73,75,76] Such surfaces can be used to produce highly reproducible cell effects. These surfaces can reduce[73] or increase cell spreading and MSC differentiation in vivo or in vitro.[77–81] Such precise nanotopographical tools will help to dissect the rules of cell-topographical interactions and how they can be useful for work with Ti more simply than using, for example, roughness or random patterns.

Types of coating

There are three ways to change the physical, chemical and mechanical properties of surfaces: (1) by adding a new layer to the surface, (2) by changing the surface itself by exposure to physical or chemical agents like plasma or wet chemicals or (3) by subtraction or attrition process to modify the mechanical surface. However, to achieve the nanoscale modifications it should be able to reach all the topography device surface, change it to reach the commercial scale to be finally industrially integrated.[2,82–84]

In mechanical modification, the changes are required to improve the adhesion, bonding and bio-mineralization by increasing the surface area.[85] Surface mechanical attrition treatment (SMAT) is a novel technique developed to provide a surface roughness at the nanoscale. This increase in the surface roughness leads to increases in the adhesion energy which have a positive reflection on cellular response.[86,87] The cons behind this technique is the flexibility limitation in controlling the intracellular response beyond local adhesion energy.[2] There are three ways for chemical modification for metal surfaces: (1) physiochemical adsorption, (2) molecule covalent binding and (3) peptide inclusion into a carrier material. However, different methods including anodization, oxidative, biochemical functionalization, acid/alkaline treatment, chemical vapour and sol-gel process can affect biologically active moieties onto the surface by controlling the relative densities or arrangement that in turn may have an effect on cell signalling.[2,88–90] Physical modification mainly involves the physical spraying of coating or atomic rearrangement with ion implantation.[91] The common techniques used to change the physical components of the substrate include plasma and vapour deposition, ion implantation, thermal oxidation and laser irradiation. Plasma is the fourth type of matter that highly excites the atoms, ions or radial species. The vacuum deposition is using vacuum condensation of a thin material to coat the substrate, while during the ion implantation, selected ions can be deposited on the material surface. Moreover, the involvement of temperature leads to alteration of the crystal structure of the Ti oxide layer which generates a superficial stress or changing in the previous surface nanostructure.[2] Previous studies showed the effect of various nanosurface modifications on enhancing osteoblast activity, spreading, proliferation, differentiation and osteoconduction.[91–97]

There are three ways of coating: organic, inorganic and combination of both. Organic coating such as polymers, biomimetic and bioinspired films like a component of natural cell surroundings and inorganic components such as calcium phosphate (CaP), HA, titanium oxide (TiO_2) and nitride coating.[82] The combination coating is also divided into many types: by their mode of action, type of biological reagent incorporated with (e.g. antibiotics), type of coating (e.g. biodegradable polymers, hydrogel or bioceramic), coating deposit (layer-by-layer, vacuum deposit or electrophoresis) and the coating function. The antibacterial combination coating was reported and discussed in detail in previous study.[98] Tobin,[99] in his review, discussed the three types of combination device coating: (1) reduced infection either by controlling the kinetics release or coating with low potential to induce microbial resistance, (2) enhanced device integration or (3) reduced infection and enhanced integration. A number of reviews have been published on the combination of different coating on orthopaedic implants[98–101] and dental implants.[46,82]

Moreover, implant coating must meet a number of significant challenging requirements to achieve a successful clinical implementation: for instance, a sufficient mechanical integrity, minimization of the local/systemic cytotoxicity and genotoxicity, sufficient amount of the pharmaceutical or biologic agent in the excipient coating matrix, optimization of diffusion kinetics that are not impeded by attachment of proteins to the implant, broad spectrum of antibiotics against biofilm formation without indication a bacterial resistance, and for the technical parts: the coating should be produced with a low coat, easy

to manufacture, easy handling, long shelf life and ability to sterilize using conventional sterilization techniques without damaging the incorporated drug or biologic agent. However, there is no current coating system that fulfils all of these requirements.[100]

Nevertheless, due to the differences in cell variability related to the species (rat, mouse or human) and cell type (stem cell, osteoblast, etc.), the ability to assess a different kind of coating on the cell structure and function could be challenging. To address this, in the next section (Table 1), we provide selected examples of the impact of different types of coating (organic/non-organic, physical and chemical coating) on Ti surfaces in vivo or in vitro studies.

Surface modification and bacteria

Ti implants are designed to last around 20–25 years but around 10% fail prematurely with the most common cause being bacterial infection in the first year of implantation.[131] Bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* for orthopaedic implants and *Prevotella intermedia, Porphyromonas gingivalis* and *Fusobacterium nucleatum* for dental implants are understood to play a major role in tissue inflammation and subsequent bone recession through peri-implantitis, osteolysis and osteomyelitis leading to premature implant failure.[132,133]

Many of these bacteria have the capability to form biofilms which can potentially occur within hours of initial bacteria attachment to an interface (Figure 2(a)). Primary colonizers such as *Streptococci* attach and proliferate to form microcolonies and secrete self-produced extracellular polymeric substances (EPS) such as proteins, extracellular DNA and exopolysaccharides to form a protective film or matrix. Once a surface has been populated, secondary colonizers, such as *Porphyromonas gingivalis*, are able to adhere to the sessile cells within the biofilm via receptors. Further aggregation, proliferation and EPS production result in a mature, multi-species biofilm containing a range of environments with varying nutrients and oxygen levels, allowing the bacteria to persist for long periods on the surface, causing chronic complications and becoming resistant to antibiotic treatment.[134]

One of the superior qualities of Ti is its ability to absorb calcium, phosphate and serum proteins that are understood to accelerate and support osseointegration. However, such beneficial characteristics may also promote unfavourable processes such as bacterial adhesion.[135]

As implants have no resident microbiota to provide colonization resistance, they are susceptible to attachment by incoming microbes. Ideally, a surface should be designed to have selective activity against different cell types, mammalian cells or bacterial cells. Antiadhesive coatings have been created to repel bacteria from the surface and prevent attachment, thus inhibiting biofilms at the first stage. If this surface was then conversely encouraging host stem cells to adhere, proliferate, mature and differentiate, producing a continual cell layer before bacteria are able attach to the surface, implant infection and biofilm growth will be reduced or inhibited altogether (Figure 2(b)). However, it is important to note that the antiadhesive coating may reduce the mammalian cell attachment; hence, surface modifications combining antiadhesive polymers with cell adhesive motifs (e.g. FN, RGD) would be the ideal solution.[102,113,126,127]

There are two arguments about the effect of surface roughness on bacterial adhesion. The first scenario is that more bacteria adhere as surface microscale roughness increases due to the increased surface area that provides more binding sites and protection. The other argument is that increasing the surface roughness on the nanoscale may provide an unfavourable situation for the bacteria to adhere since the bacteria size is in microscale.

Regarding topography, however, after seminal reports showing that high aspect ratio topographies can kill bacteria, surfaces that can promote osteogenesis and prevent infection are being sought.[136–143] The use of such high aspect features has been demonstrated in Ti in several new reports.[142,143] In fact, it is becoming clear that both physical and chemical parameters play a role in potentially controlling bacterial adhesion (Table 2).

Anti-bacterial, high aspect ratio topographies, in fact, exist in nature. For example, cicada and dragonfly wings have topography that has been shown to be able to disrupt the bacterial membrane leading to cell lysis.[136] Chemical and physical methods are now being developed to fabricate such topographies on clinically relevant materials like titanium, making the prospect of limiting implant infections while inside the body possible and reducing the rates of revision surgery and antibiotic treatment.

A titanium alloy, Ti-6Al-4V, has been developed using thermal oxidation to create a range of titanium dioxide nanostructures and through fluorescence studies, scanning electron microscope (SEM), transmission electron microscope (TEM) and focused ion beam scanning electron microscopy (FIB-SEM) has been shown to disrupt the bacterial membranes, ultimately leading to 40% *E. coli* cell death after 2-h incubation on the surface.[161,162] Furthermore, previous studies showed that the TiO_2 nanowires interact with the lipopolysaccharide and proteins, which are held together by electrostatic interactions with divalent cations. These interactions are essential to stabilize the outer membrane helping the TiO_2 nanowires to form a molecular linkage at the cell surface allowing it to disturb bacteria membrane function which lead to the lysis of the bacteria. However, this is not the case in Gram-positive bacteria, where no antimicrobial activity has been observed as there might be no interaction of TiO_2 nanowires with lipoteichoic acid that is present in the outer membrane of Gram-positive bacteria.[163–165]

Table 1. Impact of different coatings on Ti surfaces.

Coating type	Cell type	Findings	Study
Ti surfaces coated with poly(ethyl acrylate) (PEA) fibronectin (FN) and a low dose of BMP7	In vitro: hMSCs	The current coating showed an improvement in cell adhesion proliferation, differentiation and mineralization on the surfaces coated with PEA/BMP7 in comparison to those coated with BMP7 only	Al-Jarsha et al.[102]
Graphene (G) coating onto a Ti6Al4V surface	In vivo: rabbit femoral condyle defect model	G bioactivity and electrical property (asymmetric nanostructures, rigidity and roughness of a G layer) stimulate the osteogenic differentiation of G-Ti6Al4V implant that improved the initial fixation strength and long-term osteointegration of the implant/bone interface	Li et al.[103]
Polymeric bilayer on Ti, obtained by layering of poly(acrylic acid) (PAA), then chitosan (CS) and gallium (Ga)	In vitro: MG63 osteoblast-like cells	The presence of PAA/CS-Ga bilayer did not affect cell growth. Ga upregulates bone morphogenetic protein (BMP2), a marker of early osteoblastic differentiation	Bonifacio et al.[104]
Chitosan coating on Ti	In vitro: MC3T3-E1 (pre-osteoblasts) and C2C12 myoblasts	Both of cell lines spread successfully on Ti, but only C2C12 cells adhered to chitosan	Gilabert-chirivella et al.[105]
Hydroxyapatite (HA) coating by micro-arc oxidation (MAO) process on Ti	In vitro: Murine pre-osteoblasts (MC3T3-E1) In vivo: rat	MAO provided a composite coating that promoted cell proliferation In rats, the MAO increased the bonding strength between the bone tissues and implant and increased thickness of the oxide layer	Hao et al.[106]
Calcium phosphate (CaP) coatings on the surface of Ti plates	In vitro: human osteoblast-like MG-63 cell line	At concentration 6.0×10^{-3} mol of $Ca(NO_3)_2 \cdot 4H_2O$, 3.6×10^{-3} mol $(NH_4)H_2PO_4$ and 0.1 mol $NaNO_3$ the cells had a spindle shape with thick pseudopodia which provided strong adherence to the rough and porous surfaces	Sun et al.[107]
$CaTiO_3$ screws were implanted with/without HA coating	In vivo study (rabbit)	The $CaTiO_3$ screws showed a higher compatibility and osseointegration compared with the HA-coated screws	Wang et al.[108]
1. Resorbable blast media (RBM) surface treated by HA as a control 2. Calcium and magnesium ions were implanted using plasma immersion ion implantation and deposition (PIIID)	In vitro: Human bone marrow mesenchymal stem cells (hBM-MSCs)	1. PIIID technique changed the surfaces chemistry but not the surface topography 2. Ion implantation either Ca or Mg showed an increase in cell attachment. At concentration 6.0×10^{-3} mol of $Ca(NO_3)_2 \cdot 4H_2O$, 3.6×10^{-3} mol $(NH_4)H_2PO_4$ and 0.1 mol $NaNO_3$ the cells had a spindle shape with thick pseudopodia which provided strong adhesion to the rough and porous surfaces	Won et al.[109]
Ti surfaces activated with piranha solution (a mixture of 1 part 30% H_2O_2 solution) and coated with bone sialoprotein (BSP) via physisorption or covalent coupling via an aminosilane linker (APTES)	In vitro: Primary human osteoblasts (hOBs)	1. No significant difference in cell adhesion on Ti surface coated with BSP via physisorption compared to that of untreated Ti, while BSP application via covalent coupling caused reduction in cell adhesion 2. Ti surfaces coated with higher concentration of BSP increased cell migration 3. ALP activity was reduced in the BSP-coated Ti at the early stages of culture 4. Increase in calcium deposition was noted after 21 days in BSP-coated Ti	Baranowski et al.[110]

(Continued)

Table 1. (Continued)

Coating type	Cell type	Findings	Study
Ti-based Küntscher nails (K-nails) Plates with modified nanostructured coated with HA in a rat model	In vitro: human osteosarcoma cell line (Saos-2/An1) In vivo: rat	Both surface modifications significantly improved cell proliferation and alkaline phosphatase (ALP) activity compared with control Ti plates	Sirin et al.[111]
Graphene oxide–chitosan–HA (GO–CS–HA) particles deposited on Ti substrates	In vitro: human fibroblasts (MG63) *Staphylococcus aureus*	1. GO–CS–HA coatings could improve hydrophilicity of the surfaces and provide effective corrosion protection of the Ti substrate 2. After short culture (5 days), the coating showed no significant cytotoxic effects on MG63 cells 3. This coating could reduce the *Staphylococcus aureus* adhesion	Shi et al.[112]
Sandblasted Ti discs were immobilized with FN peptide: 1. FN (FN-Ti) 2. GRGDSP (Gly-Arg-Gly-Asp-Ser-Pro) (GRGDSP-Ti) 3. PHSRN (Pro-His-Ser-Arg-Asn) (PHSRN-Ti) 4. GRGDSP/PHSRN (GRGDSP/PHSRN-Ti)	In vitro: osteoblast-like cells (MC3T3-E1)	1. FN or FN-derived peptides enhanced cell adhesion and cell proliferation 2. Peptide-modified Ti surfaces provided enhanced osteogenic differentiation 3. FN and GRGDSP/PHSRN coating improved osteo-related gene expression	Pramono et al.[113]
Ca-PO nanostructure synthesis on brush-type Ti-organic nanostructured surface	In vitro: osteoblasts cell line (MS3T3-E1)	Nano-Ti surface with brush-type Ti-organic nanostructures and Ca-PO groups inclusions provided higher osteoblast adhesion	Zemtsova et al.[114]
Graphene oxide (GO)-coated titanium (GO-Ti) substrate compared to sodium titanate (Na-Ti) substrate	In vitro: human periodontal ligament stem cells (PDLSCs)	The proliferation rate, ALP activity and up-regulation of osteogenesis-related markers were higher on GO-Ti compared to Na-Ti	Zhou et al.[115]
Polytetrafluorethylene (PTFE) and Ti nitride (TiN) coatings	In vitro: MG-63 osteoblasts	Ti coated with PTFE showed a delay in cell attachment after 48 h in culture, but after 168 h the cells present had higher viability/proliferation levels, expressed more ALP and osteocalcin (OC), and osteoprotegerin (OPG)/nuclear factor-kappa-B ligand (RANKL) ratio compared to uncoated Ti surface. TiN coating showed no effect on gene expression	Fleischmann et al.[116]
Nano-coated TiO and Ca-HA-coated Ti samples by drop casting with NAFION (sulphonated tetrafluoro-ethylene based fluoropolymer-copolymer) membrane	In vitro: hOS	TiO nanoparticles surfaces showed greater cell adhesion and cell spreading compared to Ca-HA Ti surfaces	Nayar and Chakraverty[117]
Ti soaked in simulated body fluid (SBF) on different time points	In vitro: pre-osteoblast cells (MC3T3-E1)	Ti with nanotubular topography led to a significant increase in apatite-forming ability and enhanced pre-osteoblast MC3T3 cell	Wang et al.[118]
Human placental laminin or synthetic peptides	In vivo: rats In vitro: osteoblast-like cells (HOS and MG-63)	The synthetic peptide promotes bone formation without any detectable antigenic activity in rats. While, in vitro, it showed an enhancement in bone cell function	Yeo et al.[119]
Graphene oxide (Go)-Ti.	In vitro: MC3T3-E1	Go-Ti increased the ALP activity and OCN expression and improved cell differentiation	Zhao et al.[120]

(Continued)

Table 1. (Continued)

Coating type	Cell type	Findings	Study
Poly(ethylene glycol) (PEG) functionalized single-walled carbon nanotubes (SWCNTs) grafted on Ti surfaces	In vitro: Human osteosarcoma (CAL-72)	SWCNTs grafted on Ti had no cytotoxicity effect on osteoblast cells	Pan et al.[121]
Ti nanopores (20-30-50 nm) were prepared by anodization of Ti at 5, 10 and 20 V in a mixture of fluorhydric and acetic acid	In vitro: hMSCs In vivo: implantation in rat tibias	1. Ti30 and Ti50 nanostructures increased early osteoblastic gene differentiation without osteogenic supplements present 2. Ti nanopores enhanced the bone apposition and bone bonding strength in vivo in correlation with in vitro results	Lavenus et al.[80]
Ti-6Al-4V disc surfaces were coated with FN	In vitro: MC3T3-E1 cells (expect high levels of osteoblast differentiation)	At the concentration 1 nmol/L of FN, MC3T3 attachment increased to six- to eightfold compared with uncoated surfaces and increased the osteoblast gene marker expression	Rapuano et al.[122] and Rapuano and MacDonald[123]
Ti implant surfaces modified by laser beam with/without HA	In vivo study (rabbit)	Laser irradiation on Ti surfaces may increase osseointegration	Sisti et al.[124]
Acid-etched titanium (AET) and laser-sintered titanium (LST)	In vitro: human dental pulp (DPSCs) and human osteoblasts	LST drove good levels of osteoblast differentiation from DPSCs with production of bone morphogenetic proteins and growth factors	Mangano et al.[125]
Ti nanopores (30-150-300 nm) were prepared by physical vapour deposition	In vitro: hMSCs	1. The integrins expression, cell morphology and osteoblastic differentiation were affected by nanopores Ti structure 2. Ti30 had more branched cell morphology compared with other surfaces 3. Ti30 and Ti150 nanostructured showed more osteogenic differentiation, while the Ti300 had a limited effect	Lavenus et al.[81]
Ti coated with: 1. HA 2. Type I collagen 3. Arg–Gly–Asp (RGD)–containing peptides	In vitro: Human osteoprogenitor (HOP) cell	Increased HOP cell adhesion was observed	Le Guillou-Buffello et al.[126]
GRGDSP peptide derived from FN coated on to Ti surfaces	In vitro: MC3T3-E1	Peptide-coated Ti surfaces showed an increase in osteoblast-related gene markers	Yamamichi et al.[127]
HA coating on Ti	In vitro: osteoblast-like rat cells	Cellular attachment to HA surfaces was slightly higher than titanium surfaces. HA crystallinity had no influence over initial cell adhesion in treated or control surfaces	Chang et al.[128]
Calcium ion (Ca^{2+})-implanted Ti	In vivo study (rat)	New bone formed on Ti treated with Ca ions compared to untreated, with increased osteospecific gene expression	Hanawa et al.[129]
Polystyrene culture dishes were coated with a 300 Å titanium layer via electron beam evaporation followed by coating with glycine-phenylalanine-hydroxyproline-glycine-glutamate-arginine (GFOGER) peptide	In vitro: hBM-MSCs In vivo: rat cortical bone implant	1. GFOGER on Ti enhanced osteoblastic differentiation and mineral deposition in hBM-MSCs, which lead to improvement of the osteoblastic function compared to unmodified Ti 2. The current coating significantly improved in vivo peri-implant bone regeneration and osseointegration 3. GFOGER-modified implants significantly triggered osseointegration compared to surfaces modified with full-length type I collagen	Reyes et al.[130]

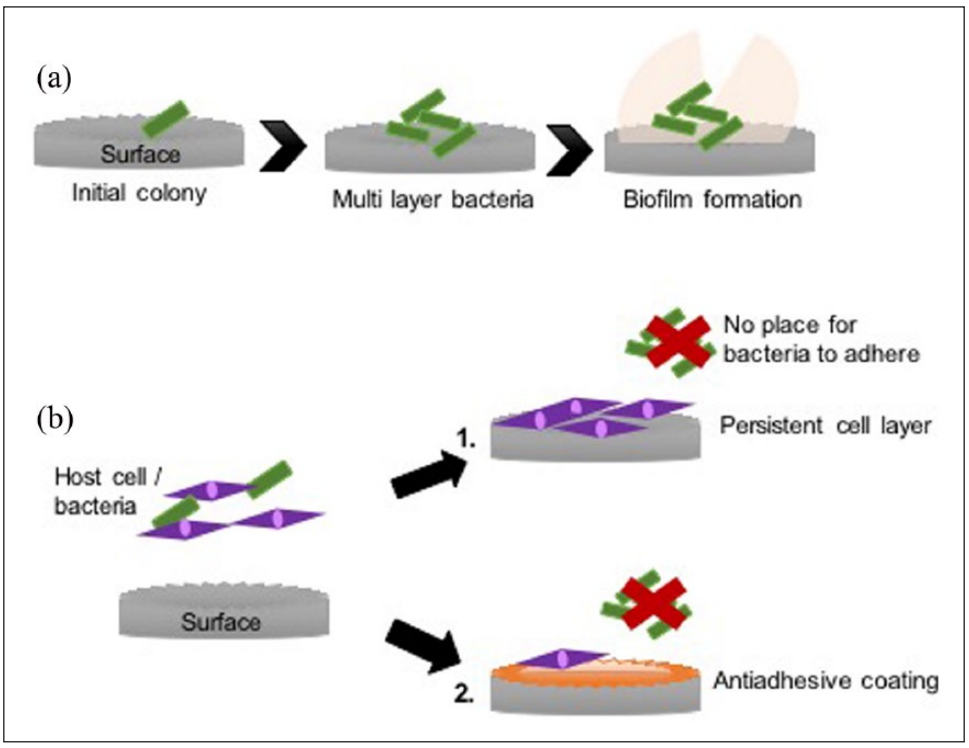

Figure 2. (a) The process of biofilm formation. Initially, cells attach, proliferate and coadhere to form microcolonies. They then continue to expand in similar fashion, together with production of EPS, to form a mature biofilm community. (b) Two possible ways to reduce implant infection: (1) provide no place for bacteria due to a continuous cell layer on the substrate and (2) use an antiadhesive coating that prevent bacterial attachment.

Hydrothermal etching has been used to create topography in the micron range to produce a hierarchically ordered array shown to physically rupture *S. aureus* and *P. aeruginosa* cells leading to loss of viability seen in *P. aeruginosa*; 47.1% death compared to *S. aureus* with 19.8% death after 18 h of incubation.[166] A chlorine base etching process has also been reported to form anisotropic nanostructures on the surface of titanium with a height of approximately 1 µm. The morphology of *P. aeruginosa* and *S. aureus* was significantly altered which correlated well with fluorescence studies showing high bactericidal activity for the Gram-negative bacteria with 98%±2% for *P. aeruginosa* and 95%±5% for *E. coli* after 4 h. For the Gram-positive bacteria *S. aureus*, there was less killing, with 22%±8% non-viable cells after 4 h, but this increased to 76%±4% after 24 h.[139]

Alkaline hydrothermal processes use sodium hydroxide, high temperatures and pressures to form titanium dioxide nano- and microscale topography on titanium substrates. Using electron microscopy, bacterial cell envelopes have been shown to be pierced by these spikes.[161] Using fluorescence microscopy, loss of viability has been reported when in contact with this nanotopography.[141,142,167] Diu et al.[167] reported that motile bacteria (*P. aeruginosa, E. coli* and *B. subtilis*) were more liable to lysis with more than 50% cell death in the first hour while non-motile bacteria (*S. aureus, E. faecalis* and *K. pneumoniae*) experienced less than 5%. Not only has membrane disruption been seen but anti-biofilm activity has also been shown. Different structures of nanotopography have also been formed using alkaline hydrothermal method, a 'spear-type' topography and 'pocket-type' topography. After 6 days, there was half as much growth on the spear-type and five times less on the pocket-type compared to a control of flat-polished titanium.[141] Tsimbouri et al.[142] reported ~30% bacterial death after 1-h incubation of *P. aeruginosa*, which increased to 58% after 18 h incubation.

Along with having a bactericidal surface, it is important to ensure mammalian cells are able to attach, proliferate, mature and differentiate into desired lineages such as osteoblasts to promote successful osseointegration. Research suggests that the nanotopography is able to support osteoblast maturation through expression of osteogenic marker proteins such as Runt-related transcription factor (RUNX-2), BMP2, osteocalcin (OCN) and osteopontin (OPN).[139,142,143,166,167]

To improve osteointegration, various coatings have been utilized; for example, integrin-binding peptidic ligands have been functionalized onto nanotopographies and shown to significantly increase human mesenchymal stem cells (hMSCs) surface area and decrease the cell's circularity evidence of improving surface interaction.[143]

Table 2 highlights various studies where titanium surfaces have been modified to reduce bacterial adhesion. Coatings with metals such as copper, gallium and silver

Table 2. Examples of the effect of different coatings on bacterial adhesion.

Ti treatment	Model bacteria	Findings	Study
A combination of silver, TiO_2 and hydroxyapatite (HA) nanocoatings	*S. sanguinis*	A dual layer of silver-HA showed a significant reduction in biofilm formation compared with uncoated Ti or TiO_2 nanocoatings	Besinis et al.[144]
Polymeric bilayers on Ti, obtained by layers of poly(acrylic acid) (PAA), then Chitosan (CS) and Gallium (Ga)	*E. coli, P. aeruginosa*	The PAA-CS-Ga coatings released Ga(III) ions which has an antimicrobial effect	Bonifacio et al.[104]
TiAl6V4 coated with multi-walled carbon nanotube (MWCNT) and impregnated with rifampicin antibiotic	*S. epidermidis*	CNTs are biologically compatible and can be utilized as drug delivery systems. MWCNT-modified surfaces showed a significant inhibition of biofilm formation up to 5 days culture	Hirschfeld et al.[145]
Ti-O or Ti-I (iodine)	*S. aureus*	Ti surfaces coated with iodine showed a significant growth inhibition compared to Ti or Ti-O	Inoue et al.[146]
Ti-copper oxide (TiCuO) coating	*S. epidermidis*	TiCuO can act as an antibacterial environment while remaining relatively nontoxic to a human osteoblast cell line	Norambuena et al.[147]
Polyhydroxybutyrate (PHB) and its copolymer, polyhydroxybutyrate-co-hydroxyvalerate (PHBV) and gentamicin antibiotic	*E. coli, S. aureus*	PHBV coatings showed a faster degradation and more stable drug release (gentamicin) than PHB	Rodríguez-Contrerasa et al.[148]
Ti surfaces coated with three layers: nanocrystalline HA, silver nanoparticles and calcium phosphate (either 150 or 1000 nm thick)	*E. coli*	An antimicrobial effect against *E. coli* was found with a 150 nm thick outer layer of the calcium phosphate	Surmeneva et al.[149]
Polydopamine coating with silver nanoparticles on TiO_2 nanotube arrays (Ag-PDA-TiO_2)	*E. coli*	The antibacterial effect of Ag-PDA-TiO_2 lasted longer than Ag-PDA-TiO or Ag-TiO_2 (UV) effect	Xu et al.[150]
Microgroove titanium functionalized with the AMP GL13K	*P. gingivalis*	Reduced the adhesion of bacteria over 72 h and promoted and adhesion and proliferation of human gingival fibroblasts	Zhou et al.[151]
Titanium nanotubes coated with calcium phosphate and phospholipid impregnated with the AMP HHC-36	*S. aureus, P. aeruginosa*	Able to kill bacteria and reduce adhesion to surface over 24 h	Kazemzadeh-Narbat et al.[152]
Chimeric peptides functionalized onto titanium surfaces	*S. oralis, S. gordonii, S. sanguinis*	Functionalized surfaced showed antibacterial and anti-biofilm capabilities along with cyto-compatibility	Geng et al.[153]
Deoxyribonuclease I (DNase I)	*S. mutans, S. aureus*	DNase I coating showed significant prevention of bacterial biofilms over a time of 24 h	Ye et al.[154]
Ti surface coated with pure magnesium	*S. epidermidis*	Colony forming unit (CFU) counts decreased over time	Zaatreh et al.[155]
Melamine (cationic peptide)	*S. aureus, P. aeruginosa*	Melamine treatment significantly inhibited biofilm formation by *P. aeruginosa* by up to 62% and *S. aureus* by up to 84% on the Ti substrates	Chen et al.[156]
Cubic yttria-stabilized zirconia (YSZ) and Ag-YSZ nanocomposite films were deposited on Ti–6Al–V	*S. aureus, S. epidermidis*	The Ag-YSZ combination is a potential candidate for clinical application due to the broad-spectrum antimicrobial activity and low risk of resistance development to silver nanoparticles	Pérez-Tanoira et al.[157]
Ti coated with covalent immobilized alkaline phosphates (ALP) on carboxymethyl chitosan (CMCS)-coated polydopamine (PDA)	*S. epidermidis*	This coating caused almost 89% reduction of the bacterial adhesion compared with uncoated surfaces	Zheng et al.[158]
Ti coated with phosphatidylcholine mixed with amikacin or vancomycin or a combination of both	*S. aureus, P. aeruginosa*	Antibiotic-loaded coatings inhibited biofilm formation	Jennings et al.[159]
Ti, zirconia and resin coated with saliva	*S. sanguinis*	Resin has a higher bacterial adhesion compared to Ti and zirconia	Lee et al.[160]

with well-documented antimicrobial properties have shown to reduce biofilm formation of various bacteria.[104,144,147,149–151] Antibiotics such as gentamicin are widely used in the treatment of both Gram-positive and Gram-negative bacteria and have been shown to have potential to be used as a coating on titanium surfaces.[141] Antimicrobial peptides (AMPs) are recognized as promising candidates as alternatives for antibiotics due to the low chance of resistance being induced. Various AMPs have been functionalized on titanium such as HHC-36, GL13K and TBP-1 and have shown potential by reducing biofilm formation of both Gram-positive and Gram-negative bacteria.[151–153]

Conclusion

The main aim of bone implant industry is to mimic the normal function of tissue by enhancing the implant biocompatibility and reduce the bacterial adhesion while providing mechanical support. Recently, the coating of Ti implants has generated much interest in order to improve osseointegration and prevent unfavourable tissue reactions such as infection, inflammation and the foreign body response. Besides that, coated implants must be shown to be safe, efficient and cost-effective prior to subsequent adoption and widespread usage. Osseointegration/biofilm reduction are required goals; coating the implant with organic/inorganic components, changing the surface topography, and so on have been shown to be efficacious. In addition, the coating composition, location, thickness, uniformity and other physico-chemical variables are important to determine the efficacy and validity of the different coating.

This article aims to provide an overview of the impact of different physical and chemical modifications on Ti surface topography. Such alterations can potentially be used to enhance bone formation, provide bacterial growth inhibition or even perhaps both. That implant surface characteristics including surface roughness, surface chemistry, nanotopography to list a few, have a significant influence on osteogenesis and microbiota inhibition is emerging. Previous studies have implicated high aspect ratio nanofeatures with bactericidal potential. While an ideal implant would be both osseoinductive and antimicrobial, many aspects of these interactions require more investigation to resolve areas of uncertainty surrounding the interaction between these surfaces and MSCs when combined with bacteria. To conclude, the potential of Ti surface modifications is largely due to the ageing population placing pressure on orthopaedic treatments and Ti being the gold standard for fabrication on dental or orthopaedic implants.

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References

1. Oldani C and Dominguez A. Titanium as a biomaterial for implants. In: Fokter S (ed.) *Recent advances in arthroplasty*. Arthroplast InTech, 2012, pp. 149–162.
2. Staruch R, Griffin M and Butler P. Nanoscale surface modifications of orthopaedic implants: state of the art and perspectives. *Open Orthop J* 2016; 10: 920–938.
3. Saini M, Singh Y, Arora P, et al. Implant biomaterials: a comprehensive review. *World J Clin Cases* 2015; 3(1): 52–57.
4. Ananth H, Kundapur V, Mohammed HS, et al. A review on biomaterials in dental implantology. *Int J Biomed Sci* 2015; 11(3): 113–120.
5. Li Y, Yang C, Zhao H, et al. New developments of Ti-based alloys for biomedical applications. *Materials* 2014; 7(3): 1709–1800.
6. Civantos A, Martínez-Campos E, Ramos V, et al. Titanium coatings and surface modifications: toward clinically useful bioactive implants. *ACS Biomater Sci Eng* 2017; 3(7): 1245–1261.
7. Neoh KG, Hu X, Zheng D, et al. Balancing osteoblast functions and bacterial adhesion on functionalized titanium surfaces. *Biomaterials* 2012; 33(10): 2813–2822.
8. De Jonge LT, Leeuwenburgh SCG, Wolke JGC, et al. Organic-inorganic surface modifications for titanium implant surfaces. *Pharm Res* 2008; 25(10): 2357–2369.
9. Braceras I, Alava JI, Goikoetxea L, et al. Interaction of engineered surfaces with the living world: ion implantation vs. osseointegration. *Surf Coat Tech* 2007; 201(19–20): 8091–8098.
10. Asri RIM, Harun WSW, Samykano M, et al. Corrosion and surface modification on biocompatible metals: a review. *Mater Sci Eng C* 2017; 77: 1261–1274.
11. Koh C and Atala A. Tissue engineering, stem cells, and cloning: opportunities for regenerative medicine. *J Am Soc Nephrol* 2004; 15(5): 1113–1125.
12. Sansone V, Pagani D and Melato M. The effects on bone cells of metal ions released from orthopaedic implants.

A review. *Clin Cases Miner Bone Metab* 2013; 10(1): 34–40.
13. Wang JX, Fan YB, Gao Y, et al. TiO2 nanoparticles translocation and potential toxicological effect in rats after intraarticular injection. *Biomaterials* 2009; 30(27): 4590–4600.
14. Daley B, Doherty AT, Fairman B, et al. Wear debris from hip or knee replacements causes chromosomal damage in human cells in tissue culture. *J Bone Joint Surg Br* 2004; 86(4): 598–606.
15. Egusa H, Ko N, Shimazu T, et al. Suspected association of an allergic reaction with titanium dental implants: a clinical report. *J Prosthet Dent* 2008; 100(5): 344–347.
16. Lin L, Wang H, Ni M, et al. Enhanced osteointegration of medical titanium implant with surface modifications in micro/nanoscale structures. *J Orthop Transl* 2014; 2(1): 35–42.
17. Bagno A and Di Bello C. Surface treatments and roughness properties of Ti-based biomaterials. *J Mater Sci Mater Med* 2004; 15(9): 935–949.
18. Xiao J, Zhou H, Zhao L, et al. The effect of hierarchical micro/nanosurface titanium implant on osseointegration in ovariectomized sheep. *Osteoporos Int* 2011; 22(6): 1907–1913.
19. Citeau A, Guicheux J, Vinatier C, et al. In vitro biological effects of titanium rough surface obtained by calcium phosphate grid blasting. *Biomaterials* 2005; 26(2): 157–165.
20. Spatz JP and Geiger B. Molecular engineering of cellular environments: cell adhesion to nano-digital surfaces. *Methods Cell Biol* 2007; 83(1): 89–111.
21. Anderson HJ, Sahoo JK, Ulijn RV, et al. Mesenchymal stem cell fate: applying biomaterials for control of stem cell behavior. *Front Bioeng Biotechnol* 2016; 4: 38.
22. McBeath R, Pirone DM, Nelson CM, et al. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev Cell* 2004; 6(4): 483–495.
23. Kuroda S, Yamada K, Deguchi T, et al. Root proximity is a major factor for screw failure in orthodontic anchorage. *Am J Orthod Dentofac Orthop* 2007; 131(4 Suppl): 68–73.
24. Shiu HT, Goss B, Lutton C, et al. Formation of blood clot on biomaterial implants influences bone healing. *Tissue Eng Part B Rev* 2014; 20(6): 697–712.
25. Kasner E, Hunter CA, Ph D, et al. Implant osseointegration and the role of microroughness and nanostructures: lessons for spine implants. *Acta Biomater* 2014; 10(8): 3363–3371.
26. Pier-Francesco A, Adams RJ, Waters MGJ, et al. Titanium surface modification and its effect on the adherence of *Porphyromonas gingivalis*: an in vitro study. *Clin Oral Implants Res* 2006; 17(6): 633–637.
27. Pye AD, Lockhart DEA, Dawson MP, et al. A review of dental implants and infection. *J Hosp Infect* 2009; 72(2): 104–110.
28. Borba M, Deluiz D, Lourenço EJV, et al. Risk factors for implant failure: a retrospective study in an educational institution using GEE analyses. *Braz Oral Res* 2017; 31: e69.
29. French D, Larjava H and Ofec R. Retrospective cohort study of 4591 Straumann implants in private practice setting, with up to 10-year follow-up. Part 1: multivariate survival analysis. *Clin Oral Implants Res* 2015; 26(11): 1345–1354.
30. Becker ST, Beck-Broichsitter BE, Rossmann CM, et al. Long-term survival of Straumann dental implants with TPS surfaces: a retrospective study with a follow-up of 12 to 23 years. *Clin Implant Dent Relat Res* 2016; 18(3): 480–488.
31. Heijdenrijk K, Raghoebar GM, Meijer HJ, et al. Two-part implants inserted in a one-stage or a two-stage procedure. A prospective comparative study. *J Clin Periodontol* 2002; 29(10): 901–909.
32. Brady RA, Calhoun JH, Leid JG, et al. Infections of orthopaedic implants and device. In: Shirtliff M and Leid JG (eds) *The role of biofilms in device-related infections*. Berlin: Springer, 2008, pp. 15–55.
33. Gottenbos B, Busscher HJ, Van Der Mei HC, et al. Pathogenesis and prevention of biomaterial centered infections. *J Mater Sci Mater Med* 2002; 13(8): 717–722.
34. Rabih O, Darouiche MD and Darouiche RO. Treatment of infections associated with surgical implants. *N Engl J Med* 2004; 350(14): 1422–1429.
35. Gray ED, Verstegen M, Peters G, et al. Effect of extracellular slime substance from *Staphylococcus epidermidis* on the human cellular immune response. *Lancet* 1984; 323(8373): 365–367.
36. Duguid IG, Evans E, Brown MRW, et al. Effect of biofilm culture upon the susceptibility of *Staphylococcus epidermidis* to tobramycin. *J Antimicrob Chemother* 1992; 30(6): 803–810.
37. Albrektsson T and Wennerberg A. Oral implant surfaces: part 1 – review focusing on topographic and chemical properties of different surfaces and in vivo responses to them. *Int J Prosthodont* 2004; 17(5): 536–543.
38. Hench LL. The story of Bioglass®. *J Mater Sci Mater Med* 2006; 17(11): 967–978.
39. Hanson ET, Lewis RL, Auerbach R, et al. Third-generation biomedical materials. *Science* 2002; 295: 1014–1017.
40. Bostman O, Hirvensalo E and Makinen J. Foreign-body reactions to fracture fixation implants of biodegradable synthetic polymers. *J Bone Joint Surg Br* 1990; 72(4): 592–596.
41. Henkel J, Woodruff MA, Epari DR, et al. Bone regeneration based on tissue engineering conceptions – a 21st century perspective. *Bone Res* 2013; 1(3): 216–248.
42. Choi C and Kim M. Wettability effects on heat transfer. In: Ahsan A (ed.) *Two phase flow, phase change and numerical modeling*. Intechopen, 2010, pp. 311–341.
43. Shafrin EG and Zisman WA. Constitutive relations in the wetting of low energy surfaces and the theory of the retraction method of preparing monolayers. *J Phys Chem* 1960; 64(5): 519–524.
44. Yuan Y and Lee TR. Contact angle and wetting properties. In: Bracco G and Holst B (eds) *Surface science techniques*. Berlin: Springer, 2013, pp. 3–34.
45. De Gennes PG. Wetting: statics and dynamics. *Rev Mod Phys* 1985; 57(3): 827–863.
46. Le Guéhennec L, Soueidan A, Layrolle P, et al. Surface treatments of titanium dental implants for rapid osseointegration. *Dent Mater* 2007; 23(7): 844–854.

47. Mendonça G, Mendonça DBS, Aragão FJL, et al. Advancing dental implant surface technology – from micron- to nanotopography. *Biomaterials* 2008; 29(28): 3822–3835.
48. Sasaki K, Osamu S and Takahashi N. Surface modification of dental implant improves implant–tissue interface. In: Sasaki K, Suzuki O and Takahashi N (eds) *Interface oral health science*. Tokyo: Springer, 2015, pp. 33–44.
49. Boyan BD, Hummert TW, Dean DD, et al. Role of material surfaces in regulating bone and cartilage cell response. *Biomaterials* 1996; 17(2): 137–146.
50. Berglundh T, Abrahamsson I, Albouy JP, et al. Bone healing at implants with a fluoride-modified surface: an experimental study in dogs. *Clin Oral Implants Res* 2007; 18(2): 147–152.
51. Isa ZM, Schneider GB, Zaharias R, et al. Effects of fluoride-modified titanium surfaces on osteoblast proliferation and gene expression. *Int J Oral Maxillofac Implants* 2005; 21(2): 203–211.
52. Dunn DS, Raghavan S and Volz RG. Anodized layers on titanium and titanium alloy orthopedic materials for antimicrobial activity applications. *Mater Manuf Process* 1992; 7(1): 123–137.
53. Shibata Y, Kawai H, Yamamoto H, et al. Antibacterial titanium plate anodized by being discharged in NaCl solution exhibits cell compatibility. *J Dent Res* 2004; 83: 115–119.
54. Tejero R, Anitua E and Orive G. Toward the biomimetic implant surface: biopolymers on titanium-based implants for bone regeneration. *Prog Polym Sci* 2014; 39(7): 1406–1447.
55. Morra M, Cassinelli C, Cascardo G, et al. Surface engineering of titanium by collagen immobilization. Surface characterization and in vitro and in vivo studies. *Biomaterials* 2003; 24(25): 4639–4654.
56. Cao H and Liu X. Activating titanium oxide coatings for orthopedic implants. *Surf Coat Technol* 2013; 233: 57–64.
57. Silvero C, Rocca MJ, de la Villarmois DM, et al. Selective photoinduced antibacterial activity of amoxicillin-coated gold nanoparticles: from one-step synthesis to in vivo cytocompatibility. *ACS Omega* 2018; 3(1): 1220–1230.
58. Sul YT, Johansson CB, Kang Y, et al. Bone reactions to oxidized titanium implants with electrochemical anion sulphuric acid and phosphoric acid incorporation. *Clin Implant Dent Relat Res* 2002; 4(2): 78–87.
59. Krishna Alla R, Ginjupalli K, Upadhya N, et al. Surface roughness of implants: a review. *Trends Biomater Artif Organs* 2011; 25(3): 112–118.
60. Mehta R, Panda S, Nanda S, et al. Implant surface modification and osseointegration – past, present and future. *J Oral Heal Community Dent* 2014; 8: 113–118.
61. Wennerberg A and Albrektsson T. On implant surfaces: a review of current knowledge and opinions. *Int J Oral Maxillofac Implants* 2009; 25(1): 63–74.
62. Wennerberg A, Albrektsson T and Wennerberg AAT. Suggested guidelines for the topographic evaluation of implant surfaces. *Int J Oral Maxillofac Implants* 2000; 15(3): 331–344.
63. Sahib A, Al-radha D, Dymock D, et al. Surface properties of titanium and zirconia dental implant materials and their effect on bacterial adhesion. *J Dent* 2011; 40: 146–153.
64. Quirynen M and Bollen CM. The influence of surface roughness and surface-free energy on supra- and subgingival plaque formation in man. *J Clin Periodontal* 1995; 22: 1–14.
65. Bolle C, Lambrechts P and Quirynen M. Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: a review of the literature. *Dent Mater* 1997; 13: 258–269.
66. Larsson C, Thomsen P, Lausmaa J, et al. Bone response to surface modified titanium implants: studies on electropolished implants with different oxide thicknesses and morphology. *Biomaterials* 1994; 15(13): 1062–1074.
67. Yang G, He F and Yang X. Bone responses to titanium implants surface-roughened by sandblasted and double etched treatments in a rabbit. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008; 106: 516–524.
68. Nishimura I, Huang Y, Butz F, et al. Discrete deposition of hydroxyapatite nanoparticles on a titanium implant with predisposing substrate microtopography accelerated osseointegration. *Nanotechnology* 2007; 18: 245101.
69. Oliveira PT, De Zalzal SF, Beloti MM, et al. Enhancement of in vitro osteogenesis on titanium by chemically produced nanotopography. *J Biomed Mater Res A* 2006; 80A: 554–564.
70. Carbone R, Marangi I, Zanardi A, et al. Biocompatibility of cluster-assembled nanostructured TiO_2 with primary and cancer cells. *Biomaterials* 2006; 27: 3221–3229.
71. Kommireddy DS, Sriram SM, Lvov YM, et al. Stem cell attachment to layer-by-layer assembled TiO2 nanoparticle thin films. *Biomaterials* 2006; 27: 4296–4303.
72. Huang H, Pan S, Lai Y, et al. Osteoblast-like cell initial adhesion onto a network-structured titanium oxide layer 2004; 51: 1017–1021.
73. Sjo T, Dalby MJ, Hart A, et al. Fabrication of pillar-like titania nanostructures on titanium and their interactions with human skeletal stem cells. *Acta Biomater* 2009; 5: 1433–1441.
74. Dalby MJ, Gadegaard N and Oreffo ROC. Harnessing nanotopography and integrin-matrix interactions to influence stem cell fate. *Nat Mater* 2014; 13(6): 558–569.
75. Sjöström T, Mcnamara LE, Meek RMD, et al. 2D and 3D nanopatterning of titanium for enhancing osteoinduction of stem cells at implant surfaces. *Adv Healthc Mater* 2013; 2: 1285–1293.
76. Sjöström T, Fox N and Su B. Through-mask anodization of titania dot- and pillar-like nanostructures on bulk Ti substrates using a nanoporous anodic alumina mask. *Nanotechnology* 2009; 20(13): 135305.
77. Mcnamara LE, Sjöström T, Seunarine K, et al. Investigation of the limits of nanoscale filopodial interactions. *J Tissue Eng*. Epub ahead of print 13 May 2014. DOI: 10.1177/2041731414536177.
78. Mcnamara LE, Sjöström T, Burgess KEV, et al. Skeletal stem cell physiology on functionally distinct titania nanotopographies. *Biomaterials* 2011; 32: 7403–7410.
79. Dalby MJ, García AJ and Salmeron-Sanchez M. Receptor control in mesenchymal stem cell engineering. *Nat Rev Mater* 2018; 3: 17091.
80. Lavenus S, Trichet V, Le Chevalier S, et al. Cell differentiation and osseointegration influenced by nanoscale

anodized titanium surfaces. *Nanomedicine* 2012; 7(7): 967–980.
81. Lavenus S, Berreur M, Trichet V, et al. Adhesion and osteogenic differentiation of human mesenchymal stem cells on titanium nanopores. *Eur Cell Mater* 2011; 22: 84–96.
82. Mandracci P, Mussano F, Rivolo P, et al. Surface treatments and functional coatings for biocompatibility improvement and bacterial adhesion reduction in dental implantology. *Coatings* 2016; 6(1): 7.
83. Varíola F, Vetrone F, Richert L, et al. Improving biocompatibility of implantable metals by nanoscale modification of surfaces: an overview of strategies, fabrication methods, and challenges. *Small* 2009; 5(9): 996–1006.
84. Variola F, Yi JH, Richert L, et al. Tailoring the surface properties of Ti6Al4V by controlled chemical oxidation. *Biomaterials* 2008; 29(10): 1285–1298.
85. Lausmaa J. Mechanical, thermal, chemical and electrochemical surface treatment of titanium. In: *Titanium in medicine: material science, surface science, engineering, biological responses and medical applications*. Berlin; Heidelberg: Springer, 2001. pp. 231–266, https://doi.org/10.1007/978-3-642-56486-4_8
86. Lu K and Lu J. Nanostructured surface layer on metallic materials induced by surface mechanical attrition treatment. *Mater Sci Eng A* 2004; 375–377(1–2): 38–45.
87. Zhang HW, Hei ZK, Liu G, et al. Formation of nanostructured surface layer on AISI 304 stainless steel by means of surface mechanical attrition treatment. *Acta Mater* 2003; 51(7): 1871–1881.
88. Ginsberg MH and Rd P. Arginyl-glycyl-aspartic acid (RGD): a cell adhesion motif. *Trends Biochem Sci* 1991; 16: 246–250.
89. Mark K, Von Der Park J and Bauer S. Nanoscale engineering of biomimetic surfaces: cues from the extracellular matrix. *Cell Tissue Res* 2010; 339: 131–153.
90. Thoneick M and Jansen JA. Effects of implant surface coatings and composition on bone integration: a systematic review. *Clin Oral Implants Res* 2009; 20: 185–206.
91. Reising A, Yao C, Storey D, et al. Greater osteoblast long-term functions on ionic plasma deposited nanostructured orthopedic implant coatings. *J Biomed Mater Res A* 2007; 87: 78–83.
92. Ogawa T, Saruwatari L, Takeuchi K, et al. Ti nano-nodular structuring for bone integration and regeneration. *J Dent Res* 2008; 87: 751–756.
93. Hovgaard MB, Rechendorff K, Chevallier J, et al. Fibronectin adsorption on tantalum: the influence of nano-roughness. *J Phys Chem B* 2008; 112: 8241–8249.
94. Herzog M, Du A and Hannig M. Focal adhesion contact formation by fibroblasts cultured on surface-modified dental implants: an in vitro study. *Clin Oral Implants Res* 2006; 17(6): 736–745.
95. Puckett SD, Taylor E, Raimondo T, et al. Biomaterials the relationship between the nanostructure of titanium surfaces and bacterial attachment. *Biomaterials* 2010; 31(4): 706–713.
96. Munirathinam B and Neelakantan L. Titania nanotubes from weak organic acid electrolyte: fabrication, characterization and oxide film properties. *Mater Sci Eng C* 2015; 49: 567–578.
97. Mariscal-muñoz E, Costa CAS, Tavares HS, et al. Osteoblast differentiation is enhanced by a nano-to-micro hybrid titanium surface created by Yb: YAG laser irradiation. *Clin Oral Investig* 2016; 20: 503–511.
98. Romanò CL, Scarponi S, Gallazzi E, et al. Antibacterial coating of implants in orthopaedics and trauma: a classification proposal in an evolving panorama. *J Orthop Surg Res* 2015; 10(1): 157.
99. Tobin EJ. Recent coating developments for combination devices in orthopedic and dental applications: a literature review. *Adv Drug Deliv Rev* 2017; 112: 88–100.
100. Goodman SB, Yao Z, Keeney M, et al. The future of biologic coatings for orthopaedic implants. *Biomaterials* 2013; 34(13): 3174–3183.
101. Zhang BGX, Myers DE, Wallace GG, et al. Bioactive coatings for orthopaedic implants-recent trends in development of implant coatings. *Int J Mol Sci* 2014; 15(7): 11878–11921.
102. Al-Jarsha M, Moulisová V, Leal-Egaña A, et al. Engineered coatings for titanium implants to present ultralow doses of BMP-7. *ACS Biomater Sci Eng* 2018; 4(5): 1812–1819.
103. Li K, Wang C, Yan J, et al. Evaluation of the osteogenesis and osseointegration of titanium alloys coated with graphene: an in vivo study. *Sci Rep* 2018; 8(1): 1843.
104. Bonifacio MA, Cometa S, Dicarlo M, et al. Gallium-modified chitosan/poly(acrylic acid) bilayer coatings for improved titanium implant performances. *Carbohydr Polym* 2017; 166: 348–357.
105. Gilabert-chirivella E, Pérez-feito R, Ribeiro C, et al. Chitosan patterning on titanium implants. *Prog Organ Coat* 2017; 111: 23–28.
106. Hao J, Li Y, Wang X, et al. Corrosion resistance and biological properties of a micro–nano structured Ti surface consisting of TiO2 and hydroxyapatite. *RSC Adv* 2017; 7(53): 33285–33292.
107. Sun Q, Yang Y, Luo W, et al. The influence of electrolytic concentration on the electrochemical deposition of calcium phosphate coating on a direct laser metal forming surface. *Int J Anal Chem* 2017; 2017: 8610858.
108. Wang Z, He R, Tu B, et al. Enhanced biocompatibility and osseointegration of calcium titanate coating on titanium screws in rabbit femur. *J Huazhong Univ Sci Technol Med Sci* 2017; 37: 362–370.
109. Won S, Huh Y-H, Cho L-R, et al. Cellular response of human bone marrow derived mesenchymal stem cells to titanium surfaces implanted with calcium and magnesium ions. *Tissue Eng Regen Med* 2017; 14(2): 123–131.
110. Baranowski A, Klein A, Ritz U, et al. Surface functionalization of orthopedic titanium implants with bone sialoprotein. *PLoS ONE* 2016; 11(4): e0153978.
111. Sirin HT, Vargel I, Kutsal T, et al. Ti implants with nanostructured and HA-coated surfaces for improved osseointegration. *Artif Cells Nanomedicine Biotechnol* 2016; 44(3): 1023–1030.
112. Shi YY, Li M, Liu Q, et al. Electrophoretic deposition of graphene oxide reinforced chitosan-hydroxyapatite nanocomposite coatings on Ti substrate. *J Mater Sci Mater Med* 2016; 27: 48.
113. Pramono S, Pugdee K, Suwanprateep J, et al. Sandblasting and fibronectin-derived peptide immobilization on titanium surface increase adhesion and differentiation of

osteoblast-like cells (MC3T3-E1). *J Dent Sci* 2016; 11(4): 427–436.
114. Zemtsova EG, Morozov PE, Valiev RZ, et al. The synthesis of titanium-organic nanostructures on nanotitanium surface for biocompatible coating development. *Rev Adv Mater Sci* 2016; 45(1–2): 59–66.
115. Zhou Q, Yang P, Li X, et al. Bioactivity of periodontal ligament stem cells on sodium titanate coated with graphene oxide. *Sci Rep* 2016; 6(1): 19343.
116. Fleischmann L, Crismani A, Falkensammer F, et al. Behavior of osteoblasts on Ti surface with two different coating designed for orthodontic devices. *J Mater Sci Mater Med* 2015; 26(1): 5335.
117. Nayar S and Chakraverty S. A comparative study to evaluate the osteoblastic cell behavior of two nano coated titanium surfaces with NAFION stabilized the membrane. *J Indian Prosthodont Soc* 2015; 15(1): 33–38.
118. Wang H, Lai YK, Zheng RY, et al. Tuning the surface microstructure of titanate coatings on titanium implants for enhancing bioactivity of implants. *Int J Nanomedicine* 2015; 10: 3887–3896.
119. Yeo IS, Min SK, Ki Kang H, et al. Adhesion and spreading of osteoblast-like cells on surfaces coated with laminin-derived bioactive core peptides. *Data Br* 2015; 5: 411–415.
120. Zhao C, Lu X, Zanden C, et al. The promising application of graphene oxide as coating materials in orthopedic implants: preparation, characterization and cell behavior. *Biomed Mater* 2015; 10(1): 15019.
121. Pan C-J, Dong Y-XD and Jandt K. Grafting carbon nanotubes on titanium surface for osteoblast cell adhesion and growth. *J Biomater Nanobiotechnol* 2012; 3(3): 353–361.
122. Rapuano B, Hackshaw K, Schniepp H, et al. Effects of coating a titanium alloy with fibronectin on the expression of osteoblast gene markers in the MC3T3 osteoprogenitor cell line. *Int J Oral Maxillofac Implant* 2012; 27(5): 1081–1090.
123. Rapuano BE and MacDonald DE. Surface oxide net charge of a titanium alloy: modulation of fibronectin-activated attachment and spreading of osteogenic cells. *Colloids Surf B Biointerfaces* 2011; 82(1): 95–103.
124. Sisti K, de Rossi R, Antoniolli-Brochado A, et al. Surface and biomechanical study of titanium implants modified by laser with and without hydroxyapatite coating, in rabbits. *J Oral Implantol* 2012; 38(3): 231–237.
125. Mangano C, De Rosa A, Desiderio V, et al. The osteoblastic differentiation of dental pulp stem cells and bone formation on different titanium surface textures. *Biomaterials* 2010; 31(13): 3543–3551.
126. Le Guillou-Buffello D, Bareille R, Gindre M, et al. Additive effect of RGD coating to functionalized titanium surfaces on human osteoprogenitor cell adhesion and spreading. *Tissue Eng Part A* 2008; 14(8): 1445–1455.
127. Yamamichi N, Pugdee K, Chang W-J, et al. Gene expression monitoring in osteoblasts on titanium coated with fibronectin-derived peptide. *Dent Mater J* 2008; 27(5): 744–750.
128. Chang YL, Stanford CM, Wefel JS, et al. Osteoblastic cell attachment to hydroxyapatite-coated implant surfaces in vitro. *Int J Oral Maxillofac Implants* 1999; 14(2): 239–247.
129. Hanawa T, Kamiura Y, Yamamoto S, et al. Early bone formation around calcium-ion-implanted titanium inserted into rat tibia. *J Biomed Mater Res A* 1997; 361: 131–136.
130. Reyes CD, Petrie T, Burns KL, et al. Biomolecular surface coating to enhance orthopaedic tissue healing and integration. *Biomaterials* 2008; 28(21): 3228–3235.
131. Tripathy A, Sen P, Su B, et al. Natural and bioinspired nanostructured bactericidal surfaces. *Adv Colloid Interface Sci* 2017; 248: 85–104.
132. Ribeiro M, Monteiro FJ and Ferraz MP. Infection of orthopedic implants with emphasis on bacterial adhesion process and techniques used in studying bacterial-material interactions. *Biomatter* 2012; 2(4): 176–194.
133. Holmberg KV, Abdolhosseini M, Li Y, et al. Bio-inspired stable antimicrobial peptide coatings for dental applications. *Acta Biomater* 2013; 9(9): 8224–8231.
134. Rickard AH, Gilbert P, High NJ, et al. Bacterial coaggregation: an integral process in the development of multi-species biofilms. *Trends Microbiol* 2003; 11(2): 94–100.
135. Yoshinari M, Oda Y, Kato T, et al. Influence of surface modifications to titanium on antibacterial activity in vitro. *Biomaterials* 2001; 22(14): 2043–2048.
136. Ivanova EP, Hasan J, Webb HK, et al. Natural bactericidal surfaces: mechanical rupture of *Pseudomonas aeruginosa* cells by cicada wings. *Small* 2012; 8(16): 2489–2494.
137. Ivanova EP, Hasan J, Webb HK, et al. Bactericidal activity of black silicon. *Nat Commun* 2013; 4: 2838.
138. Hasan J, Webb HK, Truong VK, et al. Selective bactericidal activity of nanopatterned superhydrophobic cicada *Psaltoda claripennis* wing surfaces. *Appl Microbiol Biotechnol* 2013; 97: 9257–9262.
139. Hasan J, Jain S and Chatterjee K. Nanoscale topography on black titanium imparts multi-biofunctional properties for orthopedic applications. *Sci Rep* 2017; 7: 41118.
140. Ostrikov K, Macgregor -M, Cavallaro A, et al. Influence of nanoscale topology on bactericidal efficiency of black silicon surfaces. *Nanotechnology* 2017; 28: 245301.
141. Cao Y, Su B, Chinnaraj S, et al. Nanostructured titanium surfaces exhibit recalcitrance towards *Staphylococcus epidermidis* biofilm formation. *Sci Rep* 2018; 8: 1071.
142. Tsimbouri PM, Holloway N, Fisher L, et al. Osteogenic and bactericidal surfaces from hydrothermal titania nanowires on titanium substrates. *Sci Rep* 2016; 6: 36857.
143. Fraioli R, Tsimbouri PM, Fisher LE, et al. Towards the cell-instructive bactericidal substrate: exploring the combination of nanotopographical features and integrin selective synthetic ligands. *Sci Rep* 2017; 7: 16363.
144. Besinis A, Hadi SD, Le HR, et al. Antibacterial activity and biofilm inhibition by surface modified titanium alloy medical implants following application of silver, titanium dioxide and hydroxyapatite nanocoatings. *Nanotoxicology* 2017; 11(3): 327–338.
145. Hirschfeld J, Akinoglu EM, Wirtz DC, et al. Long-term release of antibiotics by carbon nanotube-coated titanium alloy surfaces diminish biofilm formation by *Staphylococcus epidermidis. Nanomedicine* 2017; 13(4): 1587–1593.

146. Inoue D, Kabata T, Ohtani K, et al. Inhibition of biofilm formation on iodine-supported titanium implants. *Int Orthop* 2017; 41(6): 1093–1099.
147. Norambuena GA, Patel R, Karau M, et al. Antibacterial and biocompatible titanium-copper oxide coating may be a potential strategy to reduce periprosthetic infection: an in vitro study. *Clin Orthop Relat Res* 2017; 475(3): 722–732.
148. Rodríguez-Contrerasa A, García Y, Manero JM, et al. Antibacterial PHAs coating for titanium implants. *Eur Polym J* 2017; 90: 66–78.
149. Surmeneva MA, Sharonova AA, Chernousova S, et al. Incorporation of silver nanoparticles into magnetron-sputtered calcium phosphate layers on titanium as an antibacterial coating. *Colloids Surf B Biointerfaces* 2017; 156: 104–113.
150. Xu J, Xu N, Zhou T, et al. Polydopamine coatings embedded with silver nanoparticles on nanostructured titania for long-lasting antibacterial effect. *Surf Coat Technol* 2017; 320: 608–613.
151. Zhou L, Lai Y, Huang W, et al. Biofunctionalization of microgroove titanium surfaces with an antimicrobial peptide to enhance their bactericidal activity and cytocompatibility. *Colloids Surf B Biointerfaces* 2015; 128: 552–560.
152. Kazemzadeh-Narbat M, Lai BFL, Ding C, et al. Multilayered coating on titanium for controlled release of antimicrobial peptides for the prevention of implant-associated infections. *Biomaterials* 2013; 34(24): 5969–5977.
153. Geng H, Yuan Y, Adayi A, et al. Engineered chimeric peptides with antimicrobial and titanium-binding functions to inhibit biofilm formation on Ti implants. *Mater Sci Eng C* 2018; 82: 141–154.
154. Ye J, Shao C, Zhang X, et al. Effects of DNase I coating of titanium on bacteria adhesion and biofilm formation. *Mater Sci Eng C* 2017; 78: 738–747.
155. Zaatreh S, Haffner D, Strauss M, et al. Thin magnesium layer confirmed as an antibacterial and biocompatible implant coating in a co-culture model. *Mol Med Rep* 2017; 15(4): 1624–1630.
156. Chen R, Willcox MDP, Ho KKK, et al. Antimicrobial peptide melimine coating for titanium and its in vivo antibacterial activity in rodent subcutaneous infection models. *Biomaterials* 2016; 85: 142–151.
157. Pérez-Tanoira R, Horwat D, Kinnari TJ, et al. Bacterial adhesion on biomedical surfaces covered by yttria stabilized zirconia. *J Mater Sci Mater Med* 2016; 27(1): 6.
158. Zheng D, Neoh KG and Kang ET. Bifunctional coating based on carboxymethyl chitosan with stable conjugated alkaline phosphatase for inhibiting bacterial adhesion and promoting osteogenic differentiation on titanium. *Appl Surf Sci* 2016; 360: 86–97.
159. Jennings JA, Carpenter DP, Troxel KS, et al. Novel antibiotic-loaded point-of-care implant coating inhibits biofilm. *Clin Orthop Relat Res* 2015; 473(7): 2270–2282.
160. Lee B-C, Jung G-Y, Kim D-J, et al. Initial bacterial adhesion on resin, titanium and zirconia in vitro. *J Adv Prosthodont* 2011; 3(2): 81–84.
161. Jenkins J, Nobbs AH, Verkade P, et al. Characterisation of bactericidal titanium surfaces using electron microscopy characterisation of bactericidal titanium surfaces using electron microscopy. *Microsc Anal* 2018; 34: 17–22.
162. Sjöström T, Nobbs AH and Su B. Bactericidal nanospike surfaces via thermal oxidation of Ti alloy substrates. *Mater Lett* 2016; 167: 22–26.
163. Visai L, De Nardo L, Punta C, et al. Titanium oxide antibacterial surfaces in biomedical devices. *Int J Artif Organs* 2011; 34(9): 929–946.
164. Chen CZ and Cooper SL. Interactions between dendrimer biocides and bacterial membranes. *Biomaterials* 2002; 23(16): 3359–3368.
165. Munisparan T, Yang ECY, Paramasivam R, et al. Optimisation of preparation conditions for Ti nanowires and suitability as an antibacterial material. *IET Nanobiotechnol* 2018; 12(4): 429–435.
166. Bhadra CM, Khanh Truong V, Pham VTH, et al. Antibacterial titanium nano-patterned arrays inspired by dragonfly wings. *Sci Rep* 2015; 5: 16817.
167. Diu T, Faruqui N, Sjöström T, et al. Cicada-inspired cell-instructive nanopatterned arrays. *Sci Rep* 2014; 4: 7122.