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Bacterial adhesion mechanisms on dental implant surfaces and the influencing factors

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Abstract

Bacterial adhesion on dental implants may cause peri-implant disease including peri-implant mucositis and peri-implantitis. Peri-implantitis may lead to bone resorption and eventually loss of the implant. Therefore, the factors which influence bacterial adhesion are critical and revealed by many studies. The purpose of this review is to summarize the current knowledge of factors influencing the bacteria adhesion, including local factors of implant surface topography, abutment, cement and oral environment factors of saliva and protein. In addition, the corresponding strategies of surface modifications, coatings and challenges for implant materials as prevention and treatment approaches for bacteria adhesion on implant will also be discussed. We expect to give an overall picture of the bacteria adhesion on implant, and provide future perspectives, such as laser therapy, photocatalysis, plasma and bioelectric effect to inspire researchers to explore on this issue.

1. Introduction

Dental implants have been used for several decades, with undeniable benefits for patient care and are now considered as the appropriate strategy for the replacement for missing teeth [1]. After the dental implant has been placed, complex processes take place in the wounded tissue and the non-vital, mineralized cortical bone has to be remodelled. In 1981, Albrektsson and Brånemark defined osseointegration as "a direct functional and structural connection between living bone and the surface of a load carrying implant" [2]. Observing the interface between the bone and the titanium surface with light and transmission electron microscopy, it was revealed that osseointegration is present at a visible and ultrastructural level [3].

The 5- or 10-year survival rates reported for dental implants are encouraging, ranging from 82% to over 95% [1, 4]. However, the sole consideration of survival, defined by implant osseointegration (with or without peri-implantitis, or associated issues such as aesthetics) is not sufficiently representative of the global clinical picture. When considering success instead of survival, the rates decrease significantly (from 93.9 to 73.5%), becoming lower than endodontically treated teeth [1]. The complications associated with this lower success, namely peri-implant mucositis and peri-implantitis, are now increasingly reported. It was reported in 5-11 year observations that peri-implant mucositis affects 40-90% of implants in 80% of subjects, while around 20% of implants develop peri-implantitis [5-9].

Peri-implant mucositis and peri-implantitis are triggered by the presence of biofilms at the implant surface (Figure 1). Mucositis is defined as an inflammation of the soft tissue surrounding

dental implants, as evidenced by change in mucosal colour and contour, and bleeding upon gentle probing (< 0.25 N) [10]. The condition is not accompanied by bone loss around the implants, and is reversible. Characterised by the predominance of plasma and polymorphonuclear cells in the soft tissue around the implant [11, 12], mucositis may result in the proliferation of the sulcular epithelium and the degeneration of connective tissue, followed by the destruction of the mucosal seal. Once this seal is lost, the sub-gingival implant surface can be progressively colonized by pathogenic bacteria, which may be followed by inflammatory resorption of the surrounding alveolar bone [13]. Such bone loss can be observed in the radiographic images, and confirmed clinically by the presence of a peri-implant pocket [14].

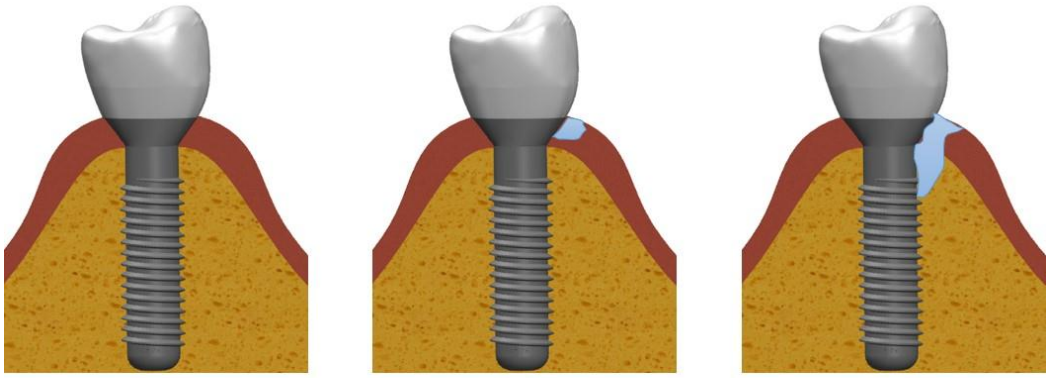

Fig.1 Progressive periodontal scenarios with the presence of biofilm at the implant surface

The frequency and/or severity of such complications depend on a large variety of factors, either before, during and after the treatment procedure. Implant failures might be divided into early and late types [15, 16]. Early failure occurs when the implants fail during the process of osseointegration, whereas late failure refers to issues occurring only after occlusal loading [15]. Peri-implant mucositis and peri-implantitis are inflammatory responses of gingival and alveolar

bone tissues triggered by the colonization of different pathogenic microorganisms on the implant surface and their organization in biofilm, and may be considered as the most common cause for late failures [14, 17, 18].

A microbial biofilm is defined as a "complex, functional community of one or more species of microbes, encased in an exopolysaccharide matrix and attached to one another or to a solid surface" [19]. Its formation is a rather complex process as well as an essential step in the development and evolution of the pathologic process [20-22]. It can also be affected by many factors: surface characteristics of implant; bacterial types and properties; serum proteins and oral environment [23]. The process of bacterial adhesion to a surface can be divided into two phases, including an initial, instantaneous, and reversible physical phase (phase one), followed by a time-dependent and irreversible molecular and cellular phase (phase two) [23-25]. In brief, following initial attachment, bacteria start to colonize and grow on the implant surface. Multilayered cellular clusters are formed due to cell proliferation, intercellular adhesion and production of an extracellular polymeric matrix [26, 27]. Subsequently, such a three-dimensional architecture develops into maturation. After that, some bacteria start to detach from the implant surface and disperse into the body fluids, leading to the spreading of biofilm across surfaces [28].

Microbiological studies in healthy peri-implant tissues demonstrated the presence of large proportions of coccoid cells, with a low proportion of anaerobic and aerobic species, a small number of Gram-negative species, and a low detection of periodontopathogenic bacteria [29-31]. Gram-positive aerobic bacteria such as *Streptococcus mitis*, *Streptococcus sanguis* and *Streptococcus oralis* were observed on dental implant surfaces surrounded by healthy oral

environment [32]. However, it was also possible to find small concentrations of anaerobic Gram-negative bacilli on some implants.

Bacteria colonized around the peri-implantitis sites are mainly Gram-negative anaerobic rods and spirochetes [29]. The microbiota species around the peri-implantitis sites are very similar to those found in the periodontal disease sites, mainly including the red and orange complexes, such as *Prevotella nigrescens, Campylobacter rectus* and *Aggregatibacter actinomycetemcomitans*. These subgingival microbiota lead to an increase in pocket depth and alveolar bone loss, which may damage the soft and hard tissue around the implant [29]. The microbiota changes in the peri-implantitis sites include an increased total bacterial quantity and the proportion of *Aggregatibacter actinomycetemcomitans*, Fusobacterium species, *Prevotella intermedia* and *Porphyromonas gingivalis*, a decrease in the percentage of cocci, and an important increase in the proportion of mobile organisms and spirochetes [31-33].

Given the inflammatory and immune response to peri-implant diseases, the response of the host immune system to the bacterial challenge will significantly affect the clinical outcome, which is similar to that for dental pulp, periapical, and periodontal inflammatory diseases [34-37]. These reviews, among others, describe the very complex interaction between immune cells, inflammatory mediators or growth factors, and bacteria. Tipping the scale on one side or the other can result either in resolution of the inflammation, or tissue destruction, since immune response to bacteria and/or their by-products can induce contradictory events by playing both protective and destructive roles. Other factors such as diabetes [38-40] ,smoking [6, 41] and previous periodontitis history [16, 42] also affect the occurrence of peri-implantitis. In any case,

regardless of the complex interplay, it is obvious that the common aetiology of peri-implant pathogens remains the microbial colonization of implant surfaces. In this context, the present work will first focus on reviewing the local (Section 2.1, 2.2 and 2.3) and oral environment (Section 2.4) factors affecting biofilm development on the implant and at its vicinity. Additionally, the review will address the main strategies to prevent infection (Section 3), and suggest possible future research avenues that may control biofilm adhesion (Section 4).

2. The effect of local factors on biofilm growth: surface topography, abutment, cement and oral environment.

2.1 Implant surface topography

Surface properties of dental implants can influence the adhesion of cells and microbial colonizers. A large number of experimental investigations have demonstrated that the influence of surface topography on bone response [43, 44], specifically surface roughness, surface free energy, and surface chemistry.

2.1.1 Surface roughness

Surface roughness is known to significantly affect osseointegration. Although various surface roughness parameters were found in different papers, R_a and S_a value were the most two frequently used parameters, which are defined as the arithmetic mean deviation of a linear profile (R_a) or a surface (S_a). R_a and S_a are considered to be robust and stable height-descriptive parameters [43]. Smooth (S_a < 0.5 mm) and minimally rough (S_a 0.5–1 mm) surfaces showed less strong bone integration than rougher surfaces. Moderately rough (S_a 1–2 mm) surfaces showed stronger bone responses than rough (S_a > 2 mm) in some studies [43]. Furthermore, previous studies recognized surface roughness as the predominant factor for biofilm formation on implant surfaces [45, 46] as more biofilm was formed on rough modified surfaces compared with the smooth group [47-49].

A literature review by Teughels et al. [45] focused on the transmucosal portion of the dental implant and concluded that a higher surface roughness increased biofilm formation and

maturation, independent of the material [50]. Further, biofilm accumulation was reported as less pronounced on flat and grooved surfaces compared with irregular topography [49]. If R_a was below 0.2 μm, the qualitative and quantitative measures of biofilms did not decrease significantly with reduced roughness. The so-called "Threshold R_a" is therefore set to $R_a < 0.2$ μm [51, 52]. However, as discussed by Matinlinna et al. [53], the roughness measurement and criteria "are very confusing and lack of unity", i.e. no general consent about the standardization of surface roughness was made in various implant studies. Thus, surface roughness values are useful as a guideline for comparison, but not an absolute value to determine the ability of osseointegration. However, there exists some debate on the influence of surface roughness on cellular adhesion and clinical outcome. A previous study claimed that the surface roughness of the implants did not significantly affect biofilm formation during the first 3 years of implant loading [54]. After this period, titanium implants, which were moderately rough, demonstrated a similar clinical outcome compared with the minimally rough machine-turned implants and no statistically significant difference in clinical, microbiological and biochemical parameters could be detected. Nevertheless, it was also suggested that longer-term follow-up researches using various different implant types are needed to confirm the conclusion [55]. Moreover, *Candida spp.* adhesion was compared on machined or cast titanium and zirconia abutments with different surface roughness. No positive correlation was detected between the cell amount and the surface roughness value [56]. It was suggested that material surface roughness did not affect fungal adhesion and subsequent biofilm formation [57].

Previous investigations also report the greater influence of surface roughness on biofilm volume at early stages of implantation, during which the rougher surface induced more bacteria adhesion,

with diminishing effects of roughness as the biofilm matured [58-60]. Therefore, further studies are needed to investigate the relationship between surface roughness and adhesion, accumulation and maturation of bacteria.

2.1.2 Surface free energy

Bacterial adhesion to implant surfaces is affected by surface free energy [47, 61, 62]. Wetting phenomena of a solid substrate is used to examine surface free energy by static measurement of the contact angle. Generally, if the water contact angle is less than 90°, the solid surface is considered hydrophilic with high surface free energy and if the water contact angle is larger than 90°, the solid surface is considered hydrophobic with low surface free energy [63].

Most of the oral microorganisms at the cell surface, e.g. strains of *Streptococcus mutans, S. sanguis* and *S. salivarius*, were reported to have high surface free energy, thus exhibit lower retention characteristics to hydrophobic surface with reduced surface free energy and superior adhesion characteristics to hydrophilic surfaces with high surface free energy [47, 61, 62]. On the contrary, a weak positive correlation was reported between contact angles of water on samples and initial adhesion, which suggested a role of hydrophobic interaction in *C. Albicans* adhesion. The reason may be due to *C. Albicans* belongs to hydrophobic strains [57].

A recent study by Villard et al. [64] investigated the effects of a novel silane coating on adhesion characteristics of *C. Albicans* to titanium and zirconia surfaces with similar surface roughness and morphology. The authors reported that, on grit-blasted titanium surface, the surface free

energy was lowered after silane application, with a statistically higher viable colony-forming unit (CFU) counts for *C. Albicans* on the uncoated surface with higher surface free energy. However, an opposite result was reported in the case of zirconia, i.e. *C. albicans* favoured zirconia surfaces with lower surface free energy without silane treatment. An in vitro study investigated the physiochemical properties of titanium and zirconia materials and compared the affinity of different bacteria to them. The affinity of *Streptococcus mitis* and *prevotella nigrescens* to modified materials, such as polished partially stabilized zirconia (PZ) and titanium blasted with zirconia (TBZ), were compared with the control group of polished titanium (PT). The results exhibited that PZ and TBZ exhibited lower surface free energy and lower percentage of bacterial adhesion compared with control PT surface[65].

Surface free energy was also modified on implant surfaces to increase cell adhesion. Such studies by Hauser et al. exhibited that plasma treatment can lead to a significant increase of surface free energy of medical implant materials. These changes strongly influence protein and increase cell adhesion on the material surface [66, 67]. Polyetheretherketone (PEEK) films which were oxygen plasma were applied to increase surface free energy in a previous study by Rochford et al. *Staphylococcus epidermidis*, *Staphylococcus aureus*, and U-2 OS cells were cocultured in a model. Competitionbetween osteoblasts and bacteria for the PEEK film surfaces occured. The study reported that more U-2 OS cells adhered to the treated surfaces compared to the untreated PEEK. The study suggested that oxygen plasma treatment of PEEK maintain the adherence ability of osteoblast-like cells, even cocultured with *S. epidermidis*, without increasing the risk of bacterial adhesion. However, in the presence of *S. aureus* contamination, cell death of the U-2 OS occurred within 10 h on all surfaces. Therefore, they concluded oxygen plasma-

treated PEEK may be a promising way to improve implant surface free energy for better osseointegration without leading to more bacteria adhesion [68].

2.1.3 Surface chemistry

Variations in implant surface chemistry can lead to enhanced fibronectin adsorption and endothelial cell adhesion and growth [69] and corneal cell migration [70]. In fact, the cell adhesion ability highly depends on the surface chemistry on the materials. The nano-thick titanium oxides at the implant surface could partially hydrolyse as titanium hydroxide (Ti-OH) (Figure 2). Indeed, Ti-OH could further react with atmospheric moisture, and yield acidic $[Ti\text{-}OH_2]^+$ and alkali $[Ti\text{-}O]^-$ through hydrolysis under different pH values (Figures 3 and 4).

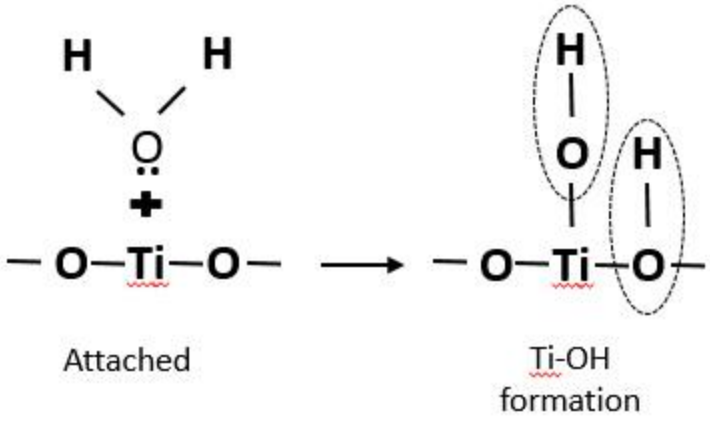

Fig.2 The process of the nano-thick titanium oxides (TiO_2) partially hydrolyse as titanium hydroxide (Ti-OH).

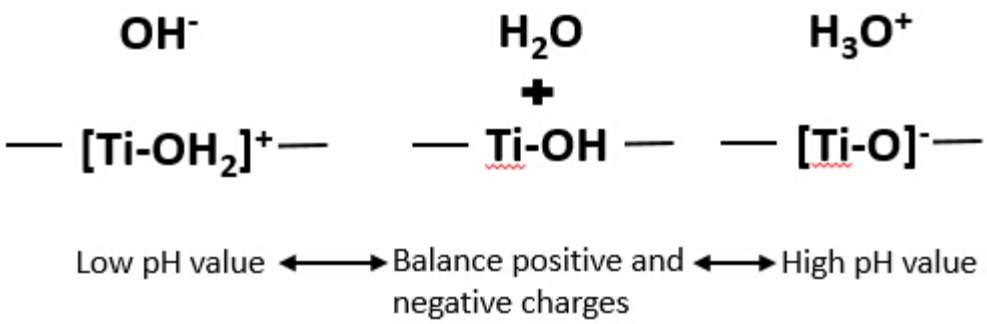

Fig. 3 Ti-OH equilibrium reaction with atmospheric moisture, and generate acidic $[Ti\text{-}OH_2]^+$ and alkali $[Ti\text{-}O]^-$ under different pH.

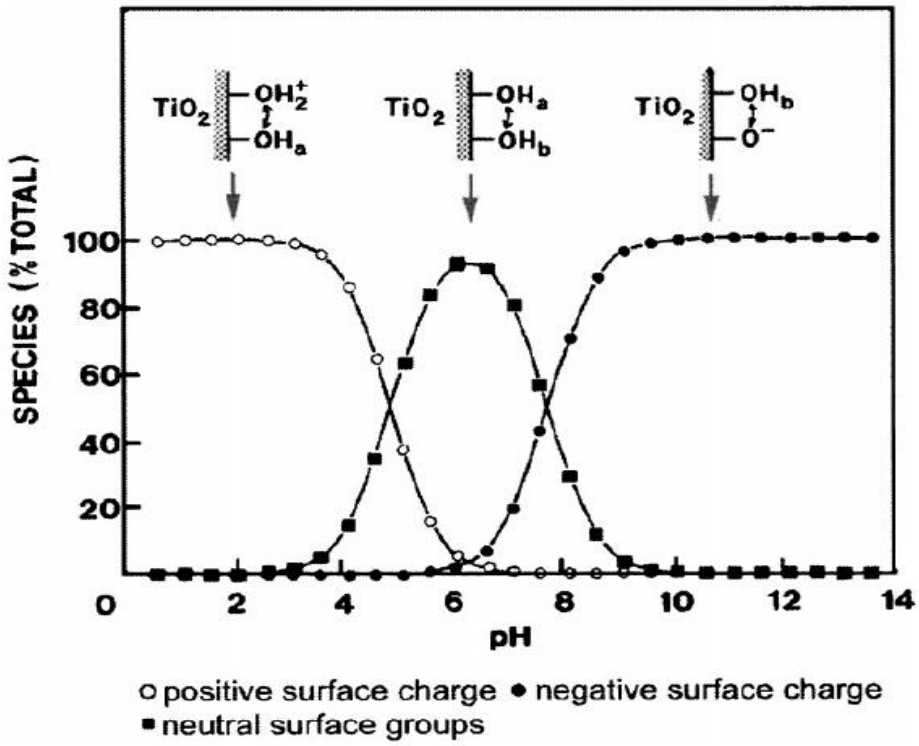

Fig.4 Concentration of the different charged and uncharged hydroxy-related species at a titanium oxide surface (%) as a function of pH of the aqueous solution. $–OH_a$ and $–OH_b$ denote acidic and basic hydroxyl group (adapted from [71]), respectively.

Oxide surfaces are electrically charged in liquid, due to the interaction of–OH groups at their terminal with hydronium and hydroxide ions in the in aqueous solutions. The net charge on the surface is pH dependent; it is zero at the isoelectric point (IEP). When pH > IEP, the surface is negatively charged; while pH < IEP the surface is positively charged [72]. Various studies [72-74] have determined the isoelectric point range from 5.0 to 6.7 on titanium surfaces. In physiological pH, i.e. 7.4, it appears that the negative-charged $[Ti\text{-}O]^-$ is the dominant specie and important to any biological reactions, since it would be able to attract proteins or biological factors (e.g. TGFβ and H34 histadine) and with positively charged polymer branches [75], forming branched or networked polymers by adsorption and further attracting cells and bacteria, which are usually negatively charged (Figure 5). Some titanium surface treatment methods, such as grit-blasting by alumina, would generate a negatively charged surface [76]. Nonetheless, the common regime to treat the titanium surface using acid, according to the equilibrium reaction (Figure 3), would not only create roughness due to etching effect but also promote the formation of acidic, hydrophilic and hydroxylated $[Ti\text{-}OH_2]^+$, which has been documented for enhancement of biological activity [77] without the assistance of proteins (Figure 6) . Thus, the acid etching or storage the implant body in acidic medium, *e.g.* SLActive® (Straumann, Basel, Switzerland) that has claimed to use an acidified (pH 4-6) saline (0.9% NaCl) to store [78], seems to be easy and effective ways to enhance the chemical interaction for osteoblastic adhesion.

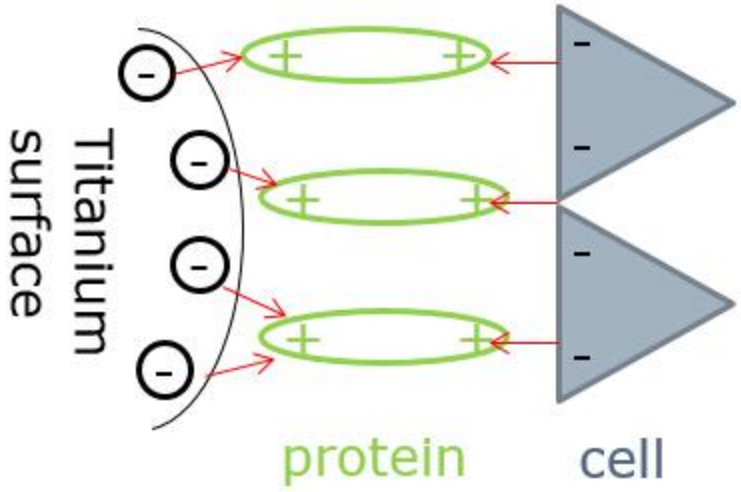

Fig.5 Biological reaction between the titanium surface (negatively-charged), adsorpted protein (positively-charged) and the cell (negatively-charged), showing the linkages for cell interaction, e.g. the bacteria and osteoblast.

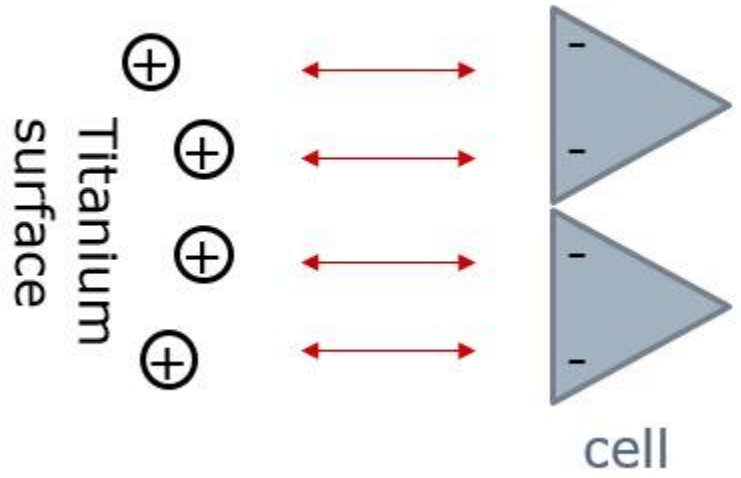

Fig.6 Biological reaction between the titanium surface (positively-charged) and the cell, showing that titanium surface with positive charge can enhance biological activity of cell attachment without the assistance of proteins

Given the modification of surface chemistry that could reduce protein binding which enable the reduction of cell adhesion, the chemistry of surface could therefore be engineered so as to affect biofilm formation [79, 80]. On different implant surfaces, with similar roughness parameters, significant differences were reported in both the amount and composition of the biofilm. This was ascribed to the antibacterial properties of implant surface after chemical modification [45], e.g. silane [64]. Even some previous studies suggested that the variation of chemical composition and nanomorphology of surface coatings have no effect on the biofilm formation of *S. epidermidis* [81]. However, the large differences in methodology between the studies, such as bacterial strains, culture conditions, and culture time, make it very difficult to compare the results from one study to another. Thus, comprehensive efforts for standardization are required to optimize the surface chemistry at various parts of the implant, such as using a coating at the neck and abutment parts, to prevent or stabilize the bacteria from adhesion and colonization. Therefore, the peri-implantitis which is the major disease for implant failure may be better controlled or eliminated.

2.1.4 Titanium purity

Previous study reported that not only implant surface characteristics, but also titanium purity, influence early bacterial colonization. An *in vitro* study compared the bacterial adhesion of a four-species oral biofilm on different purity of titanium discs of grades cp-2 and cp-4, namely Tigr2-c (cp-2, machined surface), Tigr2-t (cp-2, modified surface with Avantblast®), Tigr4-c (cp-4, machined surface) and Tigr4-t (cp-4, modified surface with Avantblast®). A significantly higher total bacterial biomass on both cp-4 titanium surfaces was reported [82]. Although the

study did not explain in detail, it may be that the higher oxygen content during the processing of cp-4 increased the availability of –OH groups and promoted more bacterial adhesion.

Conversely, another study [83] compared the osteoblast-like cell on cp-1 and cp-4 titanium, and concluded under the same surface treatment method that cp-4 had higher initial (4 h) osteoblastic adhesion than cp-1, but after 24h cp-1 demonstrated a higher osteoblastic adhesion than cp-4. In fact, after the same surface treatment, cp-1 and cp-4 titanium demonstrated different surface morphology, e.g. cp-1 (R_a ~ 553.7nm) exhibited higher roughness than cp-4 (R_a ~ 147.5 nm), and the grooves were shown to be rounded in cp-1 and sharp in cp-4. That said, the surface would promote chemical adhesion of osteoblasts at an initial stage. Subsequently, osteoblasts would cover the surface and other parameters, such as roughness and shapes of grooves, could enable (or vice versa) maturation of osteoblasts. Therefore, it seems to be the titanium purity relates to the surface chemistry that affects the initial cell adhesion, although various studies focused on osteoblast-like cells only.

2.2 Abutment

Implant abutment surface and geometry influences both bacterial and yeast colonization inside the implants as well as the torque value used to connect abutments to implants. However, the situation of bacteria adhesion on abutments is different from that on implants. A previous study found that different sites in the oral cavity are characterized by specific groups of bacteria. Higher levels of *Streptococcus* species were found in the sulcus fluid of the abutments compared with residual teeth. Although *Prevotella* and *Rothia* species were suggested as late colonizers as these species were frequently detected in the oral cavity, they were not found at the abutments

[84]. Plaque retentive sites exist in implant prostheses like crowns or bridges. Thus, a gap, i.e. the interface, between the dental abutments and prostheses can boost the biofilm formation. The gap in fact is created due to the geometry of the abutment, which evidently increase the risk of invading bacteria as shown in an *in vitro* study [85]. Therefore, biofilm accumulation around abutment sites cannot be ignored.

2.3 Cement

Cement is often used to fixed dental prosthetics on implants. However, excess cement in the peri-implant sulcus may favour biofilm formation, and result in inflammation of peri-implant tissue, which may facilitate the development of peri-implantitis [86, 87]. In a study collecting excess cement from patients and investigating bacterial in situ colonization by 16S rDNA-based methods, it was reported both *in situ* and *in vitro* that a strong association existed between bacterial invasion and methacrylate-based cement by opportunistic species and pathogens [88]. A study was conducted to analyze the effect of two different dental cements on the composition of the microbial peri-implant community [87]. Zinc oxide–eugenol cement (Temp Bond, TB) and a methacrylate resin-based cement (Premier Implant Cement, PIC) were used to fix a dental restoration above the implant. Compared with TB, PIC was found to favour the development of suppuration and the growth of periodontal pathogens [87].

Bacteria being an organic substance may preferentially adhere to organic-based chemicals due to chemical similarity. Indeed, dental resins commonly contain methacrylate monomers such as bis-GMA, UDMA and TEGDMA, as well as initiator system comprised with camphorquinone and tertiary amine. After the photocuring polymerization, the monomers would chemically bonded

and form ester. The functional groups of the resins contain C-H and N-H that are susceptible for bacterial adhesion [89]. Human saliva has been demonstrated the ability to hydrolyze (i.e. degrade) dental resins via salivary esterase such as albumin, Zn-α2-glycoprotein, α-amylase, TALDO1 protein, transferrin, lipocalin2, and prolactin-induced protein [90]. The enzymatic activity of some of these esterase, particularly para nitro-phenyl acetate-like-dependent esterase, could be enhanced by cariogenic *S. mutans* [91]. Despite the degradants might be able to slightly inhibit the growth of bacteria [92], obviously the degraded dental resin have a weaker mechanical strength and easier to debond at the resin-tooth interface. Therefore, it seems to be a proactive approach, such as anti-bacterial dental resin, could be an option for the future materials. This could be done either adding some antibacterial chemicals [93, 94] or modifying the resin structure [95].

2.4. Oral environment

2.4.1 Saliva

The surface of dental materials will be coated by salivary components immediately after being inserted into the oral cavity. Oral salivary flow over a coating is persistent, applies continuous shear forces, and supplies proteins to bacteria, accelerating bacteria metabolism and biofilm growth [96, 97].

Moreover, saliva coating changes the physicochemical property of the surface and adds specific receptors for microbial adhesion, then, influences bacteria adhesion. A previous study suggested the presence of saliva increased the biofilm volume of *S. sanguinis* and *A. naeslundii* on the

substrates of turned titanium, sol-gel nanoporous TiO_2 coated surfaces and anodized Ca^{2+} modified surfaces, almost tenfold when compared to the absence of saliva. However, no significant differences were reported between the test surfaces [98]. This might be due to the proteins in saliva, particularly albumin, which is negatively charged and the divalent Ca^{2+} ions in saliva is necessarily to bridge the electrostatic adsorption between albumin and other negatively charged species such as titanium oxides and bacteria [99].

2.4.2 Protein

It is widely accepted that the initial phase of material interaction with blood, soft tissue, or bony tissue is largely dictated by adsorbed adhesion proteins. The adhesion proteins include fibrinogen, fibronectin, vitronectin, von Willebrand's factor. Surface chemistry and adsorption conditions (e.g. mass loading, co-adsorbed proteins, residence time and temperature) influence the amount and potency of adhesion protein adsorption [100].

Understanding the behaviour of proteins at material-tissue interfaces may start from that of the simple, coiled polymers. Firstly, like the simple polymers, the ability of a protein to adsorb is increased by attaching several segments to a surface [101].The protein may leave the surface when the affinity of the molecular segments is reduced by environmental changes (e.g. temperature, pH and ionic strength). Also, protein molecules may be displaced from the surface by adding components that have a higher affinity to adsorb. Additionally, because of their ionic groups, proteins demonstrate the type of adsorption patterns that are typical for polyampholytes, which represent strong pH-dependence, with a maximum adsorbed amount at isoelectric conditions [102]. As mentioned in the previous section, the adsorbed adhesion proteins strongly

affect the adhesion of cells to any surface. There is little or no cell adhesion unless the surface has at least some adsorbed adhesion protein due to dipole-dipole interaction. Variations in the relative amounts of the adhesion proteins in the adsorbed layer on different surfaces would be expected to lead to variations in cell adhesion [100].

The mechanism of bacteria adhesion to, say, Ti surfaces is influenced by ions and proteins of the initial coating derived from the blood. A previous study suggested that although albumin coating of Ti reduced the adhesion of *S. mutans* to all surfaces, it had no influence on the adhesion of *P. gingivalis* or *F. nucleatum*. Moreover, coating Ti with fibronectin enhanced *P. gingivalis* and *F. nucleatum* adhesion [103]. Mucin seems to have decisive effects on *C. albicans* immobilization and biofilm development on the materials. Biofilm made up of *C. albicans* indicates that mucin plays an important role in biofilm formation and its rigidity and enhanced *C. albicans* accumulation, in contrast, albumin is unlikely to be involved in the adhesion process of *C. albicans* [104]. SEM observation also revealed fewer *C. albicans* cells on saliva-coated Ti than on saliva-coated hydroxyapatite or acrylic resin [57]. In the challenging oral environment, it seems that saliva and oral microbial flora are unlikely to be changed, but the surface could be modified in order decrease biofilm formation.

3. Surface modifications, coatings and challenges for implant materials

Currently, dentists used the Cumulative Interceptive Supportive Therapy (CIST) protocol that four general stages in a sequential procedure for the treatment of peri-implantitis should be followed, as suggested by Lang [105, 106]: (1) Mechanical debridement like scaling/root planning to eliminate bacteria from the inflammation site; (2) Antiseptic treatment to make the implant surface disinfect; (3) Medical antibiotics application to remove bacteria in the surrounding peri-implant tissues; (4) Regenerative surgery to reconstruct the bone formation around the implant [105, 107]. Apparently, Smeets et al. [108] commented that there is no ideal peri-implantitis therapy since standardized prospective randomized long-term follow-up studies were lacked. High variety of study design, populations, materials, sample sizes and follow-up periods were observed. Obviously, an individual therapy regime concerning multifactorial etiology and treatment options would affect the study results, and thus, Smeets et al. suggested prevention is the most important part to get rid of the peri-implantitis.

3.1 Protein adsorption resistance

As aforementioned, proteins play a key major role in cellular adhesion on implant surface. Thus, controlling protein adsorption at the initial stage of biofilm formation may be an effective strategy to protect metal surfaces from bacterial contamination not only in dental manipulations but also in orthopaedic applications [109]. The present information related to the protein may be applied as a reference for selecting materials in implant overdenture treatment from a microbiological point of view. Thus, ideally if a material could be made that resisted adsorption of all or almost all of the adhesion proteins and also remained highly resistant for long periods in

the body, it would be expected to exhibit superior biocompatibility. Such a technological challenge is essentially a huge research field not only for dentistry/medicine, but the whole industry. Various strategies have been attempted, such as anti-fouling surfaces [110-114] to load antibacterial compounds to kill the bacteria, or to interfere the protein adsorption. It seems that silane-based [53, 111] could be the best for dentistry due to the long-term use for resin-titanium adhesion. However, it is worth noting that devices with protein resistant surfaces have so far received very little testing *in vivo*, so it is not known how much improvement they will actually provide.

3.2 Antibiotics

Local delivery of antibiotics at the implant site might be an efficient way against biofilms which can have several advantages. Firstly, in case high local dose does not cause any systemic toxicity, high efficacy can be achieved at the specific local site. Additionally, local delivering of antibiotics also allows for a selection of antibiotics against specific peri-implantitis pathogens, preventing potential antibiotic resistance [115].

A variety of surface coatings have been developed to achieve the effect of controlled release of antibiotics *in vitro*. Some requirements are raised for both antibiotics and coating materials. For antibiotics, broad antibacterial spectrum and thermostable property are the most important requirements, since the coating procedures are usually conducted at high temperatures. Gentamicin is such an example of antibiotics, which has a relative broad antibacterial spectrum. Furthermore, it is one of the rare kinds of thermostable antibiotics and so it is one of the most widely used antibiotics in antibiotics-loaded coatings on titanium implants. Besides, for instance,

cephalothin, carbenicillin, amoxicillin, cefamandol, tobramycin, and vancomycin have been used in coatings on bone implants [116]. On the other hand, the way which the drug are incorporated in the coating as well as the releasing rate of the drug from the coating are two important aspects, for they can highly influence the effectiveness of the antibiotics. Materials such as polyurethane, biodegradable polymers, and calcium phosphates (including carbonated hydroxyapatite and porous hydroxyapatite) are presented as representative examples of coatings which can meet these requirements [117]. Yet, no titanium implants with antibiotic containing coatings have been found for clinical use. A major limitation of this approach is that every drug delivery method has intrinsic limitations. The positive effect will disappear since the drug is finite. Moreover, the local toxicity on surrounding tissues needs to be fully investigated.

Systemic antibiotics were given as adjuncts to mechanical debridement and/or surgical procedures on affected dental implants heavily colonized by putative bacterial pathogens. As a result, systemic antibiotic therapy is often advised as a part of peri-implantitis treatment protocols, similar to the use of systemic antibiotics in periodontitis treatment, despite an absence to date of strong supporting scientific data.

Previous study assessed the occurrence of *in vitro* antibiotic resistance among putative bacterial pathogens isolated from human peri-implantitis lesions. Peri-implantitis patients frequently yielded submucosal bacterial pathogens which are resistant to individual therapeutic concentrations of clindamycin, amoxicillin, doxycycline or metronidazole *in vitro*, but only rarely to the combination therapy of amoxicillin and metronidazole. Due to the wide variation in observed drug resistance patterns, antibiotic susceptibility testing of cultivable submucosal

bacterial pathogens may aid in the selection of antimicrobial therapy for peri-implantitis patients [117].

3.3 Silver

Silver is well-known to have antibacterial properties, and recently discovered to be due to "zombie" effect [118]. Titanium coated with silver nanoparticles (nAg) was able to kill all the planktonic bacteria in a solution releasing silver within a few days. Furthermore, the bacteria were not able to attach the surface for about 30 days after immersion. This time duration can prevent infection after operation in the early and intermediate stages. Study has demonstrated nAg could act longer on bacteria than most antibiotics [119], possibly due to the release of nAg from the coating. A study has described a method to modify Ti/TiO_2 surfaces with citrate-capped nAg. These nanoparticles spontaneously adsorb on Ti/TiO_2, forming nanometer-sized aggregates consisting of individual nAg that homogeneously cover the surface. The modified nAg-Ti/TiO_2 surface exhibits a good resistance to colonization by *Pseudomonas aeruginosa* [120]. In spite of silver is able to kill bacteria and has no cytotoxic effect on osteoblasts and epithelial cells [121] at low dose, this could not be guaranteed in high dose [122]. Therefore, a suitable coating is necessary to load and release the silver.

A study about the biocompatibility of silver-loaded coatings on human osteoblast-like cells MG63 has been conducted. One of the key findings of this research is about the effect of silver on the cell system around the implanted medical devices. The release of silver ion needs to be properly adjusted in order to obtain antibacterial activity as well as preserving osteoblasts cell attachment at the titanium interface [123]. This said, surface coating, i.e. loading carrier, on

implant surface is very important to adjust the release of silver, so that the release rate would not be too slow that could not kill the bacteria nor too fast that could kill the osteoblasts.

The antimicrobial properties of a nanocomposite coating formed by polysaccharide 1-deoxylactit-1-yl chitosan (Chitlac) and silver nanoparticles (nAg) on methacrylate thermosets were analysed. Methacrylate thermoset is a kind of biomaterials which is commonly employed for orthopaedic and dental applications. The Chitlac-nAg system showed satisfying anti-bacterial and anti-biofilm activity. *In vitro* observation, a steady silver release accompanied by antimicrobial ability lose was detected in physiological conditions as time went on. However, there was still effective protection against bacterial colonization after 3 weeks which could be explained by the residual silver. The sufficiently high level of silver content released at the beginning can kill the bacteria rapidly to prevent the development of resistant pathogens [124]. Although the silver concentration decreased after several weeks, the bactericidal effect was still effective is this system. A good biological compatibility of Chitlac-nAg-coated materials compared with implants of titanium Ti-6Al-4V alloy has been shown in bony tissue when inserted the implants in a mini-pig animal model *in vivo.* It might be arguable that aluminium and vanadium ions in the tertiary titanium alloy might also exhibit somewhat cytotoxicity and killed some bacteria, nevertheless in another study has shown bone healing patterns and biocompatibility parameters observed for nAg-treated material were comparable with those observed for control implants [125]. Therefore, the antibacterial effect due to silver seems to be effective and biocompatible in bone tissue level.

3.4 Chemotherapeutic agents

Chemical treatment is employed as a complimentary method to conventional mechanical approach, where chlorhexidine and essential oils have been found to be efficient against different oral biofilms [126].

3.4.1 Chlorhexidine

Chlorhexidine has been believed to be effective in the therapy of mucositis and peri-implantitis [127]. It was found that, with the additional use of 2% chlorhexidine, more anaerobic bacteria on the implant surface were reduced than using mechanical debridement alone. In fact, 2% chlorhexidine was shown to be the most effective concentration previously, achieving a total viable biofilm reduction ranging from 96.2% to more than 99.99%, depending on the time of exposure and the stage of biofilm development [128]. It was also reported that an oral irrigator combined with 0.2% chlorhexidine is effective in reducing biofilms attached to rough titanium surfaces immediately after cleaning [129]. However, there seemed no significant difference in reducing bleeding, suppuration, probing pocket depth and radiographic bone loss [130].

It was also reported that chlorhexidine can be adsorbed by the titanium surface [131], due to fact that chlorhexidine is a positively-charged biguanide compound. An *in vitro* study showed decreased bacteria counts on chlorhexidine diacetate (CHA) coated implant surfaces and in the surrounding medium. Unfortunately, fibroblasts were killed by CHA as well [132]. There were researchers suggested to use *poly*-(D,L-Lactide) (PDLLA) or Politerefate™ (PTF) as CHA delivering coatings due to their cytocompatibility and the good mechanical properties of the interface between implant and coating. Furthermore, these polymeric coatings could release CHA slowly, in sufficient high concentrations and in a way, which does not inhibit the

attachment of fibroblasts [132]. Nevertheless, it seems to be using chlorhexidine in implant body is not an ideal case because the chlorhexidine has been demonstrated as cytotoxic to osteoblast in dose-dependent manner, such that a significant inhibition of osteoblastic growth happened even in 0.005% concentration [133]. Therefore, the application of chlorhexidine should be focused only on abutment part which could adhere the most biofilm and initiate the peri-implantitis.

3.4.2 Essential oils

Essential oils was found to decrease biofilm activity and biomass [134, 135]. It was also reported to be antifungal and inhibitory to the adherence of *C. albicans* onto dental implants and cover screws *in vitro* [135], with commented that the essential oils were capable of promoting penetration to the deep structure of biofilm and destroy more pathogenic resistant forms. Moreover, the residual effect of essential oil is also promising, which is said to be effective even after rinsing [126].

Essential oils functioned on different implant materials can result in antibacterial and antiplaque activities immediately. There seemed to be no effect of different material type on bacterial adhesion after antimicrobial agent application. [136]. The essential oil and citronellal have proven to have antifungal activity and are able to inhibit the *in vitro* adherence of *C. albicans* [135]. No specific regime for essential oils to disrupt the implant-specific biofilm is done due to the complex mechanism for antibacterial action [137], and it seems to be the usage of essential oils could be on implant maintenance rather than therapeutic way.

3.4.3 Citric acid (CA)

To assess the effectiveness of different chemotherapeutic agents on biofilm-contaminated titanium surfaces, *Streptococcus mutans* biofilms and polymicrobial biofilms were grown on titanium discs and treated by various chemical agents. Study has found H_2O_2, Ardox-X (a topical teeth whitening gel) and CA killed significantly more *S. mutans* compared with the other treatments [138]. H_2O_2 and CA removed significantly more protein than water, whilst CA and the combination treatments of Ardox-X followed by CA, H_2O_2 followed by CA were significantly more effective against the polymicrobial biofilms than chlorhexidine, H_2O_2 and Ardox-X. Among the chemicals tested, CA demonstrated the greatest decontamination capacity with respect to both the killing and the removal of biofilm cells. Moreover, the combination of effects is clinically desirable because it promotes biocompatibility and healing around a previously contaminated implant surface [138]. Although the mechanism of biofilm removal is unknown, it could be due to the adsorption of CA on titanium surface under certain pH could form "acid clusters" (i.e. aggregation of molecules) [139], that enable the disruption of calcium-ion bridges which is the chemical binding sites within the biofilm connecting the EPS polymeric chains [140]. Further investigation is necessary.

3.5 Antimicrobial peptides (AMPs)

In order to reduce biofilm formation, several strategies focusing on the use of antimicrobial peptides (AMPs) have been studied. Antimicrobial peptides (AMPs) belong to biomolecules which have a broad spectrum of antibacterial activity against Gram-positive and Gram-negative bacteria, as well as other pathogens. The underlying antibacterial mechanism is based on their capacity to target and disrupt bacterial membrane.

Titanium substrate can be functionalized with the *hLf1-11* peptide as a potent AMP either by silanization methods or physical adsorption. An outstanding reduction in bacteria adhesion and biofilm formation of *Streptococcus sanguinis* and *Lactobacillus salivarius* was observed on the biofunctionalized surfaces compared to the control group [141]. Another antimicrobial peptide GL13K was also coated on titanium surface, and found to reduce the number of viable bacteria [142]. Thus, it seems potential to develop antimicrobial biomaterials for dental applications.

3.6 PEEK/nano-FHA biocomposite

Polyetheretherketone (PEEK) was limited in implant or even dental application due to the lack of antibacterial activity and binding ability to the bone. However, enhanced antibacterial activity and osseointegration were achieved after a polyetheretherketone/nano-fluorohydroxyapatite (PEEK/nano-FHA) biocomposite was introduced. Smooth and rough surfaces of PEEK/nano-FHA biocomposites were also prepared. Our results showed that *in vitro* initial cell adhesion and proliferation on the nano-FHA reinforced PEEK composite were improved. Furthermore, PEEK/nano-FHA biocomposite could effectively prevent the formation and proliferation of biofilm. For *in vivo* test, the volume of new bone formed bone in the PEEK/nano-FHA group was higher compared to that of bare PEEK group. Therefore, the developed PEEK/nano-FHA biocomposite has increased biocompatibility and antibacterial activity *in vitro*, and promoted osseointegration *in vivo*, which provides us the idea to apply it as dental implant material in dental tissue engineering applications [143].

3.7 Surface treatment / functionalization

3.7.1 Anodization

Discharging the surface of a titanium implant in sodium chloride solution anodizes the titanium surface by forming a superficial layer of $TiCl_3$. Subsequently, the modified surface is gradually hydrolyzed, which leads to the formation of Ti-OH and the bactericidal hypochlorous acid:

$$TiCl_3 + H_2O \rightarrow Ti\text{-}OH + HClO + HCl \quad (2)$$

Associated with the hydrolysis, the hydrophilicity of the titanium implant is increased by the formation of Ti-OH on the surface. This facilitates the adhesion of cell binding proteins and the subsequent attachment of osteoblasts. An *in vitro* investigation revealed that hypochlorous acid is released from the modified surface into a culture medium up to eight weeks [144]. The slow-released hypochlorous acid induces antibacterial properties the modified titanium surface, while the remaining Ti-OH increases the hydrophilicity and therefore the osteoconductivity.

3.7.2 Anatase-rich surfaces

Adherence of early colonizing *streptococci* to two anodically oxidized surfaces coated with saliva was compared to that on commercially pure titanium (cp-Ti). More crystalline anatase was found on the anodically oxidized surfaces than on the cp-Ti. There was less amount of bacteria adhesion after 2 h to the saliva-coated, anatase-rich surfaces than to cp-Ti [145]. Attachment of salivary proteins with different anatase concentration and/or configuration may be the underlying reason of reduced bacterial binding effect on the anatase-rich surfaces. In general, anatase-rich surfaces could reduce the overall volume of biofilm formation on dental implant abutments through diminished adherence of early colonizers, possibly via anti-biofouling [145] or

generation of hydroxyl radicals (•OH) from the oxygen and moisture from saliva, that could turn the organic biofilm substance into intermediate species and finally CO_2 and water [146]. However, the antibacterial mechanism of anatase for biofilm is still debatable.

3.7.3 Nitride coatings

To examine alterations of the microbial community structure in biofilms on different dental implant surfaces over the time, a study used zirconium nitride-coated glass (ZrN-glass) and ZrN-coated polished titanium (ZrN-Ti) disks as substrates and polished titanium was used as a control, and exposed for 24 h and 14 days of intraorally. The study revealed that there was a significant difference of microbial composition between ZrN-Ti disk surface and polished Ti surface. ZrN physical vapour deposition coatings might be further developed so that to influence the adhesion of bacteria that are less pathogenic, thereby reducing the occurrence rate of peri-implantitis [147]. However, it should be noted that the study has tested on only one human subject that the conclusion seems to be subjective. In addition, *in vitro* study [148] has shown TiN, ZrN and $(Ti_{1-x}Zr_x)N$ coatings only inhibit initial biofilm adhesion specie *S. mutans* but not *P. gingivalis* which is associated with advanced peri-implantitis. Thus, the hard nitride coatings could only delay the bacterial formation, but useless after the development of the biofilm.

3.7.4 Nano-structures

Recently Krunal et al. [149] has reported to coat TiO_2 nanotubes onto Titanium surface, and such kind of anodized nanostructure has demonstrated certain degree of antibacterial properties associated with their diameters (and contact angles), i.e. smaller the diameter has smaller the bacteria. In addition, for the same diameter, nanopores might have less bacterial adhesion than

nanotubes. Although the authors paid the attention of antibacterial adhesion onto the chemistry, which might has also been studied in other industries for the fluorine containing-TiO_2 [150], the authors also admitted that they could not explain the difference about the bacterial attachment with the variety of the substrates. In fact, obviously from the AFM figures (Fig. 7) in their study clearly showed that nanopores exhibit a biomimetic pattern similar to Dragonfly forewings [151] or Cicada wings [152]. Such a bio-inspired nano-architecture exhibits a bactericidal property via its regularly spaced nanopillar array, since the structure could physically shred the bacteria. This is a wise approach because the TiO_2, due to the chemical property of surface charge, would definitely attract cells under biological medium. After the bacteria have been actively approached to the material surface, they would be shred due the sharp nano-structure that could break the cell walls. In addition, another worth to mention bio-inspired bactericidal surface is Gecko skin. This surface surprisingly is hydrophobic and has been demonstrated self-cleaning and anti-bacterial [153] properties, due to the presence of nano-spinules (hairs) on the dome-shaped hexagonal micron-sized array. Therefore, the combination with the surface chemistry with the nano-structure should be a pioneer topic for achieving a successful surface functionalization.

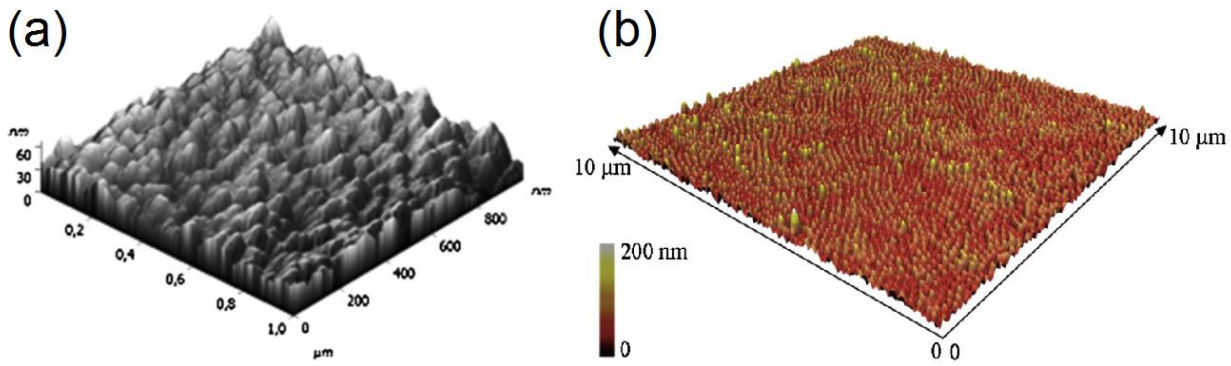

Fig. 7 AFM images showing the nano-structures that exhibit antibacterial properties. (a) TiO_2 nanopores with 15nm diameters using anodization (adapted from Krunal et al. [149]), (b) Cicada wing structure (adapted from Pogodin et al. [152]).

3.7.5 GL13K-biofunctionalized titanium

The antimicrobial activity and cytocompatibility of bio-inspired GL13K-biofunctionalized titanium make it a promising candidate for sustained inhibition of bacterial biofilm growth. GL13K, derived from parotid secretory protein, indeed has been shown to be both bactericidal and bacteriostatic [142]. This surface chemistry provides a basis for development of multifunctional bioactive surfaces to reduce patient morbidities and improve long-term clinical efficacy of metallic dental and orthopaedic implants [142]. However, GL13K is not effective in *P. gingivalis* which one of the most susceptible peri-implantitis pathogens[154], particularly for those associated with severe periodontitis [155]. Furthermore, like many other coatings, it might be easily to be removed using mechanical force such as stretching. Hence, the clinical use of such bio-inspired polymeric coating might need to be justified.

3.7.6 Polysaccharides

It has been reported that polysaccharides molecules, such as chitosan and hyaluronic acid, could inhibit the adhesion of bacteria to titanium [116] since they were claimed to interfere the surface linkage between titanium and biofilm. In fact, in one study, antibacterial multilayer coatings loaded with minocycline, which is a broad-spectrum tetracycline antibiotic, on surface of Ti substrates using chitosan and alginate has been constructed, based on layer-by-layer (*LbL*) self-assembly technique [156]. Obviously, the function of minocycline is to kill planktonic and adherent bacteria. For the chitosan and alginate coating, it claimed to have a surface charge and hydrophilicity that could be biostatic to maintain the antibacterial ability after the complete release of minocycline, as well as an improved-sustainability of minocycline release. Thus, antibacterial ability was improved. This could inhibit the immediate colonization of bacteria onto the surface of implants in the process of dental implant surgery, and thereby prevents and reduces the occurrence of peri-implantitis [156]. On the other hand, Hyaluronic acid can inhibit the attachment of bacteria onto the surface but decreases the affinity of osteoblasts at the same time. To increase the affinity without attracting bacteria, chemicals such as Sericin and argininеglycine- aspartic acid (RGD), so-called cell adhesives could be applied [116]. Such kind of coatings, similar to many polymeric coatings, has unknown effects under mechanical damage. Thus, if such coatings are applied in implant body, a careful insertion procedure without damaging the coating such as screwing might be necessary, which deemed to be impractical for implant application.

3.7.7 *Poly* (sodium styrene sulphonate) groups

A study demonstrated that cpTi coated with *poly* (sodium styrene sulphonate) does not only promote osteoblast function but also inhibits bacteria adhesion. Such modified titanium surfaces provide us a promising strategy for preventing biofilm-related infections and enhancing osteointegration of implants in dental applications [157], due to its osteoblastic selective nature by its sulphonate group [158, 159] and the negative charge might be the reason for antibacterial property [160].

3.7.8 Silane

Silanes, particularly the methacrylate-based silane, has been used in dental applications for decades usually for resin bonding promotion to metals [53], alloys [161] or ceramics [162-164] substrates. Recent studies by Matinlinna and co-workers demonstrated that silanes were able to reduce the surface free energy of titanium surfaces [53, 165], and has proven to reduce the formation of viable *C. Albicans in vitro* [64], although in the zirconia case the reduction of *C. Albicans* was also found but not due to the decrease of surface free energy as discussed in section 2.1.2. Indeed, the reported thickness of silane was ~50nm which could be regarded as self-assembled multilayers or ultra-thin film. The self-assembled nature of silane could chemically graft or pattern the surface in nanosized manner, and thus affect the initial cell adhesion and shapes, which are critical in the subsequent cellular morphogenesis, cell differentiation, growth and function [166]. For example, with the functional endgroups of NH_2 and CH_3 would exhibit a stronger efficiency pattern for fibroblasts and stress-fibre formation than Si-OH and SH, whilst Si-OH has stronger pattern for neuritogenesis than SH, and even much stronger than CH_3 and NH_2. Thus, varying the endgoups of silanes do affect and regulate

the cells in terms of their signalling pathways, and eventually their physio-adsorption, i.e. cell adhesion ability, onto the substrate [166]. Very recently, Godoy-Gallardo et al. [167] has shown certain silanes could induce osteoblast differentiation but reduce bacterial and biofilm adhesion on titanium surface. Besides the cell signalling ability, silane is thin and strong that even remain after the resin debonding. Therefore, the silane has a high potential in many biomedical application.

3.8 Challenges

3.8.1 Corrosion factors

Although titanium is a corrosion-resistant and biocompatible material, titanium surfaces are prone to bacterial colonization that could lead to inflammation, and finally to implant failure. Various corrosion features, including surface discoloration, deformation of rough and smooth interfaces, pitting attack, and severe surface rusting were present after dental implants are immersed in bacteria medium. Bacteria medium provided a sustained acidic environment for the implant. Implant surface oxidation took place even after immersed for only 2 days in bacteria medium. Metal ions and debris were dissolved into the acidic solution. Dissolution of metal ions and particles in the oral environment can facilitate the development of peri-implantitis at later stages[168].

Certain biomolecules including lipopolysaccharide (*LPS*), a component of Gram-negative bacterial cell walls and driver of inflammation have been shown to interact strongly with Ti and modify its corrosion resistance. It was found that Ti release was inhibited by *LPS* under the most

acidic conditions when pH was 2, which may develop in localized corrosion sites, but the dissolution was promoted at pH 4-7, which would be more commonly physiological position. *LPS* is found extensively on the surfaces of skin and mucosal penetrating Ti implants, therefore, the findings are relevant when considering the chemical stability of Ti implant surfaces *in vivo* [169].

3.8.2 Biofilm effects on the mechanical behaviour of implants

Friction during sliding was analyzed for titanium covered with mixed biofilms consisting of *Streptococcus mutans* and *Candida albicans*. The biofilm which is a complex structure consisting of microbial cells and their extracellular matrix, performed as a lubricant. A low level of friction in sliding contacts may have an important significance in the medical field. The decrease in friction caused by biofilm formation may lead to a loss of mechanical integrity of internal connections in dental implant. Consequently, the study of the exopolymeric matrix can be important to develop the novel joint-based systems for medical as well as engineering applications. The composition and structure of biofilms should be fully investigated to fully understand the friction behavior of dental implant connections and prosthetic joints [170].

4. Future perspectives

4.1 Laser therapy, photodynamic therapy and photobiomodulation

The use of lasers in implant dentistry has increased over recent years and varies considerably in terms of their application, laser type and irradiation dose. Applications include fabrication and modification of surface topology (e.g. selective laser sintering); ablative methods for hard and

soft tissue preparation; decontamination of the implant surface or surgical site (laser therapy, or photodynamic therapy); and therapeutic effects to reduce pain, inflammation and promote wound healing (photobiomodulation) [171].

High power surgical applications for decontamination and bactericidal effects have included the use of Nd:YAG, CO_2 and Er:YAG lasers. Although positive effects have been reported in terms of decontamination, debridement and removal of bacterial plaque and calcified deposits from the implant surface, there exists a risk of thermal side effects [172, 173] and extensive microstructural alteration of the implant material may affect stimulatory cellular responses and any further potential for osseointegration [174, 175]. There exists limited data on the clinical effectiveness of high power laser therapy, however, several studies support its use and claim at least similar results compared with mechanical methods, e.g. plastic curettes, air-polishing [176, 177]. Regardless, laser therapy remains only as an adjunct treatment option until substantive clinical effects are seen in long-term studies [178].

Photodynamic therapy (PDT) may also provide bactericidal effects following exposure of an exogenous photosensitizer dye to specific wavelengths of light. Efficient absorption of light, e.g. red wavelengths at ~650 nm by a photosensitizer, e.g. toluidine and methylene blue (λ_{max}~660nm) results in the generation of reactive oxygen species, e.g. singlet oxygen, hydroxyl radicals, which must perforate the bacterial cell wall and diffuse into the bacterial cytosol to initiate cell death. Consequently, due to differences in cell wall architecture, PDT is more effective in killing Gram-positive compared with Gram-negative bacteria [179]. Ideally, the photosensitizer should exhibit

low ‘dark’ toxicity, i.e. when not activated, exhibit high molar absorptivity and quantum yield, i.e. the ability to generate reactive oxygen species at relatively low concentrations and low irradiance, and selective uptake by the target bacterial cells. PDT has been previously used in dentistry for the treatment of caries, mucosal and endodontic infections, management of periodontal disease and peri-implantitis [180]. Specifically for the treatment of implant-related disease, previous studies have highlighted the effectiveness of PDT as a treatment option [181-184], although one study reported ineffective treatment of peri-implantitis 1-year after treatment using a light-activated disinfection technique [185].

Photobiomodulation (PBM), or “low level light therapy” is different from the aforementioned therapeutic strategies as it uses direct (without the use of a photosensitizer), non-thermal and nonionizing wavelengths (usually in the visible to near-infrared regions; ~660, 810, 940 nm) to provide inhibitory and stimulatory biological effects. Although the exact molecular mechanism is not fully understood, significant clinical effects including analgesia, reduced inflammation and accelerated wound healing are well known [186, 187]. A recent review and several previous research articles in animal models have highlighted the potential of PBM to improve dental implant stability by increasing osteocyte viability, osteoclast proliferation and improving bone repair and osseintegration [171, 188-190], although there exist limited randomized clinical trials in this area.

Currently, it would seem that PDT and PBM may offer a useful adjunct to conventional treatments of peri-implant diseases, although there is limited evidence of superior outcomes

compared with conventional therapies. A further confounding factor may also relate to the prevalence of non-standard experimental design and potential misinterpretation of results due to non- or misreporting of critical light parameter measurements in studies using light-based therapies [183, 191, 192]. Future studies should always report relevant radiometric properties of the light delivery (power, beam area, irradiance, exposure time, radiant exposure, etc) and absorption characteristics of the photosensitizer (concentration, spectral absorption, molar absorptivity, etc) in order to properly understand the beneficial effects of PDT and PBM.

4.2 Photocatalysis

Ultraviolet (UV) irradiation is known to have a photocatalytic effect on the oxide layer surface of the titanium surfaces, thus providing titanium surfaces with anti-bacteria properties. The underlying mechanisms involve generation of reactive oxygen species such as hydroxyl radicals which destroy the bacterial cell membrane and wall [193, 194]. The effects of UV-assisted TiO_2-photocatalytic oxidation (PCO) inactivation on pathogenic bacteria were determined by three UV irradiation, namely UV-A (λ=315–400 nm), UV-B (λ=280–315 nm) and UV-C (λ=100–280 nm). The bacteria of *E. coli* has structural changes of photodynamic DNA strand and membrane damage under the UV-assisted TiO_2-photocatalytic oxidation (PCO) [195].

A study by Lilja et al. assessed the effect of photocatalysis for reducing *Staphylococcus epidermidis* adhesion. Nanostructured crystalline titanium dioxide coatings evaporated on titanium implant substrates were demonstrated to exhibit UV-induced photocatalytic activity, which can provide bactericidal effects on *S. epidermidis*. A 90 % reduction of viable bacteria

was observed in 2 min with a UV dose of 2.4 J delivered at 365 nm [196] . An *in vitro* study investigated synergetic effects of TiO_2 photocatalytic surfaces with H_2O_2 against *S. epidermidis* and *S. mutans*. Viabilities of *S. epidermidis* and *S. mutans* were reduced by 99.7% and 98.9% respectively after exposure to 0.1 wt% H_2O_2 and UV light on TiO_2 surfaces for 20 mins, while he corresponding viability reduction was 86% for *S. epidermidis* and 65% for *S. mutans* without H_2O_2. This study indicated H_2O_2 can improve the efficiency of photocatalytic TiO_2 surfaces [197].

UV-A irradiation (382 nm) of titanium implants has been recently introduced as photofunctionalization method to enhance osseointegration, which possibly also provide anti-microbial function to titanium surface as with photocatalyst. *In situ* UV irradiation of pellicle-covered anatase caused a statistically significant decrease of the adsorbed salivary mass. The results suggest that the photocatalytic activity of polycrystalline anatase-modified biomaterial surfaces is able to decompose complex structured macromolecular pellicle films. The reason may be due to the superhydrophilicity of anatase upon UV irradiation [198]. In another study, it was shown that UV-C irradiation reduced the attachment and biofilm formation of wound pathogens on various topographical titanium surfaces, rivalling or surpassing UV-A irradiation in degree. The mechanism might involve superhydrophilicity and carbon elimination on the surface [199]. Therefore, this study opens the way to surface modifications supporting therapeutic approaches of biofilm removal.

A recent study [200] applied TiO_2 nanoparticles on and inside the titania nanotubes (TNT-TiO_2) surface and investigated the photocatalysis effects UV irradiation. *Streptococcus mutans*, *porphyromonas gingivalis* and stem cells were cultured on the materials to determine antibacterial and compatibility properties. After one week, due to the photocatalysis effect and related wettability change, i.e. higher the surface energy, stem cells has exhibited improved osteogenic functions on TNT-TiO_2 and both types of bacteria were lower on the surface of TNT-TiO_2 than pure Ti and TNTs. Therefore, coating TNTs with nanosized TiO_2 particles increase the surface area for photocatalysis, and increase the PCO with simultaneously improved antibacterial properties and greater cell osteogenic capacity.

4.3. Plasma

Non-thermal atmospheric pressure plasma was used for the treatment of single- and multispecies dental biofilms on titanium discs. Plasma was shown to be much more effective than 0.1% CHX against biofilms *in vitro* [201]. Thus, the development of plasma devices for the treatment of peri-implant inflammation may be promising.

4.4. Bioelectric effect

An electrical enhancement of the effect of antiseptics has recently been described. Electric currents were reported to enhance the antimicrobial effect against biofilms of many biocides. It has been confirmed the addition of low intensity direct electric currents (DC) to antimicrobial agents can improve the bactericidal efficacy significantly [202]. This phenomenon is called the "bioelectric effect". In dental research, a significant enhancement of 0.2% chlorhexidine against

P.gingivalis was observed with the application of 10mA currents [203]. Therefore, the bioelectric effects provide us a novel notion for antimicrobial treatment.

5. Conclusion

Bacterial adhesion and biofilm formation are the principle reasons that cause peri-implantitis. The adhesion is very a complicated process which can be affected by many risk factors, such as the local factors of the interaction between microorganisms and implant, systematic factors of oral environment. A large variety of studies have provided us with novel materials and methodologies to prevent bacteria adhesion on dental implant. However, mechanism of bacterial adhesion and subsequent implant inflammation need to be further investigated. To solve the problem of bacterial adhesion, in particular on dental implant, a multi-disciplinary collaboration is necessary.

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