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Peri Implant Microflora in Health and Disease

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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.[1] 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.[2]

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)[3]. 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.[4] 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.[5] 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).[6] The flora associated with peri-implantitis is mainly composed of gram negative anaerobic rods with high proportions of black-pigmented Bactericides-, Campylobacter- and Fusobacterium species. The presence of periodontal pathogens such as A. actinomycetemcomitans, P. gingivalis, T. denticola, Tanerella. Forsythia, Campylobacter gracilis and Campylobacter rectus at sites with peri-implantitis has been documented in many studies (Sanz M et al., 1990; Persson et al., 2010)[7,8]. The discrepancy 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.[9] 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 inlammatory 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, Extra cellular 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 above-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. [16,17]

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 a 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; Salcetti et al. 1997; Persson et al. 2006; Renvert et al. 2008) [22-25].

The flora associated with peri-implantitis is mainly composed of gram negative anaerobic rods with high proportions of black-pigmented Bactericides-, Campylobacter- and Fusobacterium species. The presence of periodontal pathogens such as A. actinomycetemcomitans, P. gingivalis, T. denticola, Tanerella. 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)[26]. Consequently, it was concluded that peri-implantitis and periodontitis share similar microbial profiles. However, other studies have identified species not primarily associated with periodontal diseases at sites with peri-implantitis. These species include enteric rods, fungal organisms (Leonhardt et al. 1999; Schwarz et al. 2015)[27,28], Staphylococcus aureus (Persson et al., 2010)[19] and human Cytomegalovirus as well as Epstein-Barr virus (Jankovic et al., 2011)[29], thus indicating microbiological differences between periimplantitis and periodontitis. In a culture study by Leonhardt et al. (Leonhardt et al.,1999)[54] 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 Staphylococcispecies were detected in 55 % of the diseased cases which led to the conclusion that peri-implantitis 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 diifferent detection techniques. For future 6 investigations, the authors recommended the use of megagenomic teqniques in order to avoid detection bias (Padial- Molina et al., 2016)[20]. 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 Tanerella 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.(Fig.4)

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)[30]

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.[31]

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 tnonths 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%.[32]

Rarns et al. in 1984 investigated subgingival samples taken from 13 healthy periimpiants 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. [33]

According to Slots.J et al in 1986[34],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. [35] Eusobacteria, also significantly elevated in the failing sites, are regarded as important opportunistic pathogens in oral and non-oral infections (Brook.I 1987). [36]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 Periimplantitis be regarded as a site-specific infection in which microbial pathogens, mainly belonging to the group of gram-negative anaerobic rods.[37]

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**.** [111,112] 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.[47] In an analysis of the a biofilm that forms on the surface of oral implants. Heuer and colleagues in 2007[38], 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. [39] In other studies, Mombelli et al in 1993 and colleagues stated that 80% of the bacteria cultured from around oral implants were facultative anerobic Gram-positive cocci species which are observed during the first 6 months following implant placement.[45] They also did not detect *Spirochetes* but did detect an abundance of *Fusobacterium* and also anerobic black-pigmented Gram-negative bacteria.

Apatzidou D et al in 2017, Sanz-Martin et al in 2017 reported that The genera Actinobacillus and Streptococcus were most closely associated with health.[40,41] 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, Desulfobulbus, Dialister, Eubacterium, Filifactor, Mitsukella, Porphyromonas and Pseudoramibacter.[42] 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.[43] 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.[44]

Ata-Ali J et al in 2015[45], Jervøe-Storm PM et al in 2015, Apatzidou D et al in 2017[46], Sanz-Mart et al in 2017, da Silva ES et al in 2015[47], Zheng H et al in 2014[48] reported that predominant species in peri implantitis belonged to red complex species , Eubacterium, and higher proportions of Fusobacterium nucleatum, Dialister invisus, Streptococcus sp., Filifactor alocis and Mitsuokella sp. And lower proportion of Veillonella dispar, Actinomyces meyeri, Granulicatella adiacens. Persson GR et al in 2014 found 19 species of bacteria -Aggregatibacter actinomycetemcomitans, Campylobacter gracilis, Campylobacter rectus, Campylobacter showae, Helicobacter pylori, Haemophilus influenzae, Porphyromonas gingivalis, Staphylococcus aureus, Staphylococcus anaerobius, Streptococcus intermedius,

Streptococcus mitis, Tannerella forsythia, Treponema denticola, and Treponema socranskii in peri implantitis sites. Jervøe-Storm PM et al in 2015[49] reported that P.intermedia at 4 and 12 months was associated with peri-implant bone loss at 25 months. And Ata-Ali J et al in 2015 also reported that there were increased levels of IL1,6,10 and TNF-α at diseased sites.

Jamil A et al in 2008 compared the microbial composition of supra and subgingival biofilm in subjects with and without peri-implantitis. Higher mean counts of Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia were observed in the peri-implantitis group, both supra- and subgingivally. The proportions of the pathogens from the red complex were elevated, while host-compatible beneficial microbial complexes were reduced in diseased compared with healthy implants. The microbiota associated with peri-implantitis was comprised of more periodontal pathogenic bacterial species, including the supragingival biofilm. Furthermore, peri-implant samples of group S yielded a higher proportion of coccoid cells in the darkfield microscope and demonstrated absence of large spirochetes. Porphyromas gingivalis was detected in 10% of the periodontal samples and in only one peri-implant sample. Prevotella intermedia was detected in 33% of the periodontal and in 30% of the peri-implant samples. Fusobacterium spp. had a prevalence of 58% in the periodontal samples and was recovered from 50% of the peri-implant samples. Actinobacillus actinomycetemcomitans was not detected in any dental or peri-implant sample. In 1 case, however, the organism was recovered from the internal surface of the suprastructure. These findings indicate, that the microbial leakage through the gap between the suprastructure and the abutment plays an important role in the bacterial colonization of the internal part of screw retained crowns and bridges.

Jan Cosyn et al in 2009 [49], Manisha Herekar et al in 2015[50], T.Lakkha et al in 2015, **Luigi Canullo et al in 2015** [51] reported that The restorative margin may have been the principal pathway for bacterial leakage. Contamination of abutment screws most likely occurred from the peri-implant sulcus via the implant-abutment interface and abutment-prosthesis interface and **David Penarrocha-Oltra et al in 2016**[52] aimed at investigating the microbial colonization of the peri-implant sulcus and implant connection of implants restored with cemented versus screw-retained superstructures and the results showed that cemented group presented significantly higher bacterial loads in the peri-implant sulcus but significantly lower bacterial loads at the inner portion of the implant connection.

The discrepancy in the microbial profile of peri-implantit health and disease between studies is due to the use of different detection methods. Culture analysis has long been the golden standard for microbial analysis and it has many advantages. In contrast, molecular detection methods such as qPCR and DNA-DNA hybridization and metagenomics analysis are less time consuming and more sensitive, allowing the detection of species present at low levels. Consequently, microbial data obtained from studies using 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)[116] and the prevalence of gram negative mirobes and red complex group of microorgansims 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

1. Charalampakis G. Peri-implantitis from a microbiological perspective. 2013 Mar 7.
2. Zitzmann NU, Berglundh T. Definition and prevalence of peri-implant diseases. Journal of clinical periodontology. 2008 Sep;35:286-91.
3. Lang NP, Berglundh T, Working Group 4 of the Seventh European Workshop on Periodontology. Periimplant diseases: where are we now?–Consensus of the Seventh European Workshop on Periodontology. Journal of clinical periodontology. 2011 Mar;38:178-81.
4. Silva E, Félix S, Rodriguez-Archilla A, Oliveira P, dos Santos JM. Revisiting peri-implant soft tissue–histopathological study of the peri-implant soft tissue. International journal of clinical and experimental pathology. 2014;7(2):611.
5. Froum S. Review of the treatment protocols for peri-implantitis. Dentistry IQ. 2011.
6. Jakobi ML, Stumpp SN, Stiesch M, Eberhard J, Heuer W. The peri-implant and periodontal microbiota in patients with and without clinical signs of inflammation. Dentistry journal. 2015 Mar 31;3(2):24-42.
7. Grecchi F, Di Girolamo M, Cura F, Candotto V, Carinci F. A new system of implant abutment

connection: how to improve a two piece implant system sealing. ORAL & implantology. 2017 Jul;10(3):234.
8. Griffen AL, Becker MR, Lyons SR, Moeschberger ML, Leys EJ. Prevalence of Porphyromonas gingivalisand periodontal health status. Journal of clinical microbiology. 1998 Nov 1;36(11):3239-42.
9. Lang NP, Lindhe J, editors. Clinical periodontology and implant dentistry, 2 Volume Set. John Wiley & Sons; 2015 Mar 25.
10. Sanz M, Newman MG, Nachnani S, Holt R, Stewart R, Flemmig T. Characterization of the subgingival microbial flora around endosteal sapphire dental implants in partially edentulous patients. International Journal of Oral & Maxillofacial Implants. 1990 Sep 1;5(3).
11. Persson GR, Samuelsson E, Lindahl C, Renvert S. Mechanical non-surgical treatment of peri-implantitis: a single-blinded randomized longitudinal clinical study. II. Microbiological results. Journal of clinical periodontology. 2010 Jun;37(6):563-73.
12. Padial-Molina M, López-Martínez J, O'Valle F, Galindo-Moreno P. Microbial profiles and detection techniques in peri-implant diseases: a systematic review. Journal of oral & maxillofacial research. 2016 Jul;7(3).
13. Charalampakis G, Belibasakis GN. Microbiome of peri-implant infections: lessons from conventional, molecular and metagenomic analyses. Virulence. 2015 Apr 3;6(3):183-7.
14. Becker W, Becker BE, Newman MG, Nyman S. Clinical and microbiologic findings that may contribute to dental implant failure. International Journal of Oral & Maxillofacial Implants. 1990;5(1)31-8.
15. Albrektsson T, Zarb G, Worthington P, Eriksson AR. The long-term efficacy of currently used dental implants: a review and proposed criteria of success. Int j oral maxillofac implants. 1986;1(1):11-25.
16. Güncü GN, Akman AC, Günday S, Yamalık N, Berker E. Effect of inflammation on cytokine levels and bone remodelling markers in peri-implant sulcus fluid: a preliminary report. Cytokine. 2012 Aug 1;59(2):313-6.
17. Lang NP, Lindhe J, editors. Clinical periodontology and implant dentistry, 2 Volume Set. John Wiley & Sons; 2015 Mar 25.
18. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent Jr RL. Microbial complexes in subgingival plaque. Journal of clinical periodontology. 1998 Feb;25(2):134-44.
19. de Avila E, de Molon R, Vergani C, Salih V. The relationship between biofilm and physical-chemical properties of implant abutment materials for successful dental implants. Materials. 2014;7(5):3651-62.
20. Alshehri AM. The maintenance of crestal bone around dental implants. Implants. 2011;2:20-4.
21. Costalonga M, Herzberg MC. The oral microbiome and the immunobiology of periodontal disease and caries. Immunology letters. 2014 Dec 1;162(2):22-38.
22. Wade WG. The oral microbiome in health and disease. Pharmacological research. 2013 Mar 1;69(1):137-43.
23. Mombelli A. Microbiology of the dental implant. Advances in dental research. 1993 Aug;7(2):202-6.
24. Mombelli A, Van Oosten MA, Schürch Jr E, Lang NP. The microbiota associated with successful or failing osseointegrated titanium implants. Oral microbiology and immunology. 1987 Dec;2(4):145-51.
25. Salcetti JM, Moriarty JD, Cooper LF, Smith FW, Collins JG, Socransky SS, Offenbacher S. The clinical, microbial, and host response characteristics of the failing implant. International Journal of Oral & Maxillofacial Implants. 1997 Jan 1;12(1).
26. Persson GR, Salvi GE, Heitz-Mayfield LJ, Lang NP. Antimicrobial therapy using a local drug delivery system (Arestin®) in the treatment of peri-implantitis. I: microbiological outcomes. Clinical oral implants research. 2006 Aug;17(4):386-93.
27. Renvert S, Roos-Jansåker AM, Claffey N. Non-surgical treatment of peri-implant mucositis and peri-implantitis: a literature review. Journal of clinical periodontology. 2008 Sep;35:305-15.
28. Sanz M, Newman MG, Nachnani S, Holt R, Stewart R, Flemmig T. Characterization of the subgingival microbial flora around endosteal sapphire dental implants in partially edentulous patients. International Journal of Oral & Maxillofacial Implants. 1990 Sep 1;5(3).
29. Leonhardt Å, Renvert S, Dahlén G. Microbial findings at failing implants. Clinical oral implants research. 1999 Oct;10(5):339-45.
30. Canullo L, Penarrocha-Oltra D, Soldini C, Mazzocco F, Penarrocha M, Covani U. Microbiological assessment of the implant-abutment interface in different connections: cross-sectional study after 5 years of functional loading. Clinical oral implants research. 2015 Apr;26(4):426-34.
31. 72. Silverstein LH, Kurtzman D, Garnick JJ, Schuster GS, Steflik DE, Moskowitz ME. The microbiota of the peri-implant region in health and disease. Implant dentistry. 1994;3(3):170-4.
32. Rams TE, Feik D, Listgarten MA, Slots J. Peptostreptococcus micros in human periodontitis. Oral microbiology and immunology. 1992 Feb;7(1):1-6.
33. Lekholm U, Adell R, Lindhe J, Brånemark PI, Eriksson B, Rockler B, Lindvall AM, Yoneyama T. Marginal tissue reactions at osseointegrated titanium fixtures:(II) A cross-sectional retrospective study. International journal of oral and maxillofacial surgery. 1986 Feb 1;15(1):53-61.
34. Slots J, Bragd L, Wikström M, Dahlén G. The occurrence of Actinobacillus actinomycetemcomitans, Bacteroides gingivalis and Bacteroides intermedius in destructive periodontal disease in adults. Journal of clinical periodontology. 1986 Jul 1;13(6):570-7.
35. Zambon JJ, Reynolds HS, Slots J. Black-pigmented Bacteroides spp. in the human oral cavity. Infection and Immunity. 1981 Apr 1;32(1):198-203.

36. Brook I, Frazier EH, Gher ME. Aerobic and anaerobic microbiology of periapical abscess. Oral microbiology and immunology. 1991 Apr;6(2):123-5.
37. Mombelli A, Buser D, Lang NP. Colonization of osseointegrated titanium implants in edentulous patients. Early results. Oral Microbiology and Immunology. 1988 Sep;3(3):113-20.
38. Apse P, Ellen RP, Overall CM, Zarb GA. Microbiota and crevicular fluid collagenase activity in the osseointegrated dental implant sulcus: a comparison of sites in edentulous and partially edentulous patients. Journal of Periodontal Research. 1989 Mar;24(2):96-105.
39. Quirynen M, Vogels R, Peeters W, van Steenberghe D, Naert I, Haffajee A. Dynamics of initial subgingival colonization of 'pristine'peri-implant pockets. Clinical oral implants research. 2006 Feb;17(1):25-37.
40. Heuer W, Elter C, Demling A, Neumann A, Suerbaum S, Hannig M, Heidenblut T, Bach FW, Stiesch-Scholz M. Analysis of early biofilm formation on oral implants in man. Journal of oral rehabilitation. 2007 May;34(5):377-82
41. Nakou M, Mikx FH, Oosterwaal PJ, Kruijsen JC. Early microbial colonization of permucosal implants in edentulous patients. Journal of dental research. 1987 Nov;66(11):1654-7.
42. Sanz-Martin I, Doolittle-Hall J, Teles RP, Patel M, Belibasakis GN, Hämmerle CH, Jung RE, Teles FR. Exploring the microbiome of healthy and diseased peri-implant sites using Illumina sequencing. Journal of Clinical Periodontology. 2017 Dec;44(12):1274-84.
43. Jepsen S, Berglundh T, Genco R, Aass AM, Demirel K, Derks J, Figuero E, Giovannoli JL, Goldstein M, Lambert F, Ortiz-Vigon A. Primary prevention of peri-implantitis: Managing peri-implant mucositis. Journal of clinical periodontology. 2015 Apr;42:S152-7.
44. Kumar PS, Mason MR, Brooker MR, O'brien K. Pyrosequencing reveals unique microbial signatures associated with healthy and failing dental implants. Journal of clinical periodontology. 2012 May;39(5):425-33.
45. Ata-Ali J, Flichy-Fernández AJ, Alegre-Domingo T, Ata-Ali F, Palacio J, Peñarrocha-Diago M. Clinical, microbiological, and immunological aspects of healthy versus peri-implantitis tissue in full arch reconstruction patients: a prospective cross-sectional study. BMC Oral Health. 2015 Dec;15(1):43.
46. Apatzidou D, Lappin DF, Hamilton G, Papadopoulos CA, Konstantinidis A, Riggio MP. Microbiome associated with peri-implantitis versus periodontal health in individuals with a history of periodontal disease. Journal of oral microbiology. 2017 May 31;9(sup1):1325218.
47. da Silva ES, Feres M, Figueiredo LC, Shibli JA, Ramiro FS, Faveri M. Microbiological diversity of peri-implantitis biofilm by S anger sequencing. Clinical oral implants research. 2014 Oct;25(10):1192-9.
48. Jervøe-Storm PM, Jepsen S, Jöhren P, Mericske-Stern R, Enkling N. Internal bacterial colonization of implants: association with peri-implant bone loss. Clinical oral implants research. 2015 Aug;26(8):957-63.
49. Shibli JA, Melo L, Ferrari DS, Figueiredo LC, Faveri M, Feres M. Composition of supra-and subgingival biofilm of subjects with healthy and diseased implants. Clinical Oral Implants Research. 2008 Oct;19(10):975-82.
50. Herekar M, Sethi M, Prithviraj DR, Bhat K, Fernandes A, Patil V. A clinical study evaluating changes in the microbial flora around dental implants during various stages of implant restoration. Implant dentistry. 2015 Oct 1;24(5):527-32.
51. Cosyn J, Eghbali A, De Bruyn H, Collys K, Cleymaet R, De Rouck T. Immediate single-tooth implants in the anterior maxilla: 3-year results of a case series on hard and soft tissue response and aesthetics. Journal of Clinical Periodontology. 2011 Aug;38(8):746-53.
52. Penarrocha-Oltra D, Monreal-Bello A, Penarrocha-Diago M, Alonso-Perez-Barquero J, Botticelli D, Canullo L. Microbial Colonization of the Peri-Implant Sulcus and Implant Connection of Implants Restored With Cemented Versus Screw-Retained Superstructures: A Cross-Sectional Study. Journal of periodontology. 2016 Sep;87(9):1002-11.