EVALUATION OF BACTERIAL COLONIZATION ON TITANIUM DENTAL IMPLANT SURFACES FOLLOWING DIFFERENT INSTRUMENTATION TECHNIQUES: A COMPARATIVE STUDY

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®	Registered trademark ownership
Rz	Ten points mean roughness
Ra	Roughness average

LIST OF ABBREVIATIONS

Er, Cr: YSGG	Erbium, chromium: yttrium scandium gallium garnet
CFU	Colony Forming Unit
SEM	Scanning Electron Microscope
Er, YAG	Erbium-doped yttrium aluminium garnet
KPP	Klinik Pergigian Pakar
USM	Universiti Sains Malaysia
HREC	Human Research Ethics Committee
UK	United Kingdom
USA	United States of America
rpm	revolutions per minute
BHI	Brain-Heart Infusion
ATCC	American Type Culture Collection
OD	Optical Density
TNTC	Too Numerous To Count
TFTC	Too Few To Count
PBS	Phosphate Buffer Saline
HMDS	Hexamethyldisilazane
SPSS	Statistical Package for Social Sciences
ANOVA	Analysis of Variance

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PENILAIAN KOLONISASI BAKTERIA PADA PERMUKAAN IMPLAN PERGIGIAN TITANIUM MENGIKUT TEKNIK INSTRUMENTASI BERBEZA: KAJIAN PERBANDINGAN

ABSTRAK

Penggunaan pelbagai alat pembersihan untuk penyelenggaraan implan mempengaruhi konfigurasi permukaan, yang mana mempengaruhi pelekatan bakteria pada permukaan implan titanium, seterusnya boleh menyebabkan penyakit peri-implan. Kajian ini bertujuan untuk membandingkan topografi permukaan dan pengkolonian *Streptococcus sanguinis* pada permukaan fikstur implan titanium selepas kaedah penggilapan menggunakan laser Er, Cr: YSGG, airflow, dan berus titanium dengan kumpulan kawalan/tidak dirawat menggunakan pemerhatian Pengimbasan Mikroskop Elektron (SEM) dan pengiraan Unit Pembentukan Koloni (CFU). Dua puluh fikstur implan titanium MegaGen® dibahagikan secara rawak kepada empat kumpulan. Lima fikstur telah dipilih secara rawak untuk kumpulan kawalan/tidak dirawat (C) manakala tiga kumpulan lain masing-masing dirawat dengan, laser Er, Cr: YSGG, airflow, dan berus titanium. Satu fikstur daripada setiap kumpulan diperhati di bawah SEM untuk penilaian topografi permukaan. Semua sampel lain dikulturkan dengan *Streptococcus sanguinis* untuk penilaian pengkolonian dan pelekatan bakteria. Satu sampel dari setiap kumpulan dipilih untuk pemerhatian di bawah SEM manakala sampel-sampel lain disediakan untuk pengiraan CFU. Dari analisis SEM, topografi permukaan yang dihasilkan daripada berus titanium menunjukkan permukaan yang licin diikuti oleh airflow dengan ketidaksamarataan permukaan yang lebih sedikit**,** dan kumpulan laser menunjukkan struktur yang tidak teratur berbanding dengan kumpulan kawalan. Begitu juga, untuk koloni bakteria,

berus titanium mempunyai pertumbuhan sel bakteria yang paling sedikit, dengan kehadiran yang jarang di permukaan sama seperti kumpulan kawalan, diikuti oleh kumpulan airflow yang menunjukkan rangkaian koloni berlapis, dan kumpulan laser menunjukkan koloni berkelompok. Walau bagaimanapun, untuk keputusan CFU, analisis statistik menunjukkan nilai yang signifikan ($p<0.05$) di antara semua empat kumpulan. Berus titanium disimpulkan sebagai alat instrumentasi yang paling tidak invasif dan paling berkesan untuk topografi permukaan fikstur implan dan pertumbuhan bakteria yang dinilai di bawah SEM. Namun, daripada pengiraan CFU, instrumentasi airflow didapati mempunyai jumlah bakteria yang paling sedikit berbanding dengan kumpulan yang lain.

EVALUATION OF BACTERIAL COLONIZATION ON TITANIUM DENTAL IMPLANT SURFACES FOLLOWING DIFFERENT INSTRUMENTATION TECHNIQUES: A COMPARATIVE STUDY

ABSTRACT

The use of various hygiene tools for implant maintenance affects surface configurations, which in turn affects bacterial adhesion on titanium implant surfaces, which can lead to peri-implant diseases. This study aimed to compare the surface topographies and *Streptococcus sanguinis* colonization on titanium implant fixture surfaces after polishing methods with Er, Cr: YSGG laser, airflow, and titanium brush group with control/untreated group using Scanning Electron Microscope (SEM) observation and Colony Forming Unit (CFU) counts. Twenty MegaGen® titanium implant fixtures were randomly distributed into four groups. Five fixtures were randomly selected for the control/untreated (C) group while the other three groups were treated with Er, Cr: YSGG laser, airflow, and titanium brush. One fixture from each group was observed under SEM for surface topographies evaluation. All other samples were cultured with *Streptococcus sanguinis* for bacterial colonization and adhesion evaluation. One sample for each group was selected for SEM observation while the other samples were prepared for CFU counting. From the SEM analysis, the surface topography produced by the titanium brush showed a smooth surface followed by airflow with fewer surface irregularities, and the laser group showed a haphazard structure as compared to the control group. Similarly, for bacterial colonization, the titanium brush had the least bacterial cell growth, sparsely present on the surface same as the control group followed by the airflow group showed the multilayer chains of colonies, and the laser group showed the clusters of colonies. However, for the CFU

result, statistical analysis revealed a significant value ($p<0.05$) among all four groups. Titanium brush was concluded as the least invasive and most effective instrumentation tool for implant fixture surface topographies and bacterial growth evaluated under SEM. However, from CFU counts, the airflow instrumentation was found to be the one having the least bacterial counts compared to other groups.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

For a few decades now, dental implants have served as a viable method for replacing missing teeth or even forming part of prosthetic solutions for completely edentulous jaws (Warreth et al., 2017). A systematic review highlighted that osseointegrated implants exhibit a high success rate (94.6%) and minimal marginal bone loss (1.3mm) during a follow-up period of at least 10 years (Moraschini et al., 2015). However, the placement of implants does carry the risk of exposing patients to both biological complications (Lang et al., 2000) and mechanical issues (Schwarz, 2000), which could potentially lead to implant failure.

The biological side effects of dental implants that result from infections triggered by bacterial biofilm and subsequent inflammation of the soft tissues and bone surrounding implants are known as peri-implant diseases, including peri-implant mucositis and peri-implantitis (Nascimento et al., 2014; Renvert et al., 2019). The term "biofilms" refers to the type of bacteria that often reside in mixed communities and adhere to environmental surfaces, such as dental implants. Biofilm will develop from these oral microbiota accumulations (Mombelli and Décaillet, 2011). According to Steinberg et al. (1995), peri-implant mucositis around dental implants can be largely decreased by reducing plaque build-up.

Regular maintenance of dental implants by both patients and dental healthcare professionals is essential for preventing peri-implant diseases (Mombelli, 2019). The primary goal is to minimise plaque buildup on dental implant surfaces. Patient home care involves activities like toothbrushing, using interdental cleaning tools such as

floss and interdental cleaners, as well as employing locally applied treatments like chlorhexidine gluconate and water flossers such as Hydro Floss (Gulati et al., 2014).

Professional implant hygiene care includes various methods such as laser, plastic curette, metal curette, air-powder abrasive system, and rubber cup with non-abrasive paste, lasers (Duarte et al., 2009; Gulati et al., 2014). However, these hygiene care instruments can potentially modify the implant surface. As a result, the surface profile and roughness produced by these modalities may significantly affect the newly formed biofilm thus playing an important role in peri-implant health maintenance (Louropoulou et al., 2012).

Several prior studies have examined the impact of various hygiene instruments on surface characteristics and roughness (Homiak et al., 1992; Louropoulou et al., 2012; Unursaikhan et al., 2012). Additionally, these studies have investigated the influence of these changes on bacterial adhesion (Bollen et al., 1996; Duarte et al., 2009; Quirynen and Bollen, 1995). The alteration of surface features or roughness resulting from these methods has been found to promote bacterial colonization on implant surfaces (Duarte et al., 2009; Warreth et al., 2017).

In this study, *Streptococcus sanguinis* was the selected bacteria for examination. This particular species of streptococci typically dominates implant surfaces following exposure to the oral environment. These early colonizers create a favorable environment for late colonizers to adhere to implant surfaces (Lang et al., 2015; Socransky et al., 1977). Hence, ideally preparing implant surfaces would aim to reduce the presence of early colonizers, subsequently reducing the adhesion of late colonizers and inhibiting plaque biofilm formation (Subramani et al., 2009).

Therefore, this in vitro study aimed to evaluate the surface roughness between the untreated implant fixture surfaces and those treated with implant hygiene

instrumentations (Er, Cr: YSGG laser, airflow, and titanium brush) using a Scanning Electron Microscope (SEM) before colonization of *Streptococcus sanguinis*. Additionally, the study described and compared the effects of these hygiene instruments on bacterial colonization (*Streptococcus sanguinis*) on titanium implant fixture surfaces using SEM and CFUs.

1.2 Problem statement

For the success and longevity of the implant, effective implant maintenance is one of the factors. Professional hygiene care instruments using different treatment modalities such as Er, Cr: YSGG laser, airflow, and titanium brush can achieve this. These modalities will create different surface profiles and roughness that will have effects on bacterial adhesion on titanium implant surfaces.

Since implants serve as a foundation for bacterial cells to adhere to and grow in the constantly changing oral environment, surface features and roughness of implants are critical factors in forming biofilms (Subramani et al., 2009). Therefore, any treatment that attempts to improve oral hygiene should not change the fixture surface to make it more plaque-retentive but, on the contrary, make it less susceptible to plaque and calculus accumulation (Homiak et al., 1992).

The instruments used for hygiene that resulted in smooth titanium implant surfaces had superior results in decreasing the accumulation of plaque. On the other hand, the procedures that led to more uneven implant surfaces had a reverse effect.

Furthermore, the knowledge from this study will help the dentist to choose an effective and least invasive method that reduces further bacterial adhesion and efficient, hygienic instrumentation during implant maintenance. As a result, peri-implant health can be maintained and implant survival will be increased.

Numerous studies have assessed implant decontamination methods' impacts on bacterial adhesion to implant surfaces. However, no study has definitively identified the superior method for implant maintenance as each approach carries its unique set of advantages and drawbacks.

1.3 Study rationale

This study was conducted to determine and compare the effects of *Streptococcus sanguinis* bacterial adhesion after the titanium implant fixtures were treated with Er, Cr: YSGG laser irradiation, airflow, and titanium brush. To the best of our knowledge, there was no previous study done to directly compare the bacterial adhesion between these three hygiene instruments.

The selection of hygiene instruments for implant maintenance holds significant importance in minimizing potential detrimental effects on the implant surface, thereby reducing bacterial colonization and adhesion. Consequently, this preventive approach can mitigate peri-implant diseases and potentially prolong the survival or success rate of dental implants.

1.4 Objectives

1.4.1 General Objective

To investigate the effects of *Streptococcus sanguinis* colonization on the titanium implant fixtures surfaces treated with Er, Cr: YSGG laser, airflow, and titanium brush.

1.4.2 Specific Objectives

1. To evaluate the titanium implant fixture surface topography in untreated (control), and treated (Er, Cr: YSGG laser, airflow, and titanium brush) groups using a Scanning Electron Microscope (SEM).

2. To describe the *Streptococcus sanguinis* colonization on the titanium implant fixture surfaces in groups of untreated (control), and treated (Er, Cr: YSGG laser, airflow, and titanium brush) using a Scanning Electron Microscope (SEM).

3. To compare the amounts of *Streptococcus sanguinis* colonization on the titanium implant fixture surfaces in groups of untreated (control), and treated (Er, Cr: YSGG laser, airflow, and titanium brush) using CFUs.

1.5 Research questions

1. Is there any difference between the effects of different instrumentation on surface topographies of titanium implant fixtures?

2. Is there any difference of *Streptococcus sanguinis* colonization on the titanium implant fixture surfaces in groups of untreated, and treated using SEM?

3. Is there any significant difference in the amounts of *Streptococcus sanguinis* colonization on titanium implant fixtures in groups of untreated (control), and treated (Er, Cr: YSGG laser, airflow, and titanium brush) using CFUs?

1.6 Alternative hypothesis

There is a significant difference in the amounts of *Streptococcus sanguinis* colonization on the titanium implant fixture surfaces in groups of untreated (control), and treated (Er, Cr: YSGG laser, airflow, and titanium brush) using CFUs.

CHAPTER 2

LITERATURE REVIEW

2.1 Dental implants

Dental implants are pivotal in restoring oral function and aesthetics, serving as artificial tooth roots surgically embedded into the jawbone. Their significance lies in providing a stable foundation for prosthetic teeth, ensuring natural-looking smiles and enhanced chewing ability. They prevent bone loss by stimulating the jaw, preserving facial structure, and preventing adjacent teeth from shifting. Additionally, implants offer a long-term solution over traditional alternatives like bridges or dentures, boosting confidence and overall oral health (Pjetursson et al., 2012).

Peri-implant tissues are crucial for the stability and longevity of dental implants, encompassing the gingiva and bone surrounding the implant. The health of these tissues, particularly the integrity of the peri-implant mucosa, is pivotal in preventing peri-implant diseases like peri-implant mucositis and peri-implantitis. Adequate soft tissue support and proper bone integration are essential for implant success, as outlined in research by (Berglundh et al., 2018), they emphasize the importance of healthy peri-implant tissues in maintaining long-term stability. Regular monitoring and maintenance of peri-implant tissues through professional care and patient oral hygiene are vital for preserving the integrity and function of dental implants.

Osseointegration is the critical process where dental implants form a direct and stable bond with the surrounding bone, ensuring implant stability and function. This phenomenon, elucidated (Branemark, 1983) involves the formation of a structural and functional connection at the implant-bone interface, enabling load-bearing

capabilities. Successful osseointegration, reliant on factors such as implant material, surface properties, and surgical technique, is crucial for implant longevity and patient comfort. It fosters biomechanical support and stress distribution akin to natural teeth, as emphasized in studies (Cochran, 1999), validating its significance in implant dentistry.

According to Chatzistavrianou et al. (2019), the most crucial elements to take into account for a long-term successful outcome are conservative pre-treatment planning and restoratively driven implant placement. Success in implant dentistry is determined by several factors, including patient satisfaction, osseointegration of the implant, lack of peri-implant infection, radiographic stability of alveolar bone levels, and the harmony of pink and white aesthetics (Papaspyridakos et al., 2012). However osseointegration continues to be the most important component in determining whether dental implants are placed successfully (Papaspyridakos et al., 2012).

Dental implants consists of three parts:

1. Implant Fixture (Screw): Typically made of titanium or titanium alloys, which are biocompatible, meaning they are not harmful to living tissue and can integrate with bone. Acts as the root of the artificial tooth, inserted surgically into the jawbone where the natural tooth root was located.
2. Abutment: Usually made of titanium, gold, or porcelain. Connects the implant fixture to the prosthetic tooth (crown). It is screwed onto the implant fixture.
3. Crown: Can be made from porcelain, ceramic, or a combination of materials to mimic the appearance of natural teeth. The visible part of the implant that functions as the new tooth. It is custom-made to fit the patient's mouth and match the color of their natural teeth.

2.2 Peri-implant health and diseases

Peri-implant health refers to the maintenance of soft and hard tissues surrounding dental implants, which is crucial for long-term success.

Peri-implant tissues encompass soft tissues (peri-implant mucosa and connective tissue) and hard tissues (bone) surrounding dental implants. The integrity and health of these tissues are crucial for implant stability and longevity, with soft tissue health impacting aesthetics and hard tissue support influencing implant success.

The significance of adequate keratinized mucosa around implants for maintaining peri-implant health for long term survival is very important (Lang et al., 2000; Lang et al., 2015). Moreover, preserving the peri-implant bone through proper surgical techniques and optimal loading protocols is essential for long-term implant survival. Regular monitoring and maintenance of both soft and hard peri-implant tissues are imperative for successful implant outcomes.

The absence of inflammation and the maintenance of stable soft and hard tissues surrounding dental implants is an important factors. It involves the preservation of healthy peri-implant mucosa, the absence of bleeding on probing, and the absence of suppuration. Researchers like (Berglundh et al., 2018) have highlighted specific parameters such as probing depth, absence of bleeding, and absence of radiographic bone loss as indicators of peri-implant health. Additionally, maintaining proper hygiene and regular professional maintenance is crucial for sustaining peri-implant health and preventing complications.

Peri-implant mucositis and peri-implantitis are prevalent diseases affecting the oral soft and hard tissues, characterized by inflammation and bone loss around implants. Studies like (Schwarz et al., 2018) emphasize the importance of regular monitoring and early detection of peri-implant diseases through clinical and

radiographic assessments. Management involves professional mechanical debridement, adjunctive therapies, and patient education to prevent disease progression (Renvert et al., 2019). Optimal oral hygiene and regular professional maintenance are paramount for preserving peri-implant health and preventing complications.

Peri-implant mucositis is an inflammatory condition characterized by soft tissue inflammation around dental implants without evident bone loss. Its diagnosis involves clinical signs such as bleeding on probing, increased probing depths, and visual signs of inflammation in the peri-implant mucosa. Studies like (Renvert et al., 2019) emphasize these clinical parameters for diagnosing peri-implant mucositis, highlighting the importance of regular clinical assessments.

If left untreated, peri-implant mucositis can progress to peri-implantitis, a more severe condition characterized by bone loss around the implant. This progression involves the continuation of inflammatory processes into the supporting bone, leading to radiographic evidence of bone loss, increased probing depths (greater than 4mm), often suppuration, and mobility. The conversion from mucositis to peri-implantitis involves the breakdown of soft tissue attachment and subsequent bone loss.

Diagnosing the conversion from mucositis to peri-implantitis requires careful monitoring of clinical signs, radiographic evaluation showing bone loss, and microbial analysis to identify specific pathogens involved in the progression, as highlighted in studies such as Renvert et al. (2019) and Schwarz et al. (2018).

Peri-implantitis is an inflammatory condition characterized by progressive bone loss and soft tissue inflammation around dental implants, potentially leading to implant failure. Diagnosis involves clinical signs like pain, bleeding on probing, increased probing depths, mobility, suppuration, and radiographic evidence of bone

loss around the implant. Studies such as Schwarz et al. (2018) highlighted these clinical parameters for diagnosing peri-implantitis, emphasizing the importance of thorough clinical and radiographic assessments. Additionally, microbial analysis and assessment of inflammatory markers aid in confirming the diagnosis, enabling timely intervention to prevent further complications.

2.3 Dental biofilm

Dental biofilm refers to a structured community of microorganisms that adhere to the tooth surface and other oral structures, embedded within a self-produced extracellular matrix. This biofilm formation, commonly known as dental plaque, consists of diverse bacterial species alongside fungi and viruses. The complexity and organization of dental biofilm enable microbial interactions and create a protective environment, contributing to its resistance against antimicrobial agents and host defenses. Research by Marsh and Devine (2011) delineated the stages and dynamics of dental biofilm formation, emphasizing its role in oral diseases like dental caries and periodontal conditions. Understanding biofilm structure, composition, and dynamics is crucial for developing effective preventive and therapeutic strategies in oral health care.

Dental biofilm formation occurs in stages:

a) Pellicle Formation: Within minutes of tooth exposure, salivary proteins and glycoproteins form an acquired pellicle on the tooth surface, providing an attachment site for initial bacterial adherence.

b) Bacterial Adherence: Reversible bacterial attachment begins with early colonizers (e.g., *Streptococci*) adhering to the pellicle-coated tooth surface, followed by irreversible attachment of more diverse microorganisms.

c) Biofilm Maturation: Microbial colonization intensifies, leading to biofilm growth and maturation by establishing a complex community structure embedded within an extracellular matrix, facilitating microbial interactions and protection.

d) Biofilm Maintenance: The mature biofilm persists through continuous microbial growth, detachment, and reattachment, creating a dynamic and resilient ecosystem.

After cleaning within seconds, tooth surfaces will rapidly be colonized by oral microbes together with proteins and glycoproteins. They cause the adhesion and aggregation of oral bacteria to surfaces. Colonization of bacteria to tooth surfaces started 0 to 4 hours of pellicle formation. The initial colonizers include *Streptococci sp*. (*S.sanguis, S.oralis, S.mitis*), *Actinomyces sp.*, and gram-negative bacteria. However, *S. mutans and S. sobrinus* are late colonizers.

After 2 hours, the growth and multiplication of attached bacteria forming colonies were noted. In 1 to 14 days, plaque that is dominated by *Streptococcus sp*. changes to *Actinomyces sp*. through microbial succession. In 2 to 4 weeks, there is diversion of bacterial species with more gram-negative anaerobic species. Plaque maturation causes gingivitis supra-gingivally and proceeds to periodontitis by subgingival microbes such as *Porphromonas gingivalis* and *Fusobacterium nucleatum* (Huang et al., 2011; Subramani et al., 2009; Wilson, 1996).

2.4 Microbial colonization on the implant surface

The formation of peri-implant biofilm and bacterial adhesion on implant surfaces is a critical aspect affecting the success of dental implants. Biofilm formation begins with the initial adhesion of bacteria to the implant surface, followed by colonization and biofilm maturation.

Several factors contribute to bacterial adhesion, including surface roughness, surface energy, material composition, and implant characteristics. Surface roughness, especially at the micro and nanoscale, can significantly impact bacterial adhesion and subsequent biofilm formation. Rough surfaces tend to facilitate bacterial attachment and colonization compared to smoother surfaces.

Moreover, the material composition of the implant plays a crucial role. Different materials exhibit varying degrees of bacterial adhesion and biofilm formation. For instance, titanium implants generally have better resistance to bacterial colonization compared to other materials due to their biocompatibility and ability to osseointegrate. Surface modifications and coatings have also been explored to reduce bacterial adhesion and enhance the biocompatibility of implants. Microbial colonization on implant surfaces is the same as on tooth surfaces in the same oral environment. Studies showed that multiple species harbor the gingivally healthy *(eg: Streptococcus sanguis, Streptococcus oralis and Streptococcus gondii*) and for "gingivitis" sites (*eg: Actinomyces viscosus and Actinomyces odontylicus*). Moreover, putative pathogens (e.g., *Porphyromonas gingivalis, Prevotella intermedia, Prevotella melaninogenica, and Fusobacterium sp.)* may cause symptomatic implant sites (Mombelli and Lang, 1994; Rosan and Lamont, 2000; Subramani et al., 2009).

Oral organisms that are related to periodontal diseases are the most potential etiological factors of implant treatment failures (Nascimento et al., 2014). Bacterial adhesion to dental implants is influenced by the surface roughness of dental implants (Subramani et al., 2009). This study added that it is very important to create implant surfaces that minimize the number of early colonizers (*Streptococci sp.* and *Actinomyces sp*.). The reason is that the early colonizers created a favorable environment for late colonizers later on. Bacterial adhesion to intra-oral hard surfaces

such as implants is influenced by the surface roughness of these structures and previous studies showed a remarkably higher bacterial load on rough surfaces compared to smooth surfaces (Bollen et al., 1996). The same author stated that supragingival rough surfaces not only harbor more plaque that contains pathogenic bacteria but also indicate a higher degree of inflammation of the gingiva. It was assumed that bacterial adhesion is easier but removal is more difficult on rough surfaces.

Homiak et al. (1992) studied changes created by various oral prophylactic procedures on the texture of a titanium implant fixture. The procedures that cause increased surface roughness increase the retention of plaque and calculus while smoothing the surface might create the opposite effects.

2.5 Microbiota associated with peri-implant health and infections

Healthy peri-implant sites generally exhibit a low diversity and abundance of microorganisms, predominantly comprising Gram-positive, facultatively anaerobic bacteria like *Streptococcus spp.* and *Actinomyces spp*. (Renvert et al., 2019; Schwarz et al., 2018).

In contrast, peri-implant diseases, especially peri-implantitis, are associated with a shift in microbiota characterized by an increase in Gram-negative, anaerobic species such as *Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola*, akin to the periodontal pathogens found in chronic periodontitis (Berglundh et al., 2018; Schwarz et al., 2018).

Studies suggest that a shift in the microbial profile occurs during the transition from peri-implant health to disease. Factors like poor oral hygiene, host immune

response, and implant-related factors contribute to this shift, leading to dysbiosis and subsequent inflammation (Schwarz et al., 2018).

2.6 *Streptococcus sanguinis* on tooth and implant surfaces

Streptococcus sanguinis formerly known as *Streptococcus sanguis* is a Gram-positive, facultatively anaerobic, non-spore-forming, and commensal bacteria that can be widely found in the oral cavity (Zhu et al., 2018).

Streptococcus sanguinis is one of the microbiota that is present in healthy plaque biofilm (Bik et al., 2010). As a facultative microorganism, this species is present in supragingival and subgingival plaque and prefers to colonize the teeth and implants (Aas et al., 2005; Subramani et al., 2009). This species is known to be the pioneering colonizer and has an important role in helping the other succeeding microorganisms and eventually become the key player in the development of oral biofilm (Socransky et al., 1977).

Oral streptococci colonization depends on the adhesion of bacteria to salivary components adsorbed to the tooth surfaces (Okahashi et al., 2011). Thus, the adhesion machinery of a microorganism is important for persistent colonization on saliva-coated tooth surfaces so that the biofilm can be formed (Okahashi et al., 2011).The same study by Okahashi et al. (2011) revealed that long filamentous pili of *Streptococcus sanguinis* bind to salivary α-amylase resulting in the formation of biofilm in saliva-coated tooth surfaces.

The impact factors of biofilm formation in *Streptococcus sanguinis* are shown in (Figure 2.1). The process is initiated by a single cell that is attached to the surface then followed by the formation of an initial bond when *Streptococcus sanguinis*

(orange) recognizes the tooth surface salivary pellicle receptors (pink and blue) (Zhu et al., 2018).

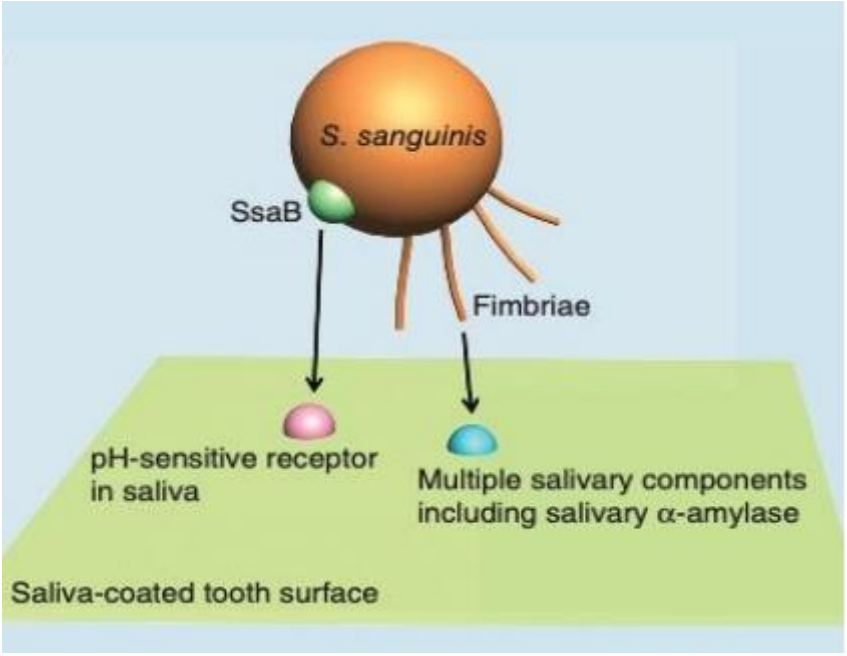

Figure 2.1 Streptococcus sanguinis biofilm formation impact factors [(adopted from (Zhu et al., 2018)]

Next is the maturation of *Streptococcus sanguinis* biofilm. The majority of the biofilm revealed that less than 10% of dry mass is composed of microorganisms while the remainder is the biofilm matrix (Flemming and Wingender, 2010). This matrix consists of polysaccharides, proteins, nucleic acids, and lipids that mediate cell-cell and cell surface adhesion forming interconnected, 3D polymetric networks (Flemming and Wingender, 2010). The microbial colonization of implants is in the same order as the teeth in the same oral cavity (Tanner et al., 1997). As in the tooth surfaces, *Streptococcus sanguinis* is noted to be one of the early colonizers that modify the environment to be convenient for the adhesion of the late colonizers to the implant surfaces (Mombelli and Lang, 1994). Furthermore, *Streptococcus sanguinis* has a similar binding capacity to the enamel and saliva-treated titanium implant surfaces

(Mombelli and Décaillet, 2011). However, this species forms biofilm on titanium implant surfaces depending on the different surface topographies (Paulo et al., 2015).

2.7 Maintenance of dental implants

From the time dental implants are inserted into the oral cavity until the end of their useful lives, maintenance is needed. At that point, a scheduled recall visit customized to the patient's needs is made.

Implant home care for the patient is even more critical than the care of a natural tooth. The biological differences between an implant and a natural tooth make the implant more susceptible to inflammation and bone loss from bacterial plaque. Indeed, dental implants do not decay, but the tissue that surrounds the implant can get infected (e.g., peri-implant disease). The peri-implant soft tissues should be the focus of the hygienist’s oral hygiene instruction with the goal of healthy keratinized tissue surrounding the implant(s). The absence of keratinized tissue has been documented to make the implant more susceptible to pathogenic bacteria, thus leaving the implant vulnerable to peri-implant disease.

The long-term success of implants predominantly depends upon long-term maintenance as well as excellent oral care. A continuous maintenance visit should include updating medical, dental, and social history, reviewing overall oral health status, radiographic examination if needed, implant stability assessment, and removal of any implant-retained plaque, including calculus. According to Mombelli (2019) maintenance care may have a better impact and be more cost-effective than primary prevention in cases following post-extensive periodontal treatment and implant therapy.

During the first year of dental implant placement, a three-month care schedule should be done with the patients especially those who have a history of tooth loss due to periodontal disease, and six months intervals after the first year (12 months) of implant placement. During the visits the clinical and radiographic examination of implants and peri-implant tissues, evaluating implant stability, removing implant retained plaque and calculus, and setting new maintenance intervals should be done (Wilson, 1996).

Diagnostic parameters used to assess peri-implant area health encompass various assessments like plaque and mucosa evaluation, bleeding on probing, depth of peri-implant probing, width of keratinized mucosa around the implant, analysis of peri-implant sulcus fluid, presence of suppuration, occlusal assessment, radiographic analysis, and examination of implant stability/mobility (Gulati et al., 2014).

Suppose mobility is observed in a stable and successful dental implant. In that case, further clinical examination of the abutment retaining screw, radiographic examination of bone loss surrounding implants, and any looseness or breakage of the prosthetic abutment collar should be performed (Kurtzman and Silverstein, 2006).

Dental implant biofilm buildup around dental implants as a result of insufficient or nonexistent access to oral hygiene can result in peri-implant diseases like peri-implant mucositis and peri-implantitis. These diseases must be prevented, detected early, and treated. Thus, the goal of maintenance therapy is to provide dental implants with a consistent, well-planned supportive implant treatment to ensure their long-term success (Newman et al., 2019). To prevent the progression from peri-implant mucositis to peri-implantitis and the conversion from peri-implant health state to peri-implant mucositis, regular biofilm removal during peri-implant supportive therapy is crucial (Heitz-Mayfield and Salvi, 2018).

The goal of implant dentistry has changed in recent years from merely achieving osseointegration to preserving the long-term health of the soft and hard tissues surrounding the implants (Kurtzman and Silverstein, 2006). In dental clinics, professional prophylaxis procedures are administered in conjunction with patients' diligent and effective oral health care to achieve co-therapy during implant maintenance therapy. The two most crucial elements for the long-term success of dental implants, aside from expert implant maintenance, are the roles and contributions of the patients (Kurtzman and Silverstein, 2006).

When dental implants are placed, personal hygiene care should be started at that time and should be adjusted using adjunctive oral hygiene tools to ensure the peri-implant area is thoroughly cleaned before, during, and after implant placement (Kurtzman and Silverstein, 2006). Brushing, interproximal cleaning (floss and interproximal cleaners), locally applied chemotherapeutics (such as chlorhexidine and phenolic compounds), and water irrigation are all examples of at-home implant oral hygiene care (Gulati et al., 2014).

In addition to treating peri-implant pathology, appropriate maintenance programs and routine professional oral hygiene care can reduce or eliminate issues that could result in implant failure (Cohen, 2003).

Scaling and curettage (plastic, metal, and ultrasonic scalers), air abrasives, rubber cups with non-abrasive polishing paste or low-abrasive dentifrices, locally applied chemotherapeutics (e.g., Arestin and PerioChip), and subgingival irrigation with antiseptic agents (e.g., Listerine and Chlorhexidine) are some examples of professional hygiene instruments that can be provided to patients with dental implants (Gulati et al., 2014). Additionally, recent studies have demonstrated that the irradiation

of Er: YAG and Er, Cr: YSGG lasers is an effective hygienic method for decontaminating dental implant surfaces (Park et al., 2020).

2.8 At-home care

1. Tooth brushing:

The primary method for maintaining endosseous dental implants at home is toothbrushing. Patients who have undergone dental implant procedures should be encouraged to use a medium-sized head, soft bristle toothbrush, employing the modified Bass technique, ideally performed at least twice daily (Kurtzman and Silverstein, 2006). Presently, mechanical toothbrushes, including rotary, circular, or sonic types, are recommended for daily use during toothbrushing routines, particularly beneficial for individuals with limited physical abilities (Gulati et al., 2014). Furthermore, in challenging-to-reach areas such as posterior regions and for adapting to the shape of implant-supported prostheses, the use of smaller-headed single-tufted toothbrushes is advised (Gulati et al., 2014; Humphrey, 2006).

2. Interproximal cleaning (floss and interproximal cleaners)

Interproximal cleaning, particularly using dental floss, proves advantageous for individuals with dental implants. Various types of dental floss, such as plastic, braided, satin, and woven floss, are available for use (Gulati et al., 2014). Alongside floss, interdental brushes play a crucial role as part of home care, aiding in interproximal cleaning for patients with dental implants (Clark and Levin, 2016). These brushes offer interchangeable tips of different sizes, necessitating careful use to prevent any damage to implant surfaces It's essential to tailor the use of these tools to each patient based on factors like implant location and angulation, length,+ and

position of transmucosal abutments, prosthetic structure, and the manual dexterity of the individual (Kurtzman and Silverstein, 2006).

3. Locally applied chemotherapeutics

Patients with dental implants can also benefit from at-home implant care using different forms (gel, foam, or solution) of local chemotherapeutic agents like chlorhexidine gluconate, phenolic compounds, and plant alkaloids (Gulati et al., 2014).

It is recommended to use antimicrobial mouth rinses, like chlorhexidine gluconate twice a day, to reduce plaque around implants; however, continued use may result in staining. (Gulati et al., 2014).

4. Water irrigation

Water irrigation, either with or without antimicrobial solutions, is a commonly used home care device for cleaning among implant patients (Kurtzman and Silverstein, 2006). However, it's crucial to ensure the correct direction of the stream to prevent any unwanted complications for the patients (Gulati et al., 2014).

2.9 Professional hygiene instrumentation/polishing methods of dental implants

2.9.1 Scaling and curettage using hand instruments

Hand instruments made of plastic, Teflon, wood, or gold coating are the best options for cleaning the area around dental implants. However, metal instruments should not be used to probe or debride the surfaces of implants; instead, they should only be used on natural teeth (Yen et al., 2022). To prevent peri-implant sulcus injury, it is imperative to use hand instruments to scale around dental implants correctly. The

blade should be closed to the abutment, engaging apically after passing the deposits, and extending the strokes coronally using short working strokes and light pressure (Gulati et al., 2014).

2.9.2 Scaling and curettage using the ultrasonic scaler

Regular stainless steel scaler tips, typically used for natural teeth, might cause abrasion on implant surfaces (Yen et al., 2022). Therefore, for professional hygiene during implant maintenance, special attachments with nylon sleeves or plastic inserts should be utilized as scaler tips (Kurtzman and Silverstein, 2006). Various commercial scaler options specifically designed for dental implants include products like Implacare®, 3i Implant Innovation®, Implant Cleaning Kits®, and Steri-Oss® scaler system (Gulati et al., 2014).

2.9.3 Rubber cup with polishing paste

Dental implant surfaces made of titanium or titanium alloy can be polished using a rubber cup and non-abrasive polishing paste such as aluminum oxide, tin oxide, or APF-free prophy paste (Gulati et al., 2014).

However, abrasive pastes like pumice flour have also been used to polish the surfaces of dental implants. Pumice is a light grey, very siliceous material with an average polishing score that is produced by volcanic activity (Sawai et al., 2015). According to a study, one of the techniques for producing the smoothest implant surfaces in reachable areas is polishing with a rubber cup and pumice flour (McCollum et al., 1992).

2.9.4 Air abrasives

Implant surfaces can also be cleaned using air abrasives or an air powder polishing unit. Various air abrasive powders, including sodium bicarbonate, glycine, and erythritol, are used by this device to clean dental implants and modify their surface (Matsubara et al., 2020). However, there is controversy surrounding the use of air abrasives due to potential side effects on patients and implant surfaces (Humphrey, 2006). These side effects encompass the uncovering of the implant surface and detachment of soft tissues at the implant's coronal portion, potentially leading to emphysema (Kurtzman and Silverstein, 2006).

2.9.5 Locally applied chemotherapeutics

One alternative approach to maintaining implant hygiene is through the application of locally administered chemotherapeutics, which include antibiotics and antimicrobials. By preventing plaque buildup and the transformation of pathogenic to non-pathogenic microorganisms around dental implants, this technique seeks to reduce or even reverse inflammation (Gulati et al., 2014; Humphrey, 2006). Many locally applied antibiotics, including Arestin®, PerioChip®, and Dentomycin®, are available for use (Gulati et al., 2014). Porras et al. (2002) found that topical application of 0.12% chlorhexidine gel reduces peri-implant mucositis.

2.9.6 Subgingival irrigation with antiseptic agents

Another hygiene instrument used for cleaning around dental implants involves subgingival irrigation using antiseptic agents like chlorhexidine gluconate or peroxide. Studies have shown that supplementing mechanical debridement with local irrigation using 0.12% chlorhexidine can reduce peri-implant mucositis by decreasing plaque,

inflammation, and probing depth while improving clinical attachment levels and suppressing pathogenic bacteria (Porras et al., 2002). However, care must be taken during cannula insertion to prevent implant surface scraping (Yen et al., 2022).

2.9.7 Titanium brushes

Titanium brushes have been developed and introduced by several companies as implant hygiene tools, particularly in cases where there are bone defects surrounding implants. A study by John et al, (2014) compared the impact of titanium brushes versus traditional metal curettes on implant surface alterations and their efficacy. According to the findings, the titanium brushes not only reduced surface roughness on titanium implant surfaces but also efficiently removed plaque biofilm (John et al., 2014). Titanium brushes were found to improve peri-implant parameters like probing depth, gingival index, and bone loss following debridement in peri-implant surgery, according to a systematic review by González et al. (2021). In addition to their superior adaptability to implant surface architectures, titanium brushes were found to have good accessibility to narrow bone defects (González et al., 2021).

2.9.8 Diode laser irradiation

The diode laser has found widespread use in peri-implant surgeries involving soft tissues, including its application in uncovering dental implants during second-stage surgery (Matys et al., 2017). Additionally, diode laser irradiation has been identified as a method for decontaminating implant surfaces. Studies suggest that diode lasers possess bactericidal effects without causing surface alterations or damage to treated implant surfaces. However, a drawback remains in the form of potential

excessive heat production in the peri-implant bone with this method (Wawrzyk et al., 2021).

Each diode laser device operates with different wavelengths of emitted photons (e.g., 445 nm and 970 nm). Moreover, the effects of this laser on irradiated tissues are influenced by operational parameters such as wavelength, power setting, continuous or pulsed mode, treatment duration, types of fiber used, and distance from treated tissues (Malmqvist et al., 2019).

2.9.9 Er: YAG laser irradiation

One technique for implant surface decontamination is the use of an erbium-doped yttrium aluminum garnet (Er: YAG) laser (AlMoharib et al., 2021). The temperature of the implant body does not rise noticeably when exposed to lasers with a wavelength of 2940 nm (Kreisler et al., 2002). Er: YAG laser is helpful for peri-implant biofilm removal without severely scratching implant surfaces, according to Duarte et al. (2009). However, several irradiation parameters are not comparable to those from other studies, including contact mode, water irrigation, angle, and time of irradiation (Duarte et al., 2009). However, compared to carbon fiber curets, a study found that the Er: YAG laser did not significantly improve the biofilm removal process (AlMoharib et al., 2021).

2.9.10 Er, Cr: YSGG laser irradiation

The use of erbium, chromium doped: yttrium, scandium, gallium, and garnet (Er, Cr: YSGG) laser, operating at a wavelength of 2780 nm, has proven effective in decontaminating biofilm around titanium surfaces of dental implants (Park et al., 2020). This method shows promise in debriding implant surfaces and finds wide