Peri Implant Microflora in Health and Disease

Dr. Sathya Ramanathan *, Dr. PSG Prakash, Dr. Devapriya Appukuttan, Dr. Sangeetha Subramanian, Dr. DJ Victor

SRM Dental college, Ramapuram, Chennai-600089

Abstract:
Implants are one of the fastest growing segments in patient care today for replacing missing teeth and/or retaining other prosthetics. With broad range molecular detection methods, more than 600 bacterial species have been identified that colonize different ecological niches in the human mouth. Microorganisms populating surfaces are gradually organized into complex biofilms. Species within the biofilm interact specifically with each other. Biofilm formation around implants is characterized by a shift from mainly gram-positive aerobic and facultative anaerobic cocci and rods to a higher proportion of periodontal pathogens. The presence of anaerobic microbiota present in the residual periodontal pockets are a plausible threat for future infection in and around dental implants. A pocket around a teeth would favour growth of opportunistic pathogens which would in turn endanger the long term peri implant health. Bacteria in such pathological environments are clear risk factors for peri implant pathology.

INTRODUCTION
The oral cavity is a single site in the human body which provides non-shedding surfaces for microbial colonization. This, and the oral environmental conditions, facilitates growth of numerous micro-organisms and development of dental biofilms. Disturbance of the balance between the oral microflora and the host immune response may result in infection and destructive inflammatory responses in the periodontal tissues. The presence of anaerobic microbiota present in the residual periodontal pockets are a plausible threat for future infection in and around dental implants. A pocket around a teeth would favour growth of opportunistic pathogens which would in turn endanger the long term peri implant health. Bacteria in such pathological environments are clear risk factors for peri implant pathology. The inflammatory condition that develop around the implant are collectively recognized as peri-implant disease. They are

1. Peri implant mucositis - An inflammatory response limited to the soft tissues surrounding a functioning oral implant.
2. Peri implantitis- An inflammatory response that involves loss of marginal bone around a functioning oral implant.

Peri implantitis is defined as bacterially induced inflammation of supporting peri implant tissues leading to non-reversible bone destruction (Lang and Berglundh et al 2011). The microbiota colonizing successfully osseointegrated dental implants was similar to that colonizing clinically similar crowned teeth in the same oral cavity. Studies suggested that peri-implant microbiota does not differ significantly from dental sulcus microbiota, neither in health nor in disease, and concluded that crossinfection of implant habitants is through bacterial transmission. Same way, all the implants unavoidably present a micro-gap between the implant and the abutment which cannot provide a complete seal at the implant abutment junction, so that bacterial leakage may occur regardless of the type of connection. When the host’s immune response, being either inefficient or excessive, fails to suppress the pathogenic flora the balance maintaining peri-implant health is altered which eventually leads to destruction of the peri-implant tissues (Griffen et al., 1998). The flora associated with peri-implantitis is mainly composed of gram negative anaerobic rods with high proportions of black-pigmented Bacteroides-, Campylobacter- and Fusobacterium species. The presence of periodontal pathogens such as A. actinomycetemcomitans, P. gingivalis, T. denticola, T. forsythia, Campylobacter graciesis and Campylobacter rectus at sites with peri-implantitis has been documented in many studies (Sanz M et al., 1990; Persson et al., 2010). The discrepency in the microbial profile of peri-implantitis between studies is in part due to the use of different detection methods. Culture analysis has long been the golden standard for microbial analysis and it has many advantages. However, non-cultivable and dead species are not detected by this method which increases the risk for false negative results. It requires sufficient sample volumes as well as adequate transportation conditions. In contrast, molecular detection methods such as qPCR and DNA-DNA hybridization are less time consuming and more sensitive, allowing the detection of species present at low levels.

GENERAL REVIEW
MICROBIAL COLONIZATION IN IMPLANTS
Implants have revolutionized dental rehabilitation, prosthetic dentistry, and maxillary reconstruction. Although dental implants survive well, infections at peri-implant sites have been widely reported. The colonization pattern on implants appears to be initially slower than on natural teeth. However, once the biofilm is established, it acts as an orchestrated microbial challenge causing, soft and hard tissue reactions in and around the implants. The bacterial products originating from the microgap and from peri implant sulcus cause the upregulation of cytokines, in healthy peri-implant macrophages, mesenchymal stromal cells and gingival fibroblasts that result in the recruitment of resorptively active osteoclasts and, ultimately, bone resorption. The bacterial endotoxins may upregulate pro-inflammatory genes in a number of resident cells found in the healthy peri-implant compartment, and, that the local synergistic action of cytokines secreted by such cells result in the genesis of resorptively active osteoclasts.

PERI IMPLANT BIOFILM FORMATION
When Transmucosal part of implant is:
1. Exposed to oral cavity
2. Rapidly colonized by microorganisms
They attach to salivary proteins and peptides and form Pellicle (which Contains receptors for adhesins on the cell surface of bacteria). Then Adhesion of early colonizers; (S. sanguinis, A. naeslundii) to salivary pellicle occurs and the early colonizers grow and modify the environment and promote the adhesion of secondary colonizers via co-aggregation.

The biofilm becomes stable and forms a protective environment for Plaque to buildup around the dental implant which results in inflammatory changes in the peri-implant tissues. The inflammatory cell infiltrate & an ulcerated epithelium which detach from the implant surface are found to be causing Peri-implant disease. 10 (Fig.2) Bacteria may exert deleterious effect on the peri-implant tissues both directly and indirectly. Implants with rough surface are particularly susceptible because the plaque can adhere and spread easily. Bacteria can elaborate Collagenases, Trypsin-like substances, Extracellular phospholipase A, Anti-chemotactic substances, IgG IgA Proteases, LPS (endotoxin ). By activating the local & systemic immune response bacteria may indirectly cause tissue damage that is mediated by macrophages & PMNS. Moreover, type and shape of the implant, connection type, abutment and suprastructure material and the type of prosthetic suprastructure also affect the peri-implant soft and hard tissues. Individuals with periodontal disease typically have a large amount of pathogenic microorganisms in the periodontal pocket. If the individuals lose their teeth, these microorganisms remain viable inside the mouth and can directly influence peri-implant microbiota. (Socransky et al,1998). 11 By adhering on the abutment implant surfaces which induces peri-implantitis causing potential destruction of the alveolar bone near to the implant threads and cause the subsequent loss of the implant. The scientific literature shows that bacterial plaque may play a prominent role as an etiologic factor responsible for implant loss after osseointegration, due to the presence of high levels of bacteria in the peri-implant sites.

The physical and chemical characteristics of the materials will determine the type and quantity of the microbiota around these surfaces. Once biomaterial surfaces have contact with biological molecules either in vitro or in vivo, the proteins present in the biological medium immediately coat the surfaces. Thereafter, salivary acquired pellicle formation takes place as the first step to biofilm formation. 12

Burgers et al. evaluated the initial biofilm formation, in vitro and in vivo, on different titanium surfaces and correlated these findings with different surface properties. He demonstrated that the rough surfaces tend to entrap bacteria into micropits, protecting them from washing forces. 13

A microgap has been described at the level of the implant-abutment connection. This results in the formation of microcracks between the implant and the abutment. Numerous studies have shown that bacterial contamination of the gap between the implant and the abutment adversely affects the stability of the peri-implant tissue. 14 If average axial forces are exerted on the implant, a pumping effect may ensue, which may then result in a flow of bacteria from the gap, causing the formation of inflammatory connective tissue in the region of the implant neck. On the other hand, they can pose a potential risk for infection once the symbiotic balance between the host and the microbiota is lost. It may be possible that the interindividual variation in microflora of the digestive tract, including the oral cavity, can be attributed to differences in host factors that modulate colonization patterns. 15 This may partly explain a clinical observation in cases where inflammation severity does not correspond with oral hygiene measures. As an example, there are patients who suffer from PIDs despite having a proper hygiene regimen and vice versa – there are cases with no clinical signs of infection despite poor oral hygiene or a history of periodontitis or smoking. PID appears to result from an inappropriate inflammatory reaction to the normal microbiota exacerbated by the presence of some disease-associated bacterial species, host-related factors, geographical factors influencing disease progression and the characteristics of the foreign body material.

Interactions between bacterial- and host-related factors lead to homeostasis breakdown, similar to the PSD(Poly microbial Synergy and Dysbiosis) model. 39

**PERI IMPLANT MICROFLORA**

**IN HEALTH**

Although dental implants survive well, infections at peri-implant sites have been widely reported. The colonization pattern on implants appears to be initially slower than on natural teeth. However, once the biofilm is established, it acts as an orchestrated microbial challenge causing, soft and hard tissue reactions in and around the implants. Recently, Lekholm et al. reported clinical and microbiologic observations in a group of patients with successful osseointegrated titanium fixtures worn from 6 months to 15 years. Darkfield microscopic results produced similar findings, namely 88.5% coccoid forms, 5.6% non-motile rods and very small proportions of all other morphotypes not exceeding 5%. 18 Several studies such as those by Apse and colleagues and Quirynen and colleagues, which indicate that the composition of the microbial flora around implants is dependent on the presence of teeth and, consequently, the bacteria present around implants and around teeth are similar. 19 Another study by Buchmann and colleagues stated that among the bacteria cultured from around implants Peptostreptococcus and Streptococcus were the least abundant, Veillonella had a variable frequency, and of the Gram-negative bacteria Fusobacterium nucleatum, Porphyromonas gingivalis, and Peptostreptococcus micros were the most abundant. 20 In an analysis of the biofilm that forms on the surface of oral implants Heuer and colleagues, showed using molecular methods that Porphyromonas gingivalis and A. actinomycetemcomitans and were more abundant than other bacteria.

Jamil A. Shibli et al in 2008 reported that the profiles of the complexes that harbor most of the beneficial species...
(purple, yellow and green) were similar between healthy and diseased implants. However, most of the pathogens from the red and orange complexes were present at higher levels in the peri-implantitis group. P. gingivalis, T. forsythia, T. denticola, Fusobacterium nucleatum ss nucleatum, Fusobacterium nucleatum ss vicentii and P. intermedia were at significantly higher levels in the subgingival biofilm of the diseased implants. 21

IN DISEASE, peri-implant inflammations represent serious diseases after dental implant treatment, which affect both the surrounding hard and soft tissue. Mucositis describes a bacteria-induced, reversible inflammatory process of the peri-implant soft tissue with reddening, swelling and bleeding on periodontal probing. These are typical signs, but they are sometimes not clearly visible. Furthermore, bleeding on probing (BOP) might be an indicator for peri-implant disease. In contrast to mucositis, peri-implantitis is a progressive and irreversible disease of implant-surrounding hard and soft tissues with bleeding on probing, deep probing depths and is accompanied with bone resorption, decreased osseointegration, increased pocket formation and purulence. The abundances of anaerobic and gram-negative bacteria were statistically higher in peri-implantitis sites that is a peri-implant pocket seems to harbor a microbiota similar to that found in periodontal disease, such as Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens, Tannerella forsythia, Campylobacter rectus and Aggregatibacter actinomycetemcomitans (Mombelli et al. 1987; Söderfalk et al. 1997; Persson et al. 2006; Renvert et al. 2007). The flora associated with peri-implantitis is mainly composed of gram negative anaerobic rods with high proportions of black-pigmented Bacteroides-, Campylobacter- and Fusobacterium species. The presence of periodontal pathogens such as A. actinomycetemcomitans, P. gingivalis, T. denticola, Tannerella forsythia, Campylobacter gracilis and Campylobacter rectus at sites with periimplantitis has been documented in many studies (Sanz M et al., 1990; Persson et al., 2010). Consequently, it was concluded that periimplantitis and periodontitis share similar microbial profiles. However, other studies have identified species not primarily associated with periodontal diseases at sites with periimplantitis. These species include enteric rods, fungal organisms (Leonhardt et al. 1999; Schwarz et al. 2015) 22,28, Staphylococcus aureus (Persson et al., 2010) 29 and human Cytomegalovirus as well as Epstein-Barr virus (Jankovic et al., 2011), thus indicating microbiological differences between periodontitis and periimplantitis. In a culture study by Leonhardt et al. (Leonhardt et al., 1999) 30 it was revealed that diseased implants harboured periodontal pathogens such as A. actinomycetemcomitans, P. gingivalis, Prevotella intermedia and Prevotella nigrescens in 60% of the cases. In addition, enteric rods, Candida- and Staphylococcus species were detected in 55% of the diseased cases which led to the conclusion that periimplantitis and periodontitis may share different microbial profiles. In a newly published systematic review 194 microbiological studies were screened of which 47 studies were included in the review. It was concluded that classic periodontal pathogens are often found in the flora of peri-implant disease, however, correlation between studies is difficult due to the use of different detection techniques. For future 6 investigations, the authors recommended the use of megagenomic techniques in order to avoid detection bias (Padial- Molina et al., 2016) 31. Methods which are used for microbial detection involve culture techniques, DNA hybridization, polymerase chain reaction (PCR), immunofluorescence and 16S RNA sequencing. The different detection methods all have advantages and disadvantages and analytical results vary depending on the technique used. Hence, comparison of results generated by different techniques in different studies vary. The management of peri-implant infections aim at reduction of inflammation, pathogenic bacterial load and the probing depths. Biofilms related to dental implants are best treated through debridement of the contaminated implant surface (mechanical/laser/photodynamics, etc.), or the antimicrobial therapy with local or systemic antibiotics. (TABLE 1)

PLATFORM SWITCHING

IAJ is a vulnerable area for biofilm-related infections. Innovative implant abutment designs have helped reducing the microleakage at the IAJ with the sequential decrease in the microbial growth at the microgap. The use of tapered implants deceases or eliminates this probable microbial ingress. Studies have reported that the connection design might also influence bacterial colonization. Canullo et al evaluated the micro biome in abutment implant interface in different connections and reported that Peptostreptococcus and Tannerella denticola was the most significant microbe in external collar and internal hexagon design. Through placement of Platform switched abutments, the horizontal and vertical distance between the implant-abutment interface and the marginal bone crest is increased and the inflammatory infiltrate is displaced away from the marginal crestal bone which provides a hermetic seal from the peri implant microflora and in turn prevents crestal bone loss. So the connections influence bacterial activity levels quantitatively and qualitatively. Finally last but not the least, Oral hygiene plays a key role the in implant survival rate. The hygiene of the implants and implant-supported prosthesis must be maintained with daily home care and with the patient adhering to a supportive maintenance program. Recent discoveries in microbiology open a completely new perspective on the etiology of peri-implant disease and the further development of metagenomics might open the way to thoroughly new therapeutic approaches. A complete knowledge of oral and peri-implant microbiota in health and disease in their full genomic composition could potentially lead to the development and thorough knowledge of this disease, supported by the concepts which will allow the clinician to better understand and prevent its occurrence and arrest its progression.
**DISCUSSION**

Dental implants have become an indispensable established therapy in dentistry in order to replace missing teeth in different clinical situations. Success rates of 82.9% after 16 years follow-up have been reported that inflammation in the soft tissues and hard tissues around an implant results in peri implant mucositis and peri implantitis. This disease process is similar to the pathological process that occurs around natural teeth and cause gingivitis and periodontal disease. If the implants are placed in patients with active periodontal disease, the microflora around the implants will become similar to microbiota around the diseased teeth. Hence it is essential to treat the periodontium before placement of dental implants. (Lee H. Silverstein et al in 1994)  

The microbiota of healthy periodontal sites and of diseased sites have been shown to differ from each other. According to Listgarten M.A. et al 1992, Small numbers of microorganisms and fewer morphotypes were found in healthy gingival sulci. 

Recently, Lekholm et al. in 1986 reported clinical and microbiologic observations in a group of edentulous patients with successful osseointegrated titanium fixtures worn from 6 months to 15 years. Darkfield microscopic results produced similar findings, namely 88.5% coccoïd forms, 5.6% non-motile rods and very small proportions of all other morphotypes not exceeding 5%. 

Rams et al. in 1984 investigated subgingival samples taken from 13 healthy periimplants and from 3 implants with advanced pocket depths in the microscope. They found significantly higher proportions of spirochetes (32.0%) in diseased sites and elevated cocci (64.2%) combined with very low spirochetal counts (2.3%) in healthy sites. According to Slots J et al in 1986, Gram-negative organisms were significantly elevated in unsuccessful periimplant areas, including black-pigmented Bacteroides which are considered as important bacteria in the pathogenesis of periodontal diseases. The occurrence of B. intermedius, the dominant Bacteroides sp. in samples of failing sites has been shown to correlate with the severity of gingival inflammation and periodontal pocket depth was reported by Zambon J.J. et al in 1981. Eubacteria, also significantly elevated in the failing sites, are regarded as important opportunistic pathogens in oral and non-oral infections (Brook 1987). 

Spirochetes are ubiquitous in plaques associated with gingivitis and periodontitis and their proportions have been correlated with severity of the disease. They may be pathogens in certain periodontal diseases, and may be indicative of a periodontal ecosystem conducive to disease. 

According to Mombelli et al. in 1988 Perimplantitis be regarded as a site-specific infection in which microbial pathogens, mainly belonging to the group of gram-negative anaerobic rods. 

Several studies such as those by Apse and colleagues in 1989 and Quirynen and colleagues in 2006 indicate that the composition of the microbial flora around implants is dependent on the presence of teeth and, consequently, the bacteria present around implants and around teeth are similar. Another study by Buchmann and colleagues stated that among the bacteria cultured from around implants Peptostreptococcus and Streptococcus were the least abundant. Veillonella had a variable frequency, and of the Gram-negative bacteria Fusobacterium nucleatum, Porphyromonas gingivalis, and Peptostreptococcus micros were the most abundant. In an analysis of the a biofilm that forms on the surface of oral implants. Heuer and colleagues in 2007 showed using molecular methods that Porphyromonas gingivalis and A. actinomycetemcomitans and were more abundant than other bacteria. 

However, a study by Nakou et al in 1987 and colleagues suggested that the presence of Spirochetes is related to the presence of inflammation around implants. In other studies, Mombelli et al in 1993 and colleagues stated that 80% of the bacteria cultured from around oral implants were facultative anaerobic Gram-positive cocci species which are observed during the first 6 months following implant placement. They also did not detect Spirochetes but did detect an abundance of Fusobacterium and also anaerobic black-pigmented Gram-negative bacteria. 

Apatazioud D et al in 2017, Sanz-Martin et al in 2017 reported that The genera Actinobacillus and Streptococcus were most closely associated with health. da Silva ES et al in 2015 reported that Healthy implants demonstrated higher proportions of Actinomyces, Atopobium, Gemella, Kingella and Rothia and lower levels of Campylobacter, Desulfovibulus, Dialister, Eubacterium, Filifactor, Mitsukella, Porphyromonas and Pseudorambacter. 

AL- Ahmed et al in 2018 reported that different distributions of taxa belonging to the phyla Firmicutes, Bacteroidetes, Fusobacteria, Actinobacteria, Proteobacteria, Synergistetes, Spirochaetae and TM 7 were detected in the healthy implant and peri implantitis group. The putative periodontal red complex (Porphyromonas gingivalis, Tannerella forsythia) was also detected at significantly higher levels in the PI group, whereas the yellow group, as well as the species Veillonella dispar, tended to be associated with the HI group. Kumar PS et al in 2012 reported that the predominant species in peri-implant health belonged to the genera Butyrivibrio, Campylobacter, Eubacterium, Prevotella, Selenomonas, Streptococcus, Actinomyces, Leptotrichia, Propionibacterium, Peptococcus, Lactococcus and Treponema. 

Consequently microbial data obtained from studies using allowing the detection of species present at low levels. Analysis are less time consuming and more sensitive, as qPCR and DNA-DNA hybridization and metagenomics advantages. In contrast, molecular detection methods such as the golden standard for microbial analysis and it has many different detection methods. Culture analysis has long been health and disease between studies is due to the use of different detection methods may be very difficult to compare.

**Conclusion:**

On the basis of this review it can be suggested, that the mean prevalence of peri-implant mucositis and peri-implantitis is 43 % and 22 %, respectively (Jepsen et al., 2015) and the prevalence of gram negative mirobes and red complex group of microorganisms would be detected at higher levels around implants with peri-implantitis and higher bacterial load comprising of gram positive cocci and rods are detected around healthy implants. The striking presence of species of the red complex in supragingival biofilm of implants with peri-implantitis suggests an environment for reservoir of pathogenic species, which is able to contribute re-infection in treated subgingival spots. The health-associated microbiome exhibits lower taxonomic diversity, but its exact composition varies significantly across patients. Hence, identification of the individual members within biofilms in healthy individuals and in patients with peri-implant infection of great significance in the development of preventive and therapeutic strategies. Hence, it is possible to conclude that:

- Peri-implant microbiome is present even before installation of dental implants;
- Microbiome established around dental implants is similar to the microbiome of periodontitis, in health, and also in cases of periodontal disease;
- Further studies are still required to find an implant with the correct surface that decreases microbial colonization and ensures bigger success in dental implant treatments.

**Reference**

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