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REVIEW ARTICLE

Biomedical engineering of dental implant infections

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Abstract

Implant surfaces have significant role in osseointegration potential of the implant. Dental implants are predominantly placed in primary care settings, commonly in general dental practice under local anesthesia. There are however, no controls legislated over the operating environment in dental clinics. Despite this and the contaminated oral surgical field through which they are placed, success rates are reported as being as high as 90-95%. Despite the high success rate of implant dentistry in recent years, implant failures due to peri-implant diseases do exist. The ability of bacteria to adhere titanium implant surfaces has been confirmed in various studies. Elimination of bacteria from the implant surface is necessary in order to terminate the source of infection and disrupt the formation of bio-film. Biomaterial therapies using fibers, gels, and beads to deliver antibiotics have been used in the treatment of peri-implantitis though clinical efficacy is not well documented. Guided tissue regeneration membranes (e.g., collagen, poly-lactic/glycolic acid, chitosan, ePTFE) loaded with antimicrobials have shown success in reosseointegrating infected implants in animal models. Future strategies include the development of surfaces that become antibacterial in response to infection and improvements in the permucosal seal. Research is still needed to identify strategies to prevent bacterial attachment and enhance normal cell/tissue attachment to implant surfaces .There is also possibility of development of recombinant protein using r-DNA technology and using the application of tissue engineering in development of coating of biomaterials using for dental implants. In the present study, general microbial status of healthy implants, infected dental implants, along with normal microflora present in the mouth have been covered as per the reports of different methods of isolation used, the mechanisms of attachment of microbes through biofilm formation and how to minimize the forces of adhesion to the surface of dental implant material has been covered to enable the exploring the applications of biomedical engineering with respect to the understanding of characteristics of microbiota (morphology and biochemical aspects) and compatibility surface characteristics of biomaterials with respect to the ossointegration and biofilm formation.

Keywords: Dental implants, peri-implantitis, osseointegration, r-DNA technology, microflora, biofilm.

Introduction

A typical implant consists of a titanium screw (resembling a tooth root) with a roughened or smooth surface. The most common type of dental implant is endosseous comprising a discrete, single implant unit (screw or cylinder-shaped are the most typical forms) placed within a drilled space within dento-alveolar or basal bone (Branemark *et al.*, 1977). Commercially pure titanium or titanium alloy are the common constituents of dental implants. However, alternative materials include ceramics such as Al_2O_3 and other alloys (gold and nickel chrome-vanadium). Implant surfaces may be modified by plasma spraying, anodizing etching, or sandblasting to increase the surface area and osseointegration potential of the implant (Palmer, 2007). Dental implants are predominantly placed in primary care settings, commonly in general dental practice under local anesthesia. There are however, no controls legislated over the operating environment in dental clinics. Despite this and the contaminated oral surgical field through which they are placed, success rates are reported as being as high as 90-95% (Adell *et al.,* 1981; Quirynen *et al.*, 2000).

Despite the high success rate of implant dentistry in recent years, implant failures due to peri-implant diseases do exist. The ability of bacteria to adhere titanium implant surfaces has been confirmed in various studies. Elimination of bacteria from the implant surface is necessary in order to terminate the source of infection and disrupt the formation of bio-film. Biomaterial therapies using fibers, gels, and beads to deliver antibiotics have been used in the treatment of peri-implantitis though clinical efficacy is not well documented. Guided tissue regeneration membranes (e.g., collagen, poly-lactic/glycolic acid, chitosan, ePTFE) loaded with antimicrobials have shown success in reosseointegrating infected dental implants in animal models but have not been proven in humans (Norowski *et al.*, 2009).Experimental approaches include the development of anti-bioadhesion coatings, coating surfaces with antimicrobial agents (vancomycin, Ag, Zn) or antimicrobial releasing coatings (calcium phosphate, polylactic acid, chitosan).

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Table 1. Colonization pattern of microbiota of periodontal, dental implants and infected implants.

Frequently occurring species in microbiota of disease, healthy and dental implants			Infrequently occurring species in microbiota of periodontal and infected implants		Species colonize the implants that were failing	Dominant species characterizing symptomatic implants	Microbiota of healthy implants (included health associated species
Gingivitis sites	Gingivally healthy sites	Dental implants	Periodontal samples	Infected implant samples	Putative periodontal pathogens including spirochetes, *Peptostreptococcus micros*, *Fusobacterium* sp. enteric gram-negative rods, and yeasts (these pathogens were found in high proportions of the microflora cultured) (Mombelli *etal.,*1987; Alcoforado *et al.,* 1990; Rams *et al.,*1991; Mombelli *et al.,* 1995).	gram-negative spp., *Bacteroides forsythus, Fusobacterium nucleatum*, subspecies *vincentii, Campylobacter gracilis, P. gingivalis,* Gram-positive spp., *Streptococcus intermedius* and *P. micros* (Mombelli *et al.*, 1987; Sordyl *et al.*, 1995).	*Streptococcus sanguis, S. oralis, S. gordonii* and gingivitis associated species such as *Actinomyces naeslundii* and *Capnocytophaga gingivalis*. Overall, the microbiota of the peri-implants and the periodontal infections was found similar (Mombelli *et al.,*1987; Sordyl *et al.,*1995; Tanner *et al.,* 1996).
Streptococcus sanguis, Actinomyces viscosus and *Actinomyces odontolyticus*) as well as putative periodontal pathogens (e.g., *Porphyromonas gingivalis, Prevotella intermedia, Prevotella melaninogenica* and *Fusobacterium* sp. (Mombelli *et al.,* 1987; Sordyl *et al.,* 1995).			*staphylococci, enteric rods, pseudomonads, enterococci* and yeasts (Rams *et al.,* 1990; Rams *et al.,*1991; Alcoforado *et al.,*1991; Leonhardt *et al.,*1992; Leonhardt *et al.,* 1993; Passariello *et al.,* 1993).				

Future strategies include the development of surfaces that become antibacterial in response to infection and improvements in the permucosal seal. Research is still needed to identify strategies to prevent bacterial attachment and enhance normal cell/tissue attachment to implant surfaces. There is also possibility of development of recombinant protein using r-DNA technology and using the application of tissue engineering in development of coating of biomaterials using for dental implants. In the present study the distribution pattern of microbiota of healthy implants, failing implants, normal diseases sites (gingivitis) and healthy sites (gigivally) was reviewed along with the bacterial adhesion processes and methods of decontamination of dental has been covered to enable the future uses of applications of biomedical engineering in respect of bacterial adhesion compatibility to the surface of implants for minimizing the forces of attachment by modification of surface property of implants to study the surface free energy of implants material for avoiding formation of bio-film. Therefore, it is necessary to characterize the reported microbiota to understand the operation of engineering concepts to minimize the bacterial attack on the dental implants. The future possibility cannot be ruled out for uses of r-DNA technology for creation of bio-films of microbes on the surface of dental implants to make it inert for any type of microbes attack.

Peri-implant diseases
Peri-implantitis is defined as an inflammatory reaction with the loss of supporting bone in the tissues surrounding a functioning implant (Klinge *et al.*, 2005). Two types of peri-implant diseases are commonly recognized namely peri-implant mucositis and peri-implantitis, both describing an inflammatory response around the peri-implant tissue.

Peri-implant mucositis is a term used to describe the inflammatory reaction around the peri-implant tissue without any radiographic loss of bone. On the other hand, peri-implantitis, one of the major causes of implant failure, is an inflammatory response around osseointegrated implants, resulting in loss of bone around an implant in function (Albrektsson and Isidor, 1994; Zitzman and Berglundh, 2008). Risk factors for peri-implantitis consist of a history of periodontitis, dental plaque, poor oral hygiene, smoking, alcohol consumption and diabetes. A clinical diagnosis indicates inflammatory signs including bleeding on probing with or without suppuration and a peri-implant pocket depth ≥ 5 mm (Tung Nguyen-Hieu, 2012). Several authors reported high rates of implant failures due to peri-implantitis. Esposito *et al.* (1997) found implant removal rates due to peri-implantitis ranged from 8-50%.

The aetiology of the disease is conditioned by the status of the tissue surrounding the implant, implant design, degree of roughness, poor alignment of implant components, external morphology and excessive mechanical load. The microorganisms most commonly associated with implant failure are spirochetes and mobile forms of gram-negative anaerobes, unless the origin is the result of simple mechanical overload (Alcoforado *et al.*, 1991; Leonhardt *et al.*, 1992).

Microbiota associated with PI
In the failing implant site, increased proportion of gram negative anaerobic rods, black pigmented bacteroides and *Fusobacterium* spp., spirochetes, fusiform bacteria, and motile and curved rods were found (Alcoforado *et al.*, 1990; Mombelli *et al.*, 1987). As for control sites (successful implants) in the same patient, coccoid cells were the predominant morphotype (Mombelli *et al.*, 1987).

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If this predominates for significant time periods then peri-implantitis and eventual implant failure may result (Mombelli, 1999). Table 1 shows the microbiota of infected and healthy implant, gingivitis sites and gingivally healthy sites. After the insertion of titanium implants, rapid colonization of bacteria has been observed at the peri-implant sulcus (Van Winkelhoff *et al.*, 2000).

Some microbiological studies have shown that implants affected by PI tend to harbor microbiota encompassing periodontal pathogen species, including *Porphyromonas gingivalis, Tannerella forsythia, Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia* and *Fusobacterium* species (Mombelli *et al.*, 1987; Tam *et al.*, 1987). Leonhardt *et al.* (1993) also reported that less common oral species, such as staphylococci, enteric species, and yeasts, were recovered from failing implants. The peri-implant microbiota differs depending on whether the individual is edentulous or partially edentulous. In particular, *P. gingivalis* was rarely isolated from edentulous individuals (Mombelli *et al.*, 1993), which makes an interesting analogy to the paucity of *P. gingivalis* isolated from patients with pericoronitis (Wade *et al.*, 1991) and those in the early stages of periodontitis (Tanner *et al.*, 1996), suggesting that in both cases the deeper periodontal pocket niches favored by *P. gingivalis* were missing. The microbiota of healthy and diseased dental implants appears to differ depending on the suspected etiology of implant symptoms. The peri-implant microbiota of implants with symptoms associated with occlusal trauma was predominated by streptococci and was similar to the microbiota of gingivally healthy sites (Rosenberg *et al.*, 1991). This situation appears to have a parallel in initial periodontitis, where some sites show loss of periodontal attachment with recession and are colonized by species associated with healthy teeth (Tanner *et al.*, 1998). Implants that were failing and that had an infectious etiology were colonized by putative periodontal pathogens including spirochetes, *Peptostreptococcus micros, Fusobacterium* sp., enteric gram-negative rods, and yeasts; these pathogens were found in high proportions of the microflora cultured. No microbiological differences were found between pure titanium and hydroxyapatite-coated implants (Rams *et al.*, 1991) or between one and two-stage implants (Mombelli *et al.*, 1995). Failing implants were identified either by increase in probing depth or suppuration or by recent increased bone loss assessed from periapical radiographs. When examined clinically, the symptomatic implants had deeper probing depths, bled more frequently on probing, and had hotter peri-implant temperature readings than healthy implants. Healthy implants did have "gingivitis," as indicated by positive plaque and redness scores. The microbiota of healthy implants included health-associated species such as *S. sanguis, S. oralis,* and *S. gordonii* and gingivitis associated sp. such as *Actinomyces naeslundii* and *Capnocytophaga gingivalis* (Tanner *et al.*, 1996).

Overall, the microbiota of the peri-implants and the periodontal infections is similar (Passariello *et al.,* 1993; Sordyl *et al.*, 1995). Interestingly, microorganisms not usually associated with periodontitis or dental abscesses such as staphylococci, coliforms and *Candida* spp. are commonly isolated from peri-implant lesions in some studies (Rams *et al.*, 1990; Leonhardt *et al.*, 1999; Salvi *et al.*, 1987). Staphylococci are present within the oral cavity and their isolation from peri-implant infection is significant as both *Staphylococcus aureus* and coagulase-negative staphylococci are frequently responsible for infections associated with metallic biomaterials and in dwelling medical infections in general (Gristina, 1987; Bisno and Waldvogel, 1989). More recently, *Staphylococcus aureus* has been demonstrated to have the ability to adhere to titanium surfaces. This may be significant in the colonisation of dental implants and subsequent infections (Harris and Richards, 2004). These findings indicate the complexity of the microbiota in PI and the species responsible for PI remain unclear. It is also possible that unknown bacteria are involved in the lesions. As pockets around the remaining teeth may act as a bacterial reservoir, the composition of the peri-implant microbiota is likely to be similar to that around teeth. However, few studies have evaluated the differences in bacterial composition between dental implants and remaining teeth in the same subjects. In a recent study, molecular techniques such as oligonucleotide probes, polymerase chain reaction (PCR), and checkerboard DNA-DNA hybridization have been applied to identify the bacteria in PI (Shibli *et al.,* 2008; Maximo, 2009). However, these approaches only detect specific target bacteria and are not practical for identifying the true diversity of potential pathogens in the pockets of PI. In contrast, PCR amplification of conserved regions of the 16S ribosomal RNA (rRNA) gene followed by clone library construction has been used to comprehensively identify various microbiota. This approach allows the detection of almost every species in a given sample and is able to indicate the presence of previously uncultivated and unknown bacteria (Aas, 2005).

A total of 335 sequences from 8 samples were subjected to sequence analysis revealed 112 species, 51(46%) were uncultivated phylotypes, of which 22 were novel. The total numbers of bacterial species identified at the sites of PI, periodontitis and periodontally healthy implants were 77, 57, and 12 respectively (Mombelli *et al.*, 1987). This type of method of using the 16r-DNA gene clone library for detecting the bacterial phyla and genera has merit over the traditional methods of culturing the microbes for identification as shown in Table 2. This study reflects that the bacterial population as well as number of bacteria phyla and genera are more at the site of dental implant failure, and require the necessary steps to check the bacterial population and density at the initial stage before going to implant the teeth.

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Table 2. Microbiota of failing implants as per reports of various authors using different methods of detection of bacteria.

Method of detection	Type of implant (No. of patients/implants)	Most prevalent microbes detected(% sites infected with bacteria)
PCR	Various (10/12) (Shibli *et al.,* 2007)	*Porphyromonas gingivalis* 67% *Campylobacter rectus* 42% *Eikonella corrodens* 42% *Treponema denticola* 42% *Prevotella intermedia* 33% *Tannerella forsythia* 33% *Actinobacillus actinomycetemcomitans* 17%
Culture/dark field microscopy	Titanium hollow cylinder implants (7/not stated) (Mombelli *et al.,* 1987)	*Porphyromonas gingivalis* 27% *Bacteroides* spp., *Fusobacterium* spp., spirochetes, *Fusiform bacilli*, motile and curved rods (% not stated)
Culture	Branemark (37/1-4 per patient) (Leonhardt *et al.,*1999)	*Prevotella intermedia/P. nigrescens* 60% *Actinobacillus actinomycetemcomitans* 60% *Staphylococci, coliforms, Candida* spp. 55%
Culture	IMZ (12/18) (Augthun and Conrads, 1997)	Bacteroides spp. 89% Actinobacillus actinomycetemcomitans 89% Fusobacterium nucleatum 22% Capnocytophaga spp. 27.8% Eikenella corrodens 17%
16S rRNA gene clone library	Various(not stated) Found 77species (PI sites); 57species (periodontitis sites); 12 species (periodontally healthy implants) (Tatsuro Koyanagi *et al.,* 2010)	*Actinobacteria* (*Actinobaculum,Actinomyces,,Atopobium,Propionibacterium, Rothia*) (% not stated) Firmicutes(*Catonella,Dialister,Eubacterium,Gemella,Granulicatella,Lachnospiraceae, Lactobacillus, MogibacteriumParvimonas,Peptostreptococcs, Pseudoramibacter, Selenomonas, Solobacterium, Streptococcus, Veillonella*) (% not stated) Proteobacteria(*Campylobacter,Cardiobacterium,Desulfobulbus,Eikenella,Hemophilus, Lautropia,Neisseria, Terrahaemophilus*) (% not stated) Bacteroidetes(*Bacteroidetes,Capnocytophaga,Porphyromonas,Prevotella*) (% not stated) Fusobacteria (*Fusobacterium, Leptotrichia*) (% not stated) Tenericutes (Mycoplasma) (% not stated) Synergistetes (Synergistes) (% not stated) TM7, Chloroflexi (% not stated)
Checkerboard DNA-DNA hybridization technique	Not stated (21/28) (Salcetti *et al.,*1997)	*P. nicrescens* *P. micros* *Fusobacterium nucleatum* (% not stated)
Culture/indirect immunofluorescence	Not stated(41/not stated) (Listgarten and Lai, 1999)	*Bacteroides forsythus* 59% *Spirochetes* 54% *Fusobacterium* spp. 41% *Peptostreptococcus micros* 39% *Porphyromonas gingivalis* 27%
Culture	Not stated (13/20) (Rams *et al.,*1990)	*Staphylococcus* spp. 55%

Peri-implants biofilms showed more complex microbiota when compared to periodontitis and periodontally healthy implants and were mainly composed of gram negative anaerobic bacteria. Previously established periodontopathic bacteria showed low prevalence and several bacteria were identified as candidate of pathogens in PI, although it is unclear whether the importance of these species is higher when compared to established periodontopathic bacteria.

Factors involved in peri-implantitis
In comparison of natural tooth and the implant, the natural tooth is endowed with certain specific protective mechanisms such as junctional epithelium, connective tissue and cells belonging to the immune system. The epithelium and the interface between the supra-alveolar connective tissue and the titanium surface of an implant differ from the interface of the dental-gingival unit. Like the connective tissue attachment, the epithelium presents a hemidesmosomal attachment to the implant surface.

The difference lies in the fact that the epithelial fibres are predominantly longitudinal to the surface of the implant and not perpendicular, as in the case of a natural tooth (Berglundh *et al.*, 1991; Lindhe *et al.*, 1992).

Implant structure
The design of the implant is an important factor in the onset and development of peri-implantitis. A specific system of implants is described according to its macroscopic morphology, its microsurface and the quality of the alignments of its different components. The implant's superficial roughness favors bacterial plaque adhesion when the surface is exposed to the oral environment, although there is no correlation between the type of surface and the selection of aggressive colonizing bacterial species (Saadoun *et al.*, 1993; Bollen *et al.*, 1996). Poor alignment of the components that comprise an implant prosthesis system may foster the retention of bacterial plaque, as well as enabling microorganisms to pass inside the tranepithelial abutment.

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As Binon *et al.* (1992) described in their study, this is possible because on average, there is a difference of between 20 and 49 micra between the components of the different types of implants currently on the market (Binon *et al.*, 1992). This space provides a point of entry for microorganisms of the oral flora measuring less than 10 micra. The external orphology of the titanium implant seems to be less relevant provided that it has been properly placed. However, the influence of the macroscopic design should be taken into account in terms of the pattern of stress transmission to the bone, which can lead to excessive mechanical stress at certain points, particularly at the junction between the bone and the cervical collar of the implant. Bone loss at this biomechanically weak spot increases the likelihood of bone defect formation at this level and subsequently becoming contaminated (Abrahamsson *et al.*, 1992). Another reported cause of peri-implantitis is the corrosion that can occur when a non-noble metal structure is connected to a titanium implant. In these cases, increased amounts of macrophages have been observed in the tissues surrounding the implant; which would favor the initial bony reabsorption due to non-infectious causes (Olmedo *et al.*, 2003). Another cause of periimplantitis, as previously mentioned, is the bacterial colonization of the periimplant pocket. The association between different microorganisms and destructive periodontal or periimplant disease is governed by the same biological parameters.

Bacteria adhesion on titanium surfaces
The composition of the bacterial environment as well as the ability of bacteria to adhere to the implant surface appears to be two risk factors of peri-implantitis (Quirynen *et al.*, 2002). The ability of bacteria to adhere titanium implant surfaces has been confirmed in various studies. Bacteria ranging from *Streptococcus sanguis* (Drake *et al.*, 1999; Pereira *et al.*, 2005), *Actinomyces viscosus* (Wu-Yuan *et al.*, 1995), *Porphyromonas gingivalis* (Amoroso *et al.,* 2006) and *Actinobacillus actinomycetemcomitans* (Yoshinari *et al.*, 2000), have been reported to have the abilities to adhere to titanium surfaces. Cimasoni and McBride (1987) also documented the successful adherence of *Treponema denticola* on modified hydroxyapatite. Each manufacturer has its own processing method, whose details may not be disclosed to the public. And although the principles of manufacturing implants may be the same, minor details may differ from company to company. Therefore, these studies may not reflect the true characteristics of implants.

Physicochemical mechanisms to bacterial adhesion
Teughels and colleagues (2006) summarized the following phases involved in bacterial adhesion process.

Phase I: Bacterial transportation to the surface
In order for bacterial cells to adhere to a given surface multiple forces and conditions need to come into play (Quirynen and Bollen, 1995).

First of all, the bacterial cells need to be transported to the vicinity of the surface through random movement of particles in the liquid environment (Brownian movement), convective transport along with liquid flow, or active movement of bacteria.

Phase II: The initial adhesion of bacteria
This initial adhesion stage involves weak and reversible adhesion through short and long range forces. Two physicochemical aspects are commonly used to describe these interactions.

Surface free energy of the interacting surfaces
Prior to bacterial adhesion, the water film between the surface and bacteria has to be removed. Thus, the energy of interaction may be calculated from an assumption that this interface between bacterium to liquid (bl) and solid to liquid (sl) has been replaced with a bacteria to solid surface (sb) interface. Absolom *et al.* (1983) proposed the formula describing this change in energy upon adhesion: $\Delta G_{adh} = \gamma_{sb} - \gamma_{sl} - \gamma_{bl}$. This formula explained the bacterial interfacial free energy of adhesion (ΔG_{adh}) and its correlation with solid-bacterium interfacial free energy (γ_{sb}), the solid-liquid interfacial free energy (γ_{sl}) and the bacterium-liquid interfacial free energy (γ_{bl}). When the ΔG_{adh} is negative, adhesion of the bacterium is favored.

The following assumption can be made from the formula mentioned above. If the SFE of liquid is greater than the SFE of bacteria, then the SFE of the substratum will increase and become hydrophilic. As a result, ΔG_{adh} will drop and discourage bacterial adhesion to the substratum. On the other hand, if the SFE of liquid is lesser than SFE of bacteria, SFE of substratum is increased becoming more hydrophobic and promoting bacterial adhesion. In the oral cavity, oral bacteria generally present with high SFE (Weerkamp *et al.*,1985) and saliva with a relatively low SFE (Glantz, 1970), increasing the SFE of the substratum and creating forces favoring bacteria adhesion to any substratum present (i.e. enamel, implant surfaces, or restorative materials) present. In addition, the above mentioned formula of free energy also suggest that bacteria with low SFE will preferentially adhere to surfaces with low SFE and bacteria with high SFE will prefer the high SFE surfaces (Quirynen and Bollen, 1995).

The interaction energies between surface and bacterium
The initial adhesion of bacteria involves multiple forces which depend upon the distance of the bacteria to the surface. Different forces that affect adhesion come into play depending upon the distance the bacterium is from a surface. When the distance is more than 50 nm, Van der Waal's forces, which is the attractive or repulsive force between molecules other than covalent bonds or electrostatic interaction is the only force. When the distance between the bacterium and the surface is about 10-20 nm, both Van der Waal's force and electrostatic forces will exert their effects.

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At this time, repulsion is maximized due to both the bacterium and surface being negatively charged. Bacteria that possess fimbrae or other polymers can be very effective in overcoming this repulsion. This is due to the small diameter of the fimbrae, which result in smaller areas of negative charge on the bacterial surface, reducing its repulsion from the surface to be adhered to.

Chemical forces (between 1-5 nm, short range forces)
Such as hydrogen bonding, ion pair formation, steric interaction, covalent bond and bridging interaction from the cellular filamentous appendages take effect. Eventually when the forces are strong enough, adhesion will turn into an irreversible adhesion (Ofek *et al.*, 2003).

Phase III: Colonization/plaque maturation
The bacteria can start growing and form biofilm after they have attached firmly onto the surface. There is negative aspect of roughened surface. Roughened surfaces produce a sheltering effect for bacterial adhesion protecting them from shearing forces and allowing them the stability and time to easily transform their adhesion from a reversible bond to an irreversible bond onto the substrate (Quirynen and Bollen, 1995). In addition, rough surfaces are capable of accumulating increased thickness/area of plaque and number of colony forming units of bacteria. As time progresses with undisturbed matured plaque, rough surfaces tend to harbor more motile organisms and spirochetes (Quirynen and Bollen, 1995).

Many studies have shown that this effect of rough surfaces on bacterial adhesion in multiple surfaces including restorative material, teeth and titanium. Quirynen *et al.* (1990) demonstrated the effect of surface roughness by applying two strips of material glued on human teeth, one rough (Ra = 2 μm) and one smooth (Ra = 170.1 μm). Only a quarter of the smooth region displayed plaque accumulation while the rough region was completely covered by bio-film after 3 d cessation of oral hygiene. Rimondini *et al.* (1997) demonstrated higher bacterial count in rough surfaces in an *in vivo* study involving titanium discs with various roughnesses after suspension of oral hygiene of 24 h. In addition, more long and short rods were noted in the rougher surfaces when compared to the smooth ones. Tanner and colleagues (2005) tested 4 different materials with Ra values ranging from 0.05 to 0.51 μm bonded to the buccal surface of a molar. After 24 h, intra-orally they found the roughest surface with highest colony forming units of total facultative bacteria and plaque formation. Subgingivally, Waerhaug (1956) demonstrated roughening of subgingival enamel in both dogs and monkey induced more deposit of plaque and calculus, and also resulted in more inflammation. From a microbiological standpoint, rough surfaces appear to harbor more oral bacteria. Rams *et al.* (1991) reported a higher percentage of *P. micros* (*P. micra*) in healthy hydroxyapatite-coated fixtures (17.4%) than in healthy pure titanium fixtures (9.8%).

Quirynen *et al.* (1993) also reported rough surface abutments harbor 25 times more bacteria subgingivally when compared to smoothed abutments. The majority of studies indicate that a rough surface in general creates a friendlier environment for microbial adhesion. Nevertheless, data on newly marketed implant surfaces are surprisingly lacking with regard to the infectious aspect of peri-implantitis.

Removal of bacteria on implant surfaces (decontamination techniques)

High-pressure sodium bicarbonate device
Elimination of bacteria from the implant surface is necessary in order to terminate the source of infection and disrupt the formation of bio-film. This aspect is important when peri-implant pathology of an infectious origin is suspected. Numerous methods of decontaminating implant surfaces have been suggested. Jovanovic *et al.* (1993) suggested a "decontamination protocol" which consists of exposure of titanium surfaces to a high-pressure sodium bicarbonate device for 1 min. under aseptic conditions. Silva *et al.* (2005) tested this method of decontamination and found no viable bacteria in all 3 titanium groups. Augthun's study (1998) also reported an air abrasive system to be the most effective decontamination method and resulted in no damage to the underlying titanium surface. However, Chairay *et al.* (1997) demonstrated altered morphology of machined implants after administration of air powder abrasion. This difference was possibly due to differences in duration of application, as Augthun's group only applied the air abrasive for 60 sec. Besides altering implant surfaces, additional concerns such as possible retained particles after administration and application of compressed air intra-orally may raise some concerns toward this treatment modality.

Laser therapy
Laser therapy had been suggested for the decontamination of implant surface. However, studies show that not all types of lasers are suitable for this purpose. Subsequent damage to the implant surface has been implicated in many cases. For instance, the Nd: YAG laser was reported to cause pitting on implant surfaces in certain settings (Bida, 1991) and also resulted in melting, loss of porosity and surface alteration of plasma coated implants. In addition, Block *et al.* (1992) reported failure of sterilization by the Nd: YAG laser after contamination by spores of *Bacillus subtilis*. In Duarte *et al.* (2009), the Er: YAG laser was not effective in removing *S. sanguinis* (Duarte *et al.*, 2009). Stübinger *et al.* (2010) demonstrated surface alterations of different degrees based on various energy settings of Er: YAG laser on both surfaces. No visible surface alterations were seen when irradiated by CO_2 and diode lasers. The Er: YAG treated SLA surfaces appeared to decrease in roughness due to melting of surface peaks, while smooth surfaces increased in roughness as a result of developing cracks after irradiation.

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Carbon-di-oxide and diode laser treated surfaces were not tested for surface roughness due to lack of visible alteration. Deppe *et al.* (2001) reported thermal changes as well as surface melting and alteration by using CO_2 lasers in different settings. No data was reported on the efficacy of bacteria removal even though the in vivo part of the study appeared successful. The application of laser and its negative effects remains uncertain and further research will be needed in this area before any solid conclusions can be drawn.

Rotating brush with pumice and cotton soaked in saline
Persson *et al.* (1999) conducted an *in vivo* study on beagle dogs utilizing a decontamination method of a rotating brush with pumice and cotton soaked in saline. The rotating brush was used to polish the surface until a "gray and frosty" appearance was noted and cotton dipped in saline was used to clean the surface of implant until no visible plaque was noted. The histological parameter of "re-osseointegration" did not reflect any advantage of either treatment; microbial parameters were not tested in this study.

Systemic antimicrobial therapy
Systemic antimicrobial therapy was administered to the dogs 2 d prior to the decontamination therapy and continued for 3 weeks. From Persson's study, no information on the direct effect of either decontamination therapy on the bacteria can be extracted. The effect of "gray frosted" surface on bacterial adhesion will require more studies.

Curette
The most direct method of physically removing bacteria, plaque or calculus from a surface in the oral cavity is the use of the curette. However the potential damaging effect of a curette to the titanium surface is of great concern. Augthun *et al.* (1998) found that the usage of a metal curette, diamond polishing device, and ultrasonic scalers all resulted in damage to the implant surface. Mengel *et al.* (1998) advised the use of a plastic curette, prophy tips and air abrasive systems because the metal curettes, sonic and ultrasonic devices with universal tips cause pronounced traces or instrumentation and remove substantial substance from titanium. Due to the potential negative effect of the above mentioned implant decontamination methods, more conservative methods have been explored. In addition, no direct proof of complete removal of bacteria was provided. It is unclear whether the systemic antibiotics, degranulation during flap surgery or the actual surface cleaning of saline decontaminated and promoted the implant site healing.

Schou *et al.* (2003) tested 4 methods of decontamination: 1) air-powder abrasive, 2) air-powder abrasive follow by citric acid, 3) gauze socked in saline following citric acid and 4) gauze soaked in 0.1% chlorhexidine and saline alternatively. Following decontamination of implant surfaces, peri-implantitis defects were treated with autogenous particulate bone fill

and covered by e-PTFE membrane. Positive results were obtained and the authors recommended the decontamination of soaked gauze with chlorhexidine and saline. Once again the bacterial aspect of results was not discussed and many variables were included in the study. Rinsing of saline and chlorhexidine was described by Hammerle *et al.* (1995). Sites of peri-implantitis were treated with flap debridement along with rinsing of sterile and 0.2% chlorhexidine digluconate. Dennison *et al.* (1994) conducted an experiment on the removal of bacterial endotoxin by means of burnishing implants with cotton pellet prepared with water, citric acid, or 0.12% CHX and air-powder abrasive. Three types of implants were used: machined, plasma sprayed and hydroxyapatite coated implants. An air abrasion system appeared to be effective in decontamination of *P. gingivalis* endotoxin in all implant types. Both air abrasion and citric acid were effective in decontamination of hydroxyapatite coated implants. For machined surface implants, all treatments including cotton pellet soaked in water can be effective in removal of endotoxin. One can speculate from this study that the application of citric acid or CHX did not provide additional benefit. In addition, the use of acid in decontamination of titanium surface could be alarming because acids are used for surface modification of titanium. Water rinsing is a non-invasive and inexpensive way to remove biofilm on teeth (Gorur *et al.*, 2009). Studies on the efficacy of water rinsing on different titanium surfaces have not been conducted. If proven effective, rinsing titanium implants with sterile saline water with an easily accessible syringe device could be a simple and inexpensive way of treatment if proven to be effective.

Treating peri-implantis
At present, there is no reliable evidence for the most successful method of treating peri-implantitis (Esposito *et al.*, 2006). Despite a variety of therapeutic options, infected implants are difficult to treat and usually require removal (Esposito, 1998). Some clinicians advise systemic antibiotics for the treatment of failing implants and a variety of drug regimens are described (Yukna, 1995).Oral agents such as doxycycline, clindamycin, co-amoxiclav, penicillin V, amoxicillin and a combination of amoxicillin and metronidazole have been recommended. Nevertheless, no double-blind, randomised, placebo-controlled trial has been undertaken. Microbiological study of periimplantitis conducted by the Barcelona School of Dentistry that determined that the antibiotic therapy proven to be most efficacious in the antibiogram was the association of amoxycillin and clavulanic acid (Sanchez-Garces, 2004).

Conclusion
The microbiota of implants is similar to that of teeth in similar clinical states. Implants that fail because of mechanical stress are colonized by species associated with healthy teeth. Infected implants are colonized by subgingival species, including *Porphyromonas gingivalis, Bacteroides forsythus, Fusobacterium nucleatum,*

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Campylobacter gracilis, Streptococcus intermedius, and *Peptostreptococcus micros*. Different patients may be colonized by different microbial complexes, indicating that optimal treatment should be directed to the specific infection. Data on failure and complications of dental implants should be collected and reported in a systematic fashion. This would enable a more detailed analysis of the microbiology, treatment outcomes and assist in the formulation of biomedical engineered materials of dental implants to minimize the risk factor of infections or to completely avoid the implant infections.

Acknowledgements

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