DECONTAMINATION OF ROUGH IMPLANT SURFACES IN VITRO USING GLYCINE POWDER AIR POLISHING

by

KYLA ELIZABETH KARR YEE LEUNG

B.Sc., The University of British Columbia, 2011
D.M.D., The University of British Columbia, 2015

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Craniofacial Sciences)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

August 2018

The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, a thesis/dissertation entitled:

Decontamination of rough implant surfaces in vitro using glycine air polishing

submitted by Kyla Elizabeth Karr Yee Leung in partial fulfillment of the requirements

the degree of Master of Science

in Craniofacial Sciences

Examining Committee:

Dr. Hannu Larjava

Supervisor

Dr. Lari Hakkinen

Supervisory Committee Member

Dr. Flavia Lakschevitz

Supervisory Committee Member

Dr. Vincent Lee

Additional Examiner

Additional Supervisory Committee Members:

Dr. George Giannelis

Supervisory Committee Member

Abstract

Objectives: The prevalence of peri-implantitis and subsequent failure of implants continues to rise. The majority of cases are bacterial in etiology. In peri-implantitis, the subsequent bone loss around dental implants exposes their micro-roughened surfaces to bacterial colonization, further aggravating inflammation, bone loss and progression of disease. The challenge of peri-implantitis treatment is the complete decontamination of roughened implant surfaces once colonized with a biofilm. Many chemical agents are not effective in decontamination of the rough-surface implants with mature biofilms. Therefore, we examined if air-flow with glycine (AFG) would be effective in decontamination of implants with mature subgingival mature biofilms.

Methods: SLA® (sand-blasted acid-etched) titanium discs were inoculated with dental plaque and anaerobically incubated at 37°C for 21 days to allow for the formation of a structurally mature oral biofilm on the disc surface. Discs were then separated into different treatment groups: AFG with or without the powder and with or without prior rinsing with 0.9% sterile saline. Control groups included no saline rinse, single rinse and double rinse controls. For assessment of decontamination, discs were imaged, and bacterial cells counted, under a scanning electron microscope (SEM) at 5000X magnification.

Results: The no-rinse control group contained multi-species undisrupted biofilm. Saline rinsing removed most of the biofilm except the rough-surface-associated microorganisms. Numerous cocci and some rods remaining on the pits of the SLA® surface. AFG without the powder was not effective in decontamination of the rough SLA® surface. However, AFG with the powder

produced practically clean SLA® surfaces. In addition, prior rinsing with saline was not necessary for this effect.

Conclusions: AFG appears be an effective method to decontaminate mature biofilm contaminated implant surfaces when used directly on the contaminated implant surface.

Lay Summary

Peri-implant diseases are typically bacterial in origin and affects a large percentage of dental implants. Peri-implantitis, in particular, can lead to implant loss. The design of implant surfaces presents a challenging situation for implant decontamination in the treatment of these diseases. Due to the microscopic roughness of the surface, multitudes of bacteria commonly colonize the surface in an organized, three-dimensional structure called a biofilm. A majority of treatments fail to completely remove the biofilm from the implant surface, preventing the complete resolution of disease. Airflow with glycine powder (AFG), an amino acid, has been increasingly popular as a modality for implant disinfection. This study found that AFG is extremely effective in removing the biofilm from the roughened implant surface.

Preface

This dissertation is an original intellectual product of the author, Kyla Leung. Dr. Jiarui Bi collected and cultivated the oral biofilm. Kyla Leung performed all experimental manipulation and processing of samples for SEM imaging. Kyla Leung and Dr. Gethin Owen carried out all SEM imaging of the samples. Kyla Leung performed image analysis to collect data for statistical analysis. Kyla Leung and Dr. Jiarui Bi prepared the corresponding figures and statistical analysis.

Table of Contents

List of Figures

List of Abbreviations

AGEs: Advanced glycated end products

AFG: Air-flow with glycine

BHI: Brain-heart infusion medium

BOP: Bleeding on probing

CFU: Colony-forming units

CHX: Chlorhexidine

CD: Cluster of differentiation

ddH_2O: Double distilled water

EtOH: Ethanol

OPG: Osteoprotegerin

PBS: Phosphate-buffered saline

PD: Pocket depths

PGE2: Prostaglandin E2

PMN: Polymorphonuclear leukocyte

Psi: Pound per square inch

RAGEs: Receptors for advanced glycated end products

RANK: Receptor activator of nuclear factor κ B

SEM: Scanning electron microscopy

SRP: Scaling and root planing

SLA: Sand-blasted acid etched

Th2: T helper 2 cells

TNFα: Tumor necrosis factor alpha

TIMP: Tissue inhibitor of matrix metalloproteinases

Acknowledgements

I would like to thank my supervisor and director, Dr. Hannu Larjava for his guidance, patience and support. I am also thankful to the members of my committee Dr. Lari Hakkinen, Dr. Georgios Giannelis and Dr. Flavia Lakschevitz for their knowledge, direction and encouragement. I would also like to acknowledge and thank Dr. Gethin Owen for his help, laboratory and SEM training, and constructive input, all of which were vital to this thesis and study. I extend my sincerest gratitude to Dr. Jiarui (Jerry) Bi who aided me so much with the laboratory work and statistical analysis. The completion of this thesis could not have been possible without all of you.

Many thanks to Mr. Edison Wrzosek of the UBC Dentistry IT Department who heroically salvaged my thesis from the wreckage of my dying hard-drive.

I also acknowledge Straumann for their kind support of this study.

Everything that I have accomplished has been through the tireless support and constant prayers of my family and friends. I can never thank them enough.

And finally: isn't the human body a wondrous thing? Is it not fearfully and wonderfully made? To me, the study of science and biology have and will always serve to unveil and glorify what God has created.

Dedication

This work, and everything it represents, is dedicated to my parents, Bob and Ada, who are exemplary figures in my life of love, wisdom, faithfulness, generosity and perseverance.

Chapter 1: Introduction

The estimated number of dental implants being placed in the United States is in excess of 2 million per year[1]. Dental implants are a very successful treatment option for the replacement of missing teeth, with long-term success rates estimated to be at least 94%[2]. In a recent study of implant survival rate in patients with various systemic comorbidities and diseases, the 25-year survival rate of implants was found to be 81%[3]. Despite this, with the increase in dental implant placement as a treatment for missing teeth, it is important to address the consequent increase in peri-implant diseases: peri-implant mucositis and peri-implantitis. The prevention and management of peri-implantitis is of particular interest as, with the subsequent loss of bone due to this inflammatory disease, there is an increased risk of loss of osseointegrated implants.

The risk of peri-implantitis is elevated in patients with a history of periodontal disease, poor plaque control, diabetes, smoking, alcohol use[4]. Indeed, the accumulation and incomplete removal of a bacterial biofilm appears to be the etiology of a majority of cases of peri-implant disease. Implant microstructure may also play a role in biofilm attachment[5]. Rough-surface implants are currently favoured over polished-surface implants due to increased bone to implant contact, cell attachment, biomechanical stability, bone strength, success rates[6]. However, some reports show that increased surface roughness over a threshold of 0.2 microns may encourage more rapid bacterial aggregation to the implant surface than on machined surfaces[5, 7, 8]. Once rough-surface implants are colonized with bacteria, they are exceedingly difficult to disinfect due to lack of access to the implant surface itself[9] and the microscopic irregularities of the surface within which bacteria escape methods for removal[10]. Chemical treatments to disinfect rough

implant surfaces have yielded poor results with studies showing that bacteria continue to persist following commonly used chemical therapies such as chlorhexidine application, tetracycline paste and phosphoric acid[10].

Air polishing was introduced in the 1970s as a method for removing stains and bacterial plaque from teeth and implants[11]. An air-flow device directs a pressurized mixture of air, water and abrasive powder toward the tooth or implant surface to remove stains and biofilm efficiently and effectively in less time than conventional rubber-cup polishing[11]. Traditionally, sodium bicarbonate powder was used in air polishing. However, most manufacturers and clinicians now favour less abrasive powders with smaller particle sizes such as calcium carbonate, calcium sodium phosphosilicate and glycine[11, 12]. Air-flow with glycine (AFG) is of particular interest with respect to implant surface disinfection with many novel studies supporting its use in conjunction with other methods of managing and treating peri-implant diseases[13, 14]. A majority of available studies assessing the efficacy of AFG on implant surface cleaning have only assessed its ability to remove stains, ink, specific bacteria and bacterial endotoxins. As such, there is a dearth of studies specifically assessing the efficacy of the disinfection of a mature, multispecies biofilm on a rough-surface implant using AFG.

As such, it was our objective to assess whether or not AFG would be efficacious in the disinfection of a mature oral biofilm from sand-blasted acid etched (SLA) titanium implant surface. We hypothesized that AFG would yield better results with respect to implant disinfection than controls.

Chapter 2: Review of the Dental Literature

2.1 Peri-implant diseases

In 1986, Albrektsson et al[15] proposed criteria for implant success and failure. That the implants remained immobile with absence of pain, suppuration, neuropathy and radiolucency, and with an annual loss of bone of 0.2mm beyond the initial bone remodeling was sufficient to classify as success[15]. The difference between early and late implant failures is an important addition to the understanding of implant success or survival. Early implant failures occur prior to the restoration of implants when the implant fails to osseointegrate due to a perturbation of healing following implant placement[16]. Late implant failures happen after the implant has been restored and subjected to occlusal forces and function[16]. These failures are associated with progressive bone loss around the implant and are caused by peri-implant disease excessive occlusal loading and fracture of the implant[16].

Initially, peri-implant diseases were poorly understood and categorized, leading to confusion with respect to their study and diagnosis. At present there are two recognized peri-implant diseases: peri-implant mucositis and peri-implantitis[4, 17-19]. According to the Consensus Report of the Sixth European Workshop on Periodontology, peri-implant mucositis is defined as inflammation exclusive to the soft tissues around an implant while peri-implantitis is diagnosed when the inflammation of tissues around an implant extends to the alveolar crest and results in bone loss[17]. Peri-implant mucositis is reversible and clinically presents with probing depths greater than 3 mm and bleeding on probing (BOP) with or without suppuration, with the absence of radiographic bone loss[17, 19]. Peri-implantitis may present clinically similar to peri-implant

mucositis but with irreversible radiographic bone loss that progresses further than normal bone remodeling[17].

2.1.1 Epidemiology

Epidemiological studies of disease incidence and prevalence are complicated by the lack of a standardized approach to diagnoses of these disease and the heterogeneity of studies. While the majority of studies define peri-implant mucositis as inflammation, resulting in BOP, of the peri-implant tissues, definitions of peri-implantitis vary greatly[20]. Methods of reporting is also inconsistent as some studies will report based on patients or subjects with disease while others will assess based on an implant-level[21]. According to the findings of the 6th European Workshop on Periodontology, the prevalence patients with peri-implant mucositis was 50% while peri-implantitis affected between 28% and 56% of patients[17].

A recent cross-sectional study assessed 96 patients and 225 implants over the course of 11 years[1]. The study authors defined peri-implant mucositis as "the presence of BOP and/or gingival inflammation with no evidence of radiographic bone loss beyond normal remodeling"[1]. Peri-implantitis was defined as "the presence of BOP and/or suppuration, with 2mm of detectable bone loss after initial remodeling, and PD greater than or equal to 4mm"[1]. The prevalence of peri-implant mucositis was 33% at an implant level and in 48% of subjects[1]. Peri-implantitis was less common; its prevalence was 16% in implants and 26% in patients[1].

Another systematic review and meta-analysis assessed 47 studies to determine the prevalence of peri-implant mucositis and peri-implantitis[20]. Defining peri-implant mucositis as

inflammation affecting the soft tissue alone and peri-implantitis as soft tissue inflammation and bone loss of at least 2mm beyond the bone level following normal modeling and remodeling, the authors conceded that the diagnoses of peri-implantitis in the assessed studies were far more subjective[20]. Peri-implant mucositis and peri-implantits prevalence was 9.25% and 29.5% respectively among implants, and 19.8% and 46.8% respectively among subjects[20].

Renvert et al[22] recently reported the prevalence of disease in a longitudinal case series over the course of 21 to 26 years following implant placement. Eighty-six patients presented at follow-up. The authors found that when implants presented with healthy peri-implant mucosa and absence of bone loss 9 to 14 years following placement, it was reasonable to anticipate future health of the implants[22]. Peri-implant mucositis was diagnosed when BOP and/or suppuration was present in absence of bone loss and peri-implantitis was diagnosed when BOP and/or suppuration was accompanied by loss of crestal bone[22, 23]. Recurrent peri-implantitis was diagnosed when three or more threads were further exposed in subsequent examinations[22]. In cases where two implants were present in the subject, the worst was included in the study and the other excluded. The study found the prevalence of peri-implant mucositis to be 54.7% and that of peri-implantitis to be 22.1%[22].

Another systematic review[24] found that the mean prevalence of peri-implant mucositis is 43% and the mean prevalence of peri-implantitis is 22%. Eleven studies were assessed in this review, all with varying definitions of peri-implant mucositis and peri-implantitis[24]. The different studies defined peri-mucositis often as inflammation, BOP and/or suppuration but differed with respect to whether or not implants with bone loss could be included under this

diagnosis[24]. Where studies included small amounts of bone loss under the diagnosis of peri-implant mucositis, a strict threshold of bone loss was set at which point if the bone loss extended beyond the threshold, the diagnosis would be changed to that of peri-implantitis. Often extend of bone loss was determined based on a threshold number of threads exposed or on a specific measurement beyond normal bone remodeling and modeling[24].

2.1.2 Etiology

Various etiologies of peri-implant diseases have been identified and studied. While factors such as occlusal overloading, implant design, improper surgical technique and implant malposition may contribute to increased bone loss and probing depths in the absence of plaque, the associated periodontal tissues do not show signs of classical signs of inflammation such as erythema, edema and BOP required for the diagnosis of peri-implant disease[25-27]. As such, these factors are not included as etiologic. The established primary etiology of peri-implant diseases is the presence of bacterial oral biofilm or plaque on the implant surface[4, 21, 26, 28, 29].

Canine models of disease have established that inflammation of tissues around implants occurs in the presence of plaque[29, 30]. A comparative study in a dog model assessed the tissue response of plaque accumulation around four different implant systems[31]. The authors found that regardless of implant used, when oral hygiene is stopped and plaque allowed to amass around implants, a significant proportion of inflammatory cells are present in the connective tissue in the vicinity of the pocket epithelium[31]. Ericsson et al[32] assessed the clinical response of peri-implant tissues around implants following the termination of plaque control in a beagle dog study. Following three months of uninterrupted plaque accumulation, sites were probed and found to

bleed upon provocation. The histological examination of the tissues confirmed the earlier findings if increased inflammation in the peri-implant tissues[32].

Similar to the experimentally-induced gingivitis studies in humans by Loë et al[33], numerous human studies have shown that plaque is sufficient to produce clinical and histological inflammatory changes in peri-implant tissues that are consistent with peri-implant mucositis[19, 29, 34-36]. Pontoriero et al[34] induced peri-implant mucositis *in vivo* by stopping oral hygiene practice and allowing plaque to accumulate around implants over the course of three weeks. The study found increases in probing depths and gingival index scores following the test period, as well as significant increases in spirochetes and motile rods in the biofilm[34]. With the reestablishment of oral hygiene practice, peri-mucositis lesions could be resolved and the state of the peri-implant mucosa returned to baseline[34]. In another study, Salvi et al[36] observed a significantly greater increase in gingival index scores around implants than teeth when plaque control was stopped for three weeks. Histologically, the immune cell response to biofilm buildup around implants was also greater than around teeth[36]. Mucosal health did not completely return to baseline during the test period following the reinstatement of plaque control, though inflammation was reduced, suggesting that a period of greater than three weeks may be required for the tissues to return to baseline[36]. Although the period of time to completely resolve peri-implant mucositis may be unclear, evidence has shown that peri-implant mucositis is reversible following the complete removal of its primary etiology: the biofilm[19, 36].

2.1.3 Microbiology

It is well understood that *in vivo*, multitudes of bacteria of different species will aggregate and adhere to one another and to the substratum, forming complex, organized and dynamic three-dimensional microbial ecosystems called biofilms[37-40]. Microorganisms which exist in a biofilm exist in cooperation or competition with each other. These microbes are well protected from the external environment by an extracellular polymeric substance or exopolysaccharide matrix which is produced by specific members of the biofilm[7, 38]. Due to the inhibition of diffusion into the biofilm and chemical inactivation as a result of the exopolysaccharide matrix, increase in gene transfer of resistance factors between members of the biofilm and the slow growth of bacteria, the biofilm provides protection from the action of antimicrobials and is resistant to disruption and removal[38]. In the case of oral microflora, bacteria adhere to the surface of the tooth or implant transiently at first, through interactions with the acquired pellicle of bioactive proteins and polysaccharides on the tooth and reversible van der Waals forces and covalent bonds, and then more permanently via ionic bonds before beginning to aggregate and mature into a biofilm[41, 42]. The process of bacterial attachment to the implant surface appear similar to teeth. The acquired pellicle facilitates the primary adhesion of bacteria to the implant surface[42-44]. Indeed, it is suspected that the implant surface properties, in addition to the properties of the fluids and environment of the oral cavity, may affect the composition of the acquired pellicle which may then favour the attachment of certain microorganisms over others[42, 44, 45]. According to the "ecological plaque hypothesis", the healthy biofilm consists mainly of commensal bacteria which pose no harm to the host[39, 40]. However, when pathogenic species of the biofilm begin to increase in number, the biofilm too becomes pathogenic to the host and the etiology for infectious diseases[38-40].

Around healthy implants and teeth, the oral microflora is characterized by Gram positive, aerobic cocci and short rods[46, 47]. The early colonizers of implants are similar to those of natural teeth[46, 48]. *Actinomyces* species and *Streptococcus* species such as *Streptococcus sanguinis, S. oralis, S. infantis,* and *S. thermophilus* attach first to the tooth or implant[7, 42, 49, 50]. Together with *Neisseria pharynges* and *Gemella haemolysans*, these bacteria form a "core group" of microbes that prove foundational for the biofilm consisting primarily of anaerobes[42]. The biofilm associated with healthy implants consists mainly of *Streptococci*, *Veillonella*, *Rothia*, and *Haemophilus* species as well as *Actinomyces naeslundii, Acntinomyces oris* and *Actinomyces meyeri*[42, 51-53]. While certain bacteria exhibit more pathogenic behavior are more closely associated with diseased sites, these so-called "periodontopathogens" may be found in the biofilms at healthy sites but at low concentrations and in equilibrium with the otherwise healthy oral microflora[7, 39, 40, 54]

Periodontitis and peri-implantitis lesions which are both considered infectious diseases dominated by Gram negative, anaerobic bacteria[37]. An understanding of the microbiology of such diseases is imperative to their management. In 1998, Socransky identified various complexes thought to be implicated in periodontal disease[55, 56]. The red complex of periodontopathogens is comprised of *Porphyromonas gingivals*, *Tannerella forsythia*, and *Treponema denticola*[55, 56]. Other periodontopathogens include *Aggretibacter actinomycetemcomitans* serotype B, *Fusobacterium nucleatum*, *Prevotella intermedia, Prevotella nigrescens* and *Parvimonas micros*[56]. In periodontitis, *P. gingivalis* is considered a keystone pathogen[54, 57]. An increase in *P. gingivalis* signifies a shift in the equilibrium of the

oral microflora to that which may favour an increase in concentration and presence of other periodontopathogens[57].

Biofilms associated with peri-implant diseases are more heterogeneous and anaerobic than healthy implant sites[51, 58]. While some reports do conflict, a majority of studies show that sites of peri-implant disease exhibit a greater number of red and orange complex bacteria[51, 59]. A greater number of enteric rods are also found in peri-implant diseased sites[59]. The core biofilm shares some similarities with that of periodontitis, consisting of *Tannerella forsythia*, *Treponema denticola*, *Filifactor alocis*, *Treponema maltophilum* and *Freitibacterium fastidiosum*, together with the keystone pathogen, *Porphyromonas gingivalis*[51]. In a study by Tabanella et al[60], *T. forsythia*, *Parvimonas micras* and *Camplyobacter* species were found in association with peri-implantitis. Other studies have shown that there is also an increase in *Fusobacterium nucleatum* and *Aggretibacter actinomycetemcomitans* [61]. *Staphylococci* species such as *S. aureus and S. epidermidis* are also found in higher concentration in peri-implant disease[58]. Thurnheer et al[62] assessed the effects on a subgingival biofilm model grown in vitro on titanium when *S. epidermidis* or *S. aureus* or both were added. The original population of *Actinomyces oris, Fusobacterium nucleatum, Streptococcus oralis, Veillonella dispar, Campylobacter rectus, Prevotella intermedia, Streptococcus anginosus, Porphyromonas gingivalis, Tannerella forsythia* and *Treponema denticola*, generally remained unchanged in all test groups[62]. However, population shifted to favour *S. aureus* when both *S. epidermidis* and *S. aureus* were added to the particular biofilm[62]. It is suspected that *S. aureus* outcompetes *S. epidermidis* in a peri-implantitis biofilm and even prevents the growth and division of *S. epidermidis*[62]. Numerous studies have identified *Staphylococcus aureus*, *Enterobacteriacae, Candida albicans*

and *Pseudomonas aeruginosa* in peri-implantitis biofilms, microbes that are not usually found in periodontitis lesions[63].

The microbiota of peri-implantitis disease is difficult to study due to the intra- and inter-patient variation and identification and culturing of bacteria. Reports conflict between different studies but the general consensus is that in the mixed anaerobic biofilm of peri-implant disease, red complex pathogens as well as *S. aureus* predominate. Much like in periodontitis, the primary etiology of peri-implant diseases is considered to be bacterial in nature. As such, it is prudent to understand the microbiota of such diseases in order to optimize treatment options.

2.1.4 Risk indicators

Considering the morbidity of peri-implant diseases, it is important to understand how to manage such conditions proactively. As such, good patient selection is increasingly vital to treatment success. Risk factors are defined as patient or environment related factors which may increase the possibility of disease development[64]. Risk factors are identified through longitudinal studies of a population which suffers from a particular disease[64]. The heterogeneity of studies on peri-implant diseases and their risk factors and the dearth of longitudinal studies result in the lack of established risk factors for disease[65]. Therefore, the available literature has only been able to identify putative risk factors, risk indicators, for disease. In the case of peri-implant disease, risk indicators may be divided into local and general or systemic.

Risk indicators of peri-implant diseases include poor oral hygiene[17, 63, 66], a history of periodontitis[17, 63, 66, 67], lack of regular hygiene visits[66], genetics[19] and rough implant surfaces[4, 17].

Smoking[17, 19, 67, 68] and diabetes[1, 17] have been implicated in the past as potential risk indicators for peri-implant disease. In contrast to a majority of studies, Renvert et al failed to prove that smoking or diabetes mellitus increased the risk for peri-implantitis after 21-26 years[22]. According to the most recent consensus by the 2017 World Workshop on Periodontitis and Peri-implant Diseases and Conditions, there is still inadequate evidence to conclude that smoking or diabetes may be included among the above risk indicators for peri-implant disease[66, 69].

As in the cases of gingivitis and periodontitis, it is clear that poor oral hygiene and lack of professional maintenance increase the risk of peri-implant diseases[66]. Poor plaque control carries the strongest evidence of all putative risk factors. Numerous studies have shown that inadequate patient-performed oral hygiene vastly increases the risk of developing peri-implant disease[70]. A study by Schwarz et al found that the correlation between poor plaque control and peri-implantitis had an odds ratio of 9.2529[70]. In a longitudinal study of patients diagnosed with peri-implant mucositis, subjects who did not receive regular maintenance were at an elevated risk of peri-implantitis[71]. After five years, 44 % of patients who did not receive regular maintenance developed peri-implantitis while only 18 % of patients who received regular maintenance developed further disease[71].

Numerous longitudinal studies have shown that a history of periodontal disease increases the risk of greater longitudinal peri-implant bone loss and peri-implant disease[4, 17, 72, 73]. In a study assessing patient factors which may increase the risk of peri-implant diseases over a follow-up period of 9 to 14 years, patients with a history of periodontitis were found to have a greater tendency to develop peri-implant disease[67]. Matarasso et al[72] performed a 10-year

retrospective study of alterations in bone level around implants in periodontally healthy patients compared to that of periodontally compromised patients. The authors found that over the study period, patients with a history of periodontal disease experienced greater bone loss around implants than did periodontally healthy patients[72]. In a 10-year prospective cohort study, patients with a history of moderate to severe periodontal disease had 11.2 % - 15.1 % bone loss compared to 4.7 % in patients with no history of periodontal disease[73]. Implant loss in patients with histories of severe periodontal diseases was 10 %, compared to 2 - 3.4 % in periodontally healthy patients[73]. In a prospective long-term cohort study of implant success and failure in patients with a history of generalized aggressive periodontitis, Swierkot et al found that such patients experienced greater risk of implant failure and greater prevalence of peri-implant mucositis and peri-implantitis[74].

Implant surface treatment also appears to influence the nature of peri-implant disease and biofilm formation on the implant surface[7, 42]. The objective of treating implant surfaces is to increase surface roughness in order to facilitate more rapid osseointegration of the implant[6]. Surface roughness in the case of implant dentistry is calculated and expressed in S_a values, a three-dimensional parameter representing the difference in height of each point of roughness as compared to the surface average[6]. Implants are generally categorized into smooth (S_a values less than 0.5 μm), minimally rough (S_a values between 0.5 – 1.0 μm), moderately rough (S_a values between 1.0 – 2.0 μm), and rough surfaces (S_a values greater than 2.0 μm)[6]. Some studies have shown that increased roughness may be correlated with increased bone modelling and peri-implant bone loss following implant installation[6]. When exposed to bacterial in the oral cavity, increased surface roughness on an implant greater than a threshold of 0.2 microns may

encourage the attachment and aggregation of a biofilm to the implant surface[7]. Two dog studies by Berglundh et al[75] and Albouy et al[76] assessed the progression of bone loss around rough-surface and polished-surface implants in separate ligature-induced peri-implantitis models. Both studies compared the rate of bone loss as well as the size of the inflammatory cell infiltrate. Peri-implantitis was induced by the positioning of ligatures around implants and left in place until the bone loss was 40%[75, 76]. At this point, the ligatures were removed and plaque allowed to accumulate[75, 76]. Block biopsies were then taken and histologically assessed following five to six months[75, 76]. Bone loss and plaque accumulation around rough surface implants was significantly greater than around smooth implants[75, 76]. The biofilm accumulation appeared to extend further apically around rough implants than smooth implants[75, 76].

Smoking has been proven to be a true risk factor for periodontitis[77]. Smokers are at a four-times greater risk of developing periodontitis than non-smokers[78]. Nicotine exposure results in the alterations of the microvasculature of the periodontium by reducing the proportion of large diameter blood vessels and increasing the density of small diameter blood vessels[79]. Cigarette smoking also leads to alterations in PMN chemotaxis and immune function[80, 81]. Nicotine has a profoundly negative impact on periodontal wound healing[82]; delaying or inhibiting the adhesion of gingival fibroblasts to the root surface[83], and altering the pattern of collagen turnover, as well as promoting greater osteoclastogenesis[84]. Numerous studies have shown that smoking has a negative effect on implant health and that cigarette smoke is directly related to peri-implant disease[67, 85]. Smokers exhibit an elevated risk for inflammatory peri-implant disease than nonsmokers[85, 86]. A recent cross-sectional study showed that smoking may increase the risk of peri-implant diseases by three times[87]. As discussed previously, however, numerous studies

have failed to conclude an increase in incidence or prevalence of peri-implant disease in smokers[66, 88].

Uncontrolled diabetes mellitus has been shown to have a bidirectional relationship with periodontitis[89]. In chronic hyperglycemia, various cells and cellular products may become glycosylated, forming advanced glycated end products (AGEs)[90]. AGEs form high-affinity bonds with receptors for advanced glycated end products (RAGEs), which are found notably on monocytes and endothelium as well as other cells[90]. Uncontrolled diabetes negatively impacts collagen turnover and wound healing as well as resulting in immune dysfunction[90]. Hyperglycemic conditions lead to PMN dysfunction and increased proinflammatory cytokine production[90]. AGE formation at collagenous wound edges and on the extracellular matrix, and the interactions of AGE and RAGE at the vascular endothelium impair extravasation of products and cells from the blood into the extravascular and extracellular matrix. Collagen turnover is impaired and slowed, leading to dysfunctional wound healing[90]. While a majority of studies have not been able to prove the association between diabetes and peri-implant disease, one study found that in patients with diabetes, peri-implant mucositis prevalence was 64.6 % and peri-implantitis prevalence was 8.9 %[91]. For ethical reasons, it is likely impossible to study the effect of uncontrolled diabetes mellitus on implant survival and health. However, due to the established relationship between periodontal disease and uncontrolled diabetes, it is not inconceivable that a similar relationship exists between peri-implant disease and poor glycemic control.

2.1.5 Histopathology and pathogenesis

There are several differences between the attachment of the oral soft tissues to teeth and to implants. The most obvious of these differences is the presence of the periodontal ligament and cementum which connect natural teeth to the bone, whereas implants are connected to the bone via osseointegration alone. The attachment of the junctional epithelium via hemidesmosomes and basal lamina to teeth and implants are traditionally believed to be similar, although more recent animal studies have refuted this notion[92, 93]. In a more recent study found that instead of adhering directly to the implant surface, the peri-implant epithelium only is in contact against the implant surface[93, 94]. In fact, the authors found that the epithelium adjacent to the implant surface is structurally distinct compared to the junctional epithelium of a natural tooth[93, 94]. On the other hand, the connective tissue fiber attachment around implants and teeth differ significantly. While the connective tissue fibers extend apically, horizontally, and coronally from the teeth to the attachment apparatus, the same fibers run parallel to implant surfaces[95]. Because of the supposed strength of the junction epithelial attachment, some authors consider the soft tissue seal around implants to be adequate despite alterations in connective tissue fiber apparatus[92], however in light of more recent studies, this assumption is controversial. Indeed, the soft tissue seal around implants, while adequate for the maintenance of peri-implant health, may be significantly weaker than that around teeth[93].

It has been well-established by the literature that while periodontal disease is a result of the bacteria or biofilm found in the plaque around teeth, plaque alone is not sufficient to lead to the development of periodontitis[96]. In their classic paper, Page and Schroeder[97] describe the histopathology of gingivitis and periodontitis and separated the pathogenesis of the diseases into

four different stages. The first stage, called the *initial lesion*, though not clinically visible, can be histologically observed in the gingiva after 2 to 4 days of undisturbed plaque accumulation[97]. This stage is characterized by an inflammatory cell infiltrate, edema and the initial degradation of perivascular collagen[97, 98]. The permeability of the junctional epithelium increases with the accumulation of bacterial enzymes and metabolic end products allowing for further invasion of bacteria into the connective tissue[98]. The presence of bacterial products in the sulcus and mucosa activates the complement cascade via the alternative pathway stimulating the mast cell release of vasoactive amines which further increase permeability of the gingival vasculature and the chemotaxis of PMNs to the site[98]. The resultant inflammatory cell infiltrate is therefore dominated by PMNs and localized to the gingival sulcus[97, 98]. These PMNs are the frontline for the immune response to bacterial invasion and are involved in phagocytosis of the bacterial products[98]. In a process called "abortive phagocytosis" the lysosomal contents of PMNs are released into the sulcus[98]. While this is meant to serve a protective purpose, the released enzymes can be taken back up into the tissues leading to local destruction of the connective tissue[98].

While there is some overlap between the *initial lesion* and the following *early lesion*, the early lesion is observed after 4 to 7 days of undisrupted plaque accumulation[97]. At this point, the early clinical signs of gingivitis are apparent: changes in colour, contour, consistence but no BOP[97]. This stage is characterized by a larger inflammatory cell infiltrate to the gingival tissues which predominantly consists of PMNs and cluster of differentiation-4 (CD4) T lymphocytes[97]. The elevated expression of cell adhesion molecules and interleukin-8 (IL-8) results in a more profound and rapid increase of these inflammatory cells to the gingiva resulting in perivascular

lesions of lymphocytes and macrophages which expand, coalesce and merge[98]. Edema, connective tissue degradation and vascular dilation and changes are markedly increased[97]. Soluble breakdown products from bacterial cells, antigens, are phagocytosed by resident Langerhan cells and transported to regional lymph nodes where they sensitize T cells[98]. Thus, the protracted acute inflammatory response leads to chronic gingivitis.

Established chronic gingivitis clinically presents with bleeding on probing and possibly pocketing but without bone loss[97]. This stage, the *established lesion*, occurs following 2 to 3 weeks of undisturbed plaque and involves a dense accumulation of inflammatory cells to the connective tissue[97]. Dominated by B lymphocytes or plasma cells, immunoglobulins appear in larger quantities at the connective tissue and junctional epithelium[97]. An increase in inflammatory cytokines, particularly of IL-1, IL-6, tumor-necrosis factor alpha (TNFα) and prostaglandin E2 (PGE2), also occurs[98]. With the extensive loss of a majority of the connective tissue, the junctional epithelium apically migrates and extends laterally and may lead to the formation of a periodontal pocket[98].

Clinical periodontitis corresponds to the *advanced lesion*, whereupon resorption of the alveolar bone is observed as well as a dense inflammatory cell infiltrate consisting mainly of plasma cells[97-99]. Gingivitis and periodontitis do not exist on a continuum; the shift from gingivitis to periodontitis depends almost entirely on the patient's own host response to bacterial insult. Immunoregulation plays an integral role in the pathogenesis of periodontal disease. The development of polyclonal B cell activation is favoured by T helper 2 cells (Th2) and gives rise to polyclonal B cell activation, nonprotective antibodies and the progression of periodontal

disease[98]. These immune cell types will favour the production of inflammation cytokines such as IL-1, IL-6, TNFα and PGE2 which activate matrix metalloproteinases (MMPs) and subsequent connective tissue destruction and bone resorption[98]. These mediators of the immune response also contribute to the regulation of tissue inhibitors of matrix metalloproteinases (TIMPs) which inhibit the activity of MMPs as well as the receptor activator of nuclear factor κ B (RANK) to osteoprotegerin (OPG) ratio which affects the rate of resorption of the bone by osteoclasts[100].

While all the players and interactions of the immune response leading to periodontitis have not yet been elucidated, it is clear that an uncontrolled host inflammatory and immune response to bacterial presence is the root cause for the destruction observed in periodontal disease[96]. The complex interplay between immune cells, regulators as well as host factors and the biofilm manifest in periodontitis.

Numerous studies have confirmed that gingivitis and peri-implant mucositis do not differ with respect to the composition of the inflammatory cell infiltrate, both consisting of T cell and B cell lymphocytes[101, 102]. The heightened cytokine response in peri-implant mucositis involves an increase in IL-6 and IL-1β in the gingival crevicular fluid is associated with ailing implants compared to health[103]. A number of early reports of experimentally-induced peri-implant mucositis describe an inflammatory cell infiltrate that is significantly more robust and extends than in gingivitis by almost threefold[26, 32, 34, 36, 102]. Comparisons between gingivitis and peri-implant mucositis found no differences in expression of surface adhesion molecules in the microvascular[104]. Subtle differences in cell surface molecule expression with respect to CD3/CD19 ratios were noted in studies[101, 102]. Despite these observations, the general consensus

is that peri-implant mucositis closely resembles gingivitis with respect to histopathology and pathogenesis[102].

Berglundh et al[105] compiled the results of several human biopsy studies as well as experimentally-induced peri-implantitis models to determine the difference between periodontal and peri-implant diseased tissues. The inflammatory cell infiltrate in peri-implantitis lesions exhibit a further lateral and apical expansion than in periodontitis and is directly exposed to the pocket environment and the biofilm instead of being covered by a barrier of connective tissue or epithelium[105]. As is the case for periodontitis lesions, plasma cells and lymphocytes make up a large proportion of the infiltrate, however peri-implantitis lesions also exhibit a larger proportion of PMNs and macrophages compared to periodontitis[105]. In a mouse model, peri-implantitis and periodontitis were induced by ligatures[106]. The study showed greater bone loss in peri-implantitis lesions than around teeth and greater incidence of implant loss than tooth loss[106]. Histological evidence showed a greater amount of MMP-8 and nuclear factor kappa B (NF-κB) in peri-implantitis, corresponding with the greater osteoclastic activity evidenced in those lesions[106]. Experimental ligature-induced peri-implantitis lesions also do not exhibit the same response to removal of the ligatures as in ligature-induced periodontitis[105, 107].

2.1.6 Treatment options

Similar to treatment options available in periodontitis sites, treatment of peri-implantitis can be divided into non-surgical and surgical treatments[108]. A study by Renvert et al[109] compared mechanical non-surgical treatment with titanium hand-instruments and ultrasonic devices in a double-blind randomized longitudinal clinical study. The study of 31 patients with

peri-implantitis showed no significant change in PD was observed between baseline and 6 months following treatment in both groups, although a decrease in bleeding and plaque scores was observed[109]. In addition, no significant changes or decreases in bacterial counts were observed[109]. This indicates that mechanical debridement with hand instruments and/or ultrasonic instruments result in limited clinical improvements and are therefore not effective in the treatment of peri-implantitis[108]. Further studies into non-surgical treatment of peri-implantitis delved into the prospect of repeated local antimicrobial delivery systems. In such cases, local antimicrobials serve as adjuncts to debridement[108]. A randomized control trial studying the use of minocycline compared to chlorhexidine gel in pockets of 31 patients was assessed with a follow up at 30, 90 and 180 days after treatment[110]. While there were significant improvements observed in minocycline treated sites with respect to reductions in PD in comparison to chlorhexidine, it appears that such treatments require repetition over time[110]. Overall, with adjunctive antimicrobials in sites following debridement, some improvements have been observed in the literature in terms of PD and reduction in BOP[108]. It is suggested that patients in whom surgery is not an option for treatment, debridement with adjunctive therapy may serve well as treatment of peri-implantitis[108]. Systemic antibiotics as adjuncts to debridement have not be convincingly shown to effectively treat peri-implantitis lesions[108].

Other adjunctive treatments that can be provided together with non-surgical treatment include the use of air-abrasion, Er:YAG laser and carbon dioxide laser and hydrogen peroxide conditioning[108]. A systematic review by Schwarz et al[13] assessed five studies of the efficacy of air polishing together with debridement in the treatment of peri-mucositis and peri-implantitis. While air polishing appeared to have a significantly positive effect on the treatment of peri-

mucositis, sites with peri-implantitis did not appear to show significant differences between tests and controls after 12 months[13]. The study concluded that for the treatment of peri-implantitis, air polishing only appeared to reduce BOP but not result in disease resolution of peri-implantitis[13]. Studies assessing the efficacy of the Er:YAG laser have shown that while it may adequately and safely remove calculus and degranulate the implant surface, beneficial effects of such treatment appear limited[108]. *In vitro* studies of carbon dioxide laser and hydrogen peroxide conditioning treatment of titanium surfaces have shown promising results in terms of treating peri-implantitis[111]. However, dog studies showed that the levels of re-osseointegration between the test and controls (saline) were essentially the same, indicating no apparent effect on the re-osseointegration around peri-implantitis sites[111].

In the surgical treatment of peri-implantitis, excluding removal of the implant, treatment options include open flap debridement and use of adjuncts (locally placed antimicrobials, systemic antibiotic, air-abrasion at site or even laser decontamination), decontamination followed by implantoplasty with or without bone grafting, and guided bone regeneration[108]. Overall, it does appear that surgical therapy is more effective in the reduction of PD, BOP and suppuration than non-surgical therapy. Such decontamination, by titanium rotary brush, citric acid, chlorhexidine, antimicrobials and acids etc has been studied with no agent producing superior results with respect to disinfection of implant surfaces[108]. Decontamination of the implant surface alone appears not to be sufficient to induce re-osseointegration of the implant[112]. Guided bone regeneration with enamel matrix derivative or platelet-derived growth factor following decontamination has shown promising results[108, 113]. A case series of 170 implants showed that with the use of EMD or PDGF together with GBR and covered with a resorbable membrane

and/or tissue graft reduced PD by 5.1 mm, increased bone level by 1.77 mm, and increased ST margin gain by 0.52 mm with a 98.8% survival rate between 2 to 10 years of follow-up[113]. In cases with more exposure of the implant surface, combination therapy has been studied as well. A case study by Schwarz et al reported five cases of peri-implantitis treated with implantoplasty with bone grafting[114]. Schwarz described several defects with the conclusion that the combination of implantoplasty and bone graft appeared effective in treatment of even buccal dehiscence, while in circumferential defects, bone grafting together with removal of exposed threads of the implant proved to exhibit more success in re-osseointegration[13, 114-116]. The risk to the patient when taking a bur to the implant and heating the implant slightly as a result of implantoplasty was assessed and, while there was found to be a slight increase in temperature, the temperature appeared to reduce to baseline after ten seconds[117]. Such combined resective and regenerative therapy as described by Schwarz et al[114] was assessed in a prospective case series[118]. A 100% survival rate of treated implants was observed together with reduction of PD by 4.1 mm, BOP by 13%, and radiographic fill of the intrabony component of the defect of 93.3% after 12 months[118]. Indeed, such combined therapy shows promising results[118].

The study of treatment efficacy and effectiveness of peri-implantitis has been difficult and inconclusive. Much of this difficulty and confusion arises mainly from the heterogeneity of case definitions of the disease. As such, treatment of peri-implantitis continues to be largely empirical[119]. Prevention of peri-implantitis, proper case selection, proactive treatment planning and stringent recall and hygiene schedules for the patient are key to managing such cases[108]. Further long-term, randomized controlled trial studies of the treatment of peri-implantitis are likely required before a consensus can be reached.

2.2 Air-flow with Glycine

Airflow with abrasive powders was introduced in the 1970s and 1980s for the removal of stains and biofilm from teeth[11]. Considered to be more efficient than conventional rubber cup polishing, airflow introduces a high-pressure slurry of abrasive powders to the tooth surface in order to mechanically remove the biofilm[120]. The abrasive capacity of the airflow is dependent on a number of variables including particle mass, shape, size and hardness; air pressure and volume of water; the distance between the airflow nozzle and surface, angulation of the spray, and duration of airflow treatment[12]. Increasing the particle mass, size, hardness and angularity of shape will increase the abrasiveness[12]. The removal of substances from a surfaces is also expedited by increased water flow and air pressure[12]. While angulation of the nozzle to surface appears to have little effect on efficacy of biofilm removal, distance between the airflow source and surface is inversely proportional to efficiency[12]. Traditionally, sodium bicarbonate powders have been used and found to be very effective in the removal of stains[120]. While these powders, particles of which range up to 250 μm, have not been found to abrade enamel, extended use of such powders may damage dentin and cementum, as well as restorative materials[11, 120]. As such, powders of a smaller particle size such as glycine have been introduced to the market as alternatives to sodium bicarbonate.

Glycine is a water-soluble, non-essential amino acid which is nonallergenic and nontoxic[12]. Generally, the size of glycine particles used in AFG are less than 45 μm in size and approximately 2.16 g per cm^3 in density[12, 121].

Classic studies have assessed the effectiveness of conventional non-surgical periodontal therapy in the complete debridement of subgingival pockets and have found reduced plaque-free and calculus-free zones in deeper pockets[122, 123]. In a study on extracted teeth, scaling and root planing (SRP) effectiveness in relation to probing or pocket depths was assessed[122]. In PD of up to 3 mm, SRP was shown to be very effective with 83% of plaque-free sites following treatment[122]. The effectiveness of SRP dramatically reduces in deeper PD with 39% of plaque-free sites in PD of 3 to 5 mm and 11% in PD of greater than 5 mm[122]. The same was proven regarding calculus removal from tooth surfaces. Another study assessed the effectiveness of conventional SRP on calculus removal and found again that in PD less than 3 mm deep, SRP was 86% effective based on calculus free sites[123]. In PD between 4 to 6 mm deep, SRP was only 43% effective and in PD greater than 6 mm, SRP was 32% effective[123].

It has been shown in the literature that AFG can effectively and efficaciously reduce or remove the supragingival and subgingival biofilm from tooth surfaces. The effectiveness of AFG was assessed in one study based upon PD and surface area of root to be debrided[124]. In PD of up to 3 mm, AFG application for 5 seconds was found to be as effective as SRP[124]. Petersilka et al[125] performed a split-mouth study comparing airflow using low-abrasive powder to the control, SRP using hand instrumentation. The effectiveness of biofilm removal was assessed by enumerating the number of colony-forming units (CFU)[125]. Following different treatments, interdental pockets between 3 to 5 mm in depth were sampled and cultured[125]. The number of CFU cultured from tested sites was significantly decreased compared to the control SRP groups, indicating that air-flow polishing effectively reduces the biofilm in PD up to 5 mm[125]. Another study by Flemmig et al[126], subgingivally applied AFG was found to effectively reduce the

subgingival biofilm in deep pockets between 4 to 9 mm in depth. In addition, AFG reduced counts of *P. gingivalis* in treated patients[126].

The use of airflow has also been studied *in vitro* and shown to be useful in the decontamination of implant surfaces. An *in vitro* study assessed the efficacy of various treatment modalities in the removal of bacterial endotoxin from machined and hydroxyapatite-coated titanium cylinders and found airflow polishing with abrasive powders to be the most effective[127]. Zablotsky et al[128] also studied the removal of bacterial endotoxins, but from variously treated or roughened titanium surfaces *in vitro*, comparing different methods of detoxification. Air powder polishing was found to be superior in removing lipopolysaccharide from the titanium surfaces when compared to other methods of chemical detoxification such as application of citric acid, antibiotics, chlorhexidine gluconate, and hydrogen peroxide, and mechanical removal with ultrasonic scalers[128]. Another study assessed the removal of bacteria from implant surfaces and also found airflow polishing to be superior to plastic scalers, Gracey curets, ultrasonic scalers and chlorhexidine application[129]. A recent randomized controlled clinical trial showed that airflow polishing effectively reduced levels of *P. aeruginosa* and *S. aureus*, two pathogens found in peri-implant disease[130].

Clinical evidence of treatment effectiveness in implant disinfection using AFG is still lacking. A recent systematic review found that while some studies found that the adjunctive use of AFG was beneficial in reducing gingival index scores and BOP around ailing implants, this treatment alone was insufficient to prevent disease progression of peri-implant mucositis, as were all other adjuncts to conventional SRP[13]. Indeed, there is conflicting evidence in the

literature as to whether or not AFG is a useful adjunct in the treatment and decontamination of infected implants exhibiting peri-implant mucositis or peri-implantitis. Because of this, more research and understanding of the use of AFG to decontaminate ailing implant surfaces is warranted.

2.2.1 Effect of glycine on oral tissues

Numerous studies have assessed the effect of AFG on oral tissues. Conventional powders such as sodium bicarbonate have fallen out of favour due to their size and resultant abrasiveness on hard tissues, such as cementum, dentin and enamel, and soft tissues, like gingiva[12]. A recent systematic review assessed the results of seventeen studies and concluded that glycine powder results in less damage to intraoral hard and soft tissue structures than sodium bicarbonate or calcium carbonate powders[131].

A clinical study assessed the effect of AFG against that of airflow with sodium bicarbonate or hand instrumentation on the gingiva[132]. Teeth with PD of at least 5 mm were randomly assigned to each treatment group[132]. Assessments were done via light microscopy and histological score on gingival biopsies which were taken immediately following treatment as well as after 14 days[132]. While following a healing period of 14 days, all sites appeared to exhibit the same amount of healing, immediately after treatment, AFG resulted in significantly less erosions of the soft tissue compared to hand instrumentation and airflow with sodium bicarbonate[132].

Numerous studies have also investigated the effect of airflow or air abrasion with abrasive powders on hard tissues in the oral cavity. Many studies have found that with increasing treatment time, decreased treatment distance and increased power setting, there is an elevated risk of abrading the tooth surface to a greater degree[12, 131]. In the systematic review by Bühler et al[131], a majority of studies appeared to assess defect depth, volume, amount of removal of tooth structure and degree to which the tooth surface was roughened following treatment. Of the studies assessed, glycine appeared to be the safest and least traumatic to hard tissues, resulting in the least amount of tooth structure removal and defect formation[131].

Another study assessed the effect of airflow with abrasive powders on implant abutment surfaces, notably: grade II titanium disks[133]. Using scanning electron microscopy (SEM), the disks were separated into different treatment groups and assessed for signs of abrasion[133]. Treatment groups included no treatment, AFG and airflow with sodium bicarbonate powder[133]. AFG was shown to be less damaging to the titanium surfaces compared to airflow with sodium bicarbonate[133]. In addition, treatment with glycine powder was shown to actively inhibit bacterial growth on the surfaces when left for a 24-hour period in the oral cavity following treatment, indicating that glycine treatment may result in longer-term bacteriostatic effects[133].

Overall, it has been established in the literature that AFG is safe and reliable for use in the oral cavity and results in less soft and hard tissue trauma than airflow with other abrasive powders such as sodium bicarbonate.

2.2.2 Antibacterial properties of glycine

Aside from being effective when used as an abrasive powder to remove biofilm, staining and plaque, glycine may have bacteriostatic or antibacterial properties[134]. Glycine-rich polypeptides have been found to inhibit outer membrane protein synthesis in bacteria[134]. In excess, glycine may act as a cation and promote permeability of the bacterial cytoplasmic membrane[135, 136]. A more recent study showed that when used in conjunction with antibiotics, glycine-containing compounds may serve to increase osmotic stress / pressure on the bacterial cell wall, in the cases of *Escherichia coli* and *Helicobacter pylori*[137]. However, no studies appear to have assessed the potential for a bacteriostatic or bactericidal effect on pathogens commonly implicated in periodontal and peri-implant diseases.

2.2.3 Current consensus for the clinical use of AFG

Cobb et al[138] published a recent consensus for the use of AFG around teeth. According to the authors, AFG can be safely used around teeth and restorative materials to remove supragingival and subgingival biofilm[138]. At PD up to 9mm, AFG is more effective in biofilm removal than conventional SRP with hand instruments[138]. The is little to no evidence of soft tissue trauma when using AFG[138].

At the European Federation of Periodontology Meeting in 2015, Schwarz et al[121] presented several recommendations for the clinical use of AFG around ailing implants. The committee and resultant consensus concluded that while AFG effectively removed bacterial biofilms from implant and implant abutment surfaces, it did so without damaging the physical structure of the implant surfaces[121]. Although the use of AFG, even as an adjunct to

conventional SRP, has not been shown to completely resolve the disease processes of peri-implant mucositis or peri-implantitis, it may effectively reduce the level of inflammation of peri-implant tissues as measured by BOP[121]. The incidence of post-operative air emphysema was also noted in one case report of AFG[139].

Currently in North America, only the nonsurgical application of AFG has been approved by the Food and Drug Administration (FDA) and Health Canada.

Due to the heterogeneity of the studies and the protocols used for AFG, further studies are necessary to better understand the effects of AFG on ailing implants as well as the management of peri-implant diseases.

Chapter 3: Aims and Hypothesis

3.1 Aims

Numerous studies have assessed the clinical applications of AFG for management of peri-implant diseases. A majority of these studies have assessed the effect of AFG on the decontamination of an ink-stained or specific endotoxin-infected titanium surface, the qualitative changes in bacterial composition in patients as a result of AFG treatment, or clinical effects on BOP or gingival index scores following treatment with AFG as compared to other adjuncts to conventional SRP. No studies have cultured a mature biofilm on rough implant surfaces *in vitro* and assessed the efficacy of AFG in decontamination. We aim to contribute further to the current body of knowledge regarding the use of AFG in implant decontamination in order to further support or refute its use in the treatment of peri-implant diseases.

3.2 Hypotheses

1. The application of AFG to a biofilm-contaminated SLA® surface will more effectively reduce or eliminate bacteria than the control.
    1.1. The null hypothesis is that AFG will not remove bacteria from the SLA® surface more effectively than the control.
2. The use of AFG on a biofilm-contaminated rough surface titanium implant disk will be significantly more effective at reducing bacteria than the application of air-flow alone (AF).
    2.1. The null hypothesis is that AFG and AF will perform similarly with respect to reducing bacteria.

3. Increasing the distance between AFG source and SLA® surface would decrease the effectiveness of decontamination of the SLA® surface.
    3.1. The null hypothesis is that varying distances between source and surface would have insignificant effect on decontamination effectiveness.

Chapter 4: Materials and Methods

4.1 Mature oral biofilm grown on SLA® surface

Bi et al[140], Dostie et al[10] and Schuldt[141] described the *in vitro* oral biofilm model and method for incubation used in the present study. The SLA® implant surface is considered to be moderately rough with a S_a value of approximately 1.78 μm[6]. Sterile SLA® implant disks measuring 5 mm in diameter and 1 mm thick (Straumann®, Basel, Switzerland) were inoculated with a dispersed subgingival dental plaque extracted from healthy volunteers. The disks were first washed with phosphate-buffered saline (PBS) (Sigma-Aldrich, Saint Louis, MO) before being individually placed in a 24-well tissue culture plate, each containing 2 mL bovine dermal collagen type 1 solution (10 μg/mL collagen in 0.012 N HCL in water) (Cohesion, Palo Alto, CA). The disks were incubated overnight at 4°C to allow for coating with the collagen solution. Following this, the disks were again rinsed with 2 mL PBS before being individually placed into separate wells of a new 24-well tissue culture plate, each containing 2 mL brain-heart infusion medium (BHI). The dispersed dental plaque, at a minimum concentration of 3.2×10^7 CFU/mL, was then added to each well and anaerobically incubated (AnaeroGen, Oxiod, UK) for 21 days at body temperature, 37°C. Bi et al[140] showed that the biofilms from different donors showed no difference in morphology compared to biofilms from a single donor when cultured to maturity in the conditions used in the present study. As such, subgingival plaque was collected from a single healthy donor. The media was changed weekly. The resultant biofilm was a mature, subgingival biofilm consisting of coccoid, rod and spirochete type bacteria.

4.1 Treatment Protocols

Following the complete incubation period, the biofilm-contaminated discs (Figure 1) were separated into triplicates and then into different treatment regimens or control groups. Therefore, each treatment regimen was repeated three different times using three different biofilms from the same donor[140]. The control groups included a no-rinse control with an uninterrupted biofilm (*n=6*), a double-rinse control (*n=6*) where the contaminated discs were rinsed twice with 6 mL 0.9% sterile saline, and a single-rinse control (*n=6*) where the contaminated discs were rinsed once with 6 mL 0.9% sterile saline. Rinsing with sterile saline was completed in 1 mL increments using a pipet to directly rinse each disc. For testing of AFG, the Air-Flow Master Piezon® (EMS SA, CH-1260 Nyon, Switzerland) was used (Figure 2). Using this device, glycine contained within a separate, dry canister would be directed through the nozzle and mixed with pressurized water to create the slurry of water and glycine. At maximum power, when set to "PERIO" mode, the pressure generated by the machine ranges from 42 – 50.8 pounds per square inch (psi). The recommended treatment time per pocket is 5 seconds, as such, a duration of 5 seconds was selected per sample. The different treatment regimens were (Figure 4):

1. AFG (50% power, corresponding to 21 – 25.4 psi) for 5 seconds with prior rinsing with 0.9% sterile saline (*n=6*)
2. AFG (50% power) for 5 seconds with no prior rinsing with 0.9% sterile saline (*n=6*)
3. Airflow with no glycine powder (50% power) for 5 seconds with prior rinsing with 0.9% sterile saline (*n=6*)

In additional experiments, a probe was used to standardize distances between the air-flow device and the disc (Figure 3). As well, differences in AFG power setting were also tested (Figure 4):

1. AFG (50% power) for 5 seconds at a distance of 3mm from SLA® surface (*n=9*)
2. AFG (50% power) for 5 seconds at a distance of 6mm from SLA® surface (*n=9*)
3. AFG (25% power, corresponding to 10.5 – 12.7 psi) for 5 seconds at a distance of 3mm from SLA® surface (*n=9*)
4. Airflow with no glycine powder (50% power) for 5 seconds at a distance of 3mm from SLA® surface (*n=9*)

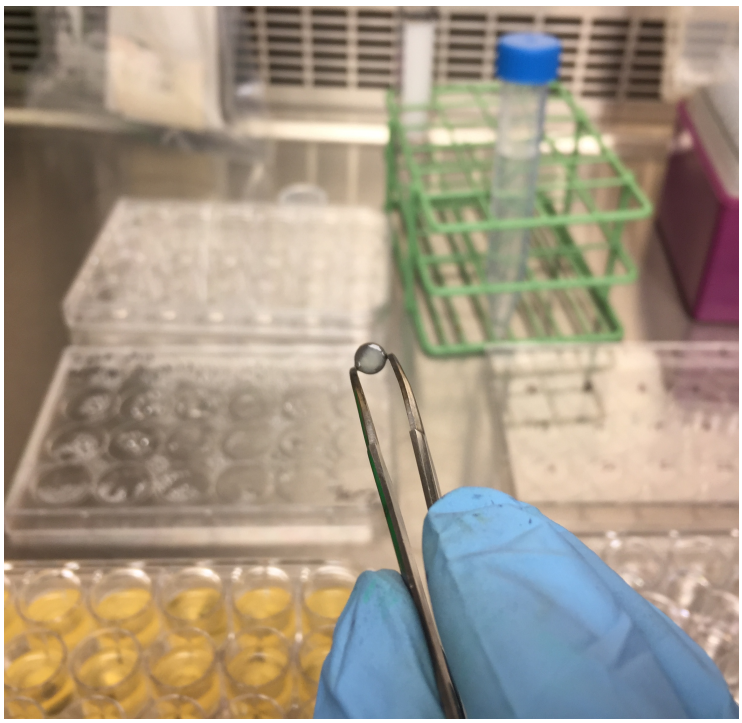

Figure 1: Titanium SLA discs with untreated mature dental biofilm following 3 week incubation period under anaerobic conditions at body temperature

Figure 2: Hu-Friedy EMS Air-Flow Master Piezon (EMS SA, CH-1260 Nyon, Switzerland)

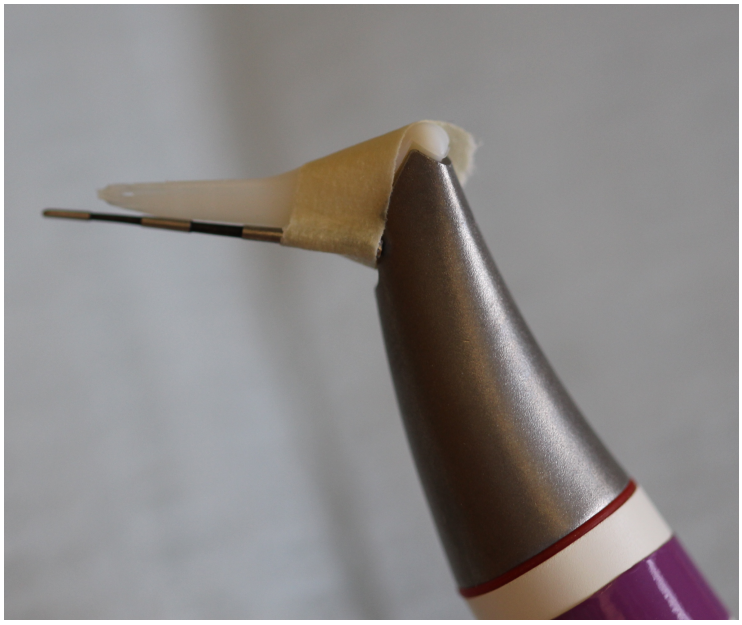

Figure 3: Use of probe attached to nozzle to standardize distance between air-flow device and disc in Experiment 2.

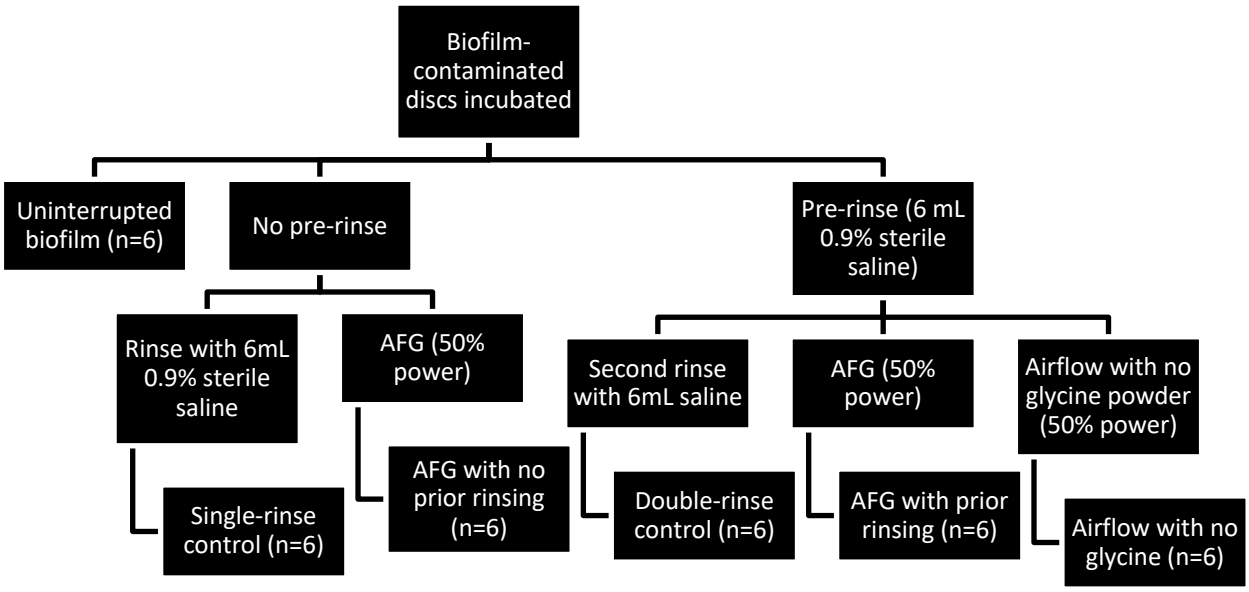

Figure 4: Workflow of experimental procedure for Experiment 1

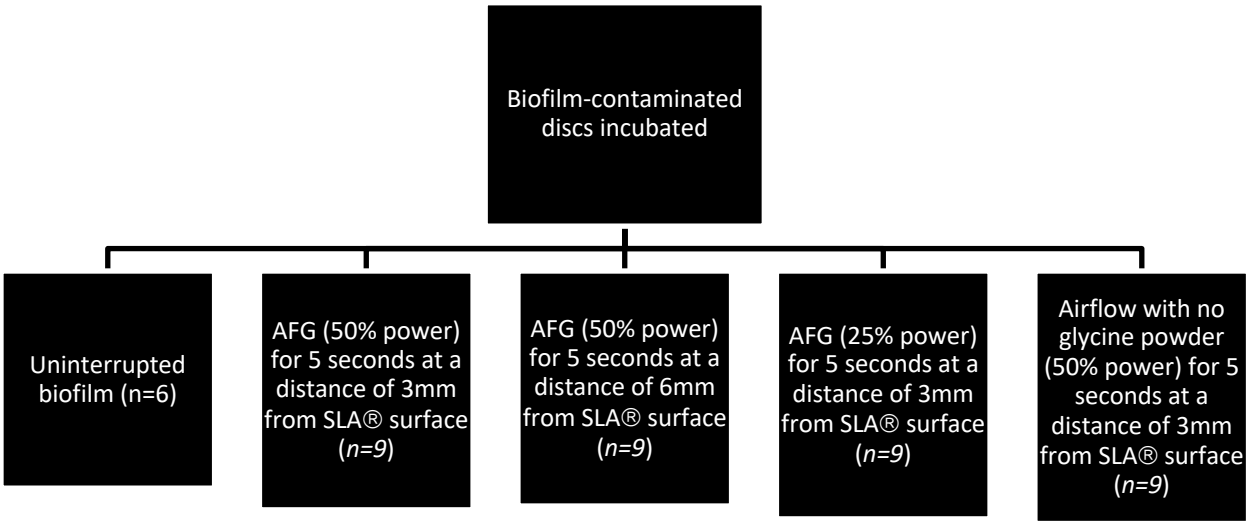

Figure 5: Workflow of experimental procedure for Experiment 2

4.2 Processing and preparation of samples for scanning electron microscopy (SEM)

Following treatments, samples underwent protein and lipid fixation, dehydration and iridium coating in preparation for SEM. To this end, each disk was first placed into a separate well in 1 mL of 0.1 M piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES) (Sigma-Aldrich, Saint Louis, MO) formulated to a pH of 7.4. After 2 minutes, the PIPES was exchanged from 1 mL 2.5% glutaraldehyde in 0.1 M PIPES (pH 7.4) for the fixation of proteins. Protein fixation was completed in 30 minutes. Disks were then placed for 5 minutes in 1 mL 0.1 M PIPES. Following this, lipid fixation was accomplished using osmium tetroxide in 0.1 M PIPES (pH 6.8). The samples were left in the osmium tetroxide for 60 minutes. Following lipid fixation, the disks were rinsed via submerging in 2 mL of double distilled water (ddH_2O) for 5 minutes and repeating the rinse three times.

The dehydration of the disks was accomplished by soaking the disks in their individual wells for 5 minutes at a time in increasing concentrations of a 200-proof ethanol (EtOH) and ddH_2O solution starting at 50% EtOH and increasing the concentration of EtOH in 10% increments. At 100% EtOH, the disks were soaked for 5 minutes in new 100% EtOH three times. After, the disks were further dehydrated in a critical point drier (Tousimis Samdri®-795 Critical Point Dryer, Rockville MA, USA).

The disks were then mounted on metallic studies via a conductive double-sided adhesive. The perimeters of each disk and the entirety of exposed adhesive on the stud at the periphery of each disk was then coated in colloidal silver. After the samples were dried overnight in a

dehydrator, they were coated with 10 nm of iridium using the Leica EM MED020 Coating System (Leica Microsystems, Wetzlar, Germany).

4.3 Scanning electron microscopy and data analysis

Imaging of each sample for assessment was done under scanning electron microscopy (Helios Nanolab 650 Focussed Ion Beam SEM, Oregon, USA). The SEM images were taken at the center of each disk at a voltage of 1 kV and at a magnification of 5000 times. The field of view in each detailed image was 30 μm by 20 μm and represented a surface area of 600 μm^2, approximately 3 % of the total surface area of each disk.

The samples were reimaged for data analysis. Three sites of each sample were randomly selected and imaged under SEM (Hitachi SU3500 Scanning Electron Microscope) at a voltage of 10 kV and at a magnification of 5000 times. Again, the field of view in each image represented a surface area of 600 μm^2. The number of bacterial cells from the three fields of view was counted and averaged. The resulting means from each triplicate experimental set were again averaged to provide a single average value of bacterial cells from each test group. ImageJ 1.51m9 software (National Institute of Health, Bethesda, MD, USA) was used to perform the bacterial cell count in each image using the Cell Counter function.

4.4 Statistical analysis

Statistical analysis was done using a two-way analysis of variance (ANOVA) and post hoc Tukey test using data collected from the mean bacterial counts pooled from each test group. Only factors with a $P < 0.05$ were considered significant).

Chapter 5: Results

5.1 Untreated control

Following the complete three-week incubation period of the biofilm onto the SLA® disks, a thick, multi-species, undisrupted biofilm covered each of the disks, completely obscuring the SLA® surfaces and resembling oral biofilms found *in vivo*. In the untreated control groups, the mature oral biofilm model on SLA® disks was imaged using SEM (Figure 6). The majority of bacteria were cocci which appeared to aggregate together in clusters and with rods, resembling corncob formations, similar to the conformations of bacteria in oral plaque. Spiral- and filamentous-shaped organisms were relatively few, but still observed amid the rods and cocci. With various layers of bacterial cells superimposed, imaging of deeper layers of bacteria was evidently obscured. The number of bacteria observed was too large to accurately enumerate.

5.1.1 Rinsed controls

The saline rinse controls, a single-rinsed ($n=6$) and double-rinsed ($n=6$) controls were observed under SEM. Saline rinsing alone appeared to remove the superficial bulk of the biofilm, leaving behind the deepest layer of cocci-shaped bacteria. The primary colonizers appeared to remain on the SLA® surfaces within the grooves and pits, attached via more permanent bonds to the surface. Between the single-rinsed (Figure 7) and double-rinsed controls (Figure 8), little difference in quantity of remaining bacteria was noted. The mean bacterial counts per field in single-rinsed and double-rinsed controls were 198.9 and 171.1 cells, respectively.

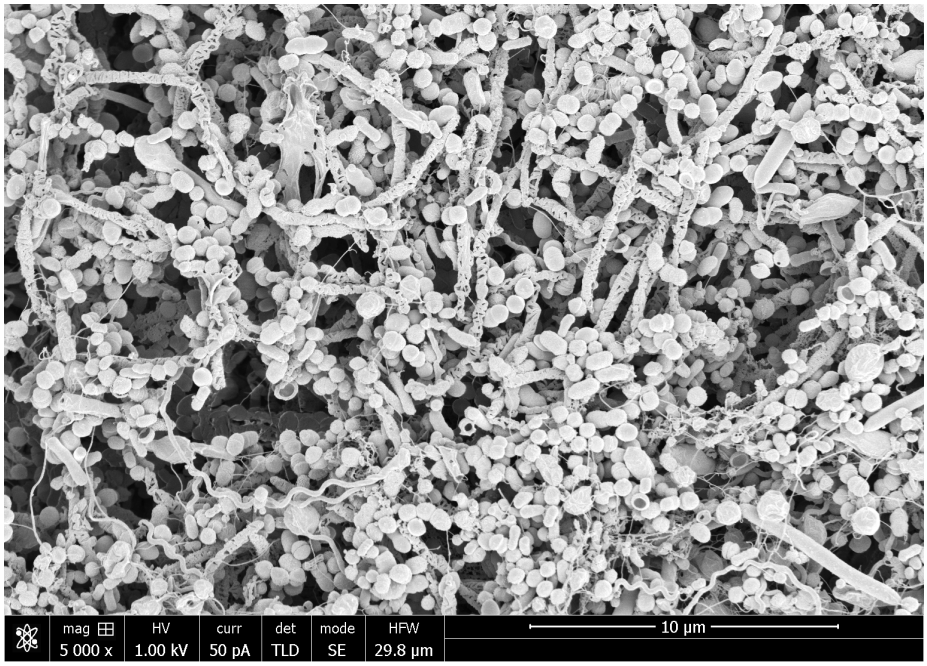

Figure 6: Untreated control; mature oral biofilm grown for 3 weeks on SLA□ surface as visualized on SEM at 5000x magnification (Helios Nanolab 650 Focussed Ion Beam SEM, Oregon, USA).

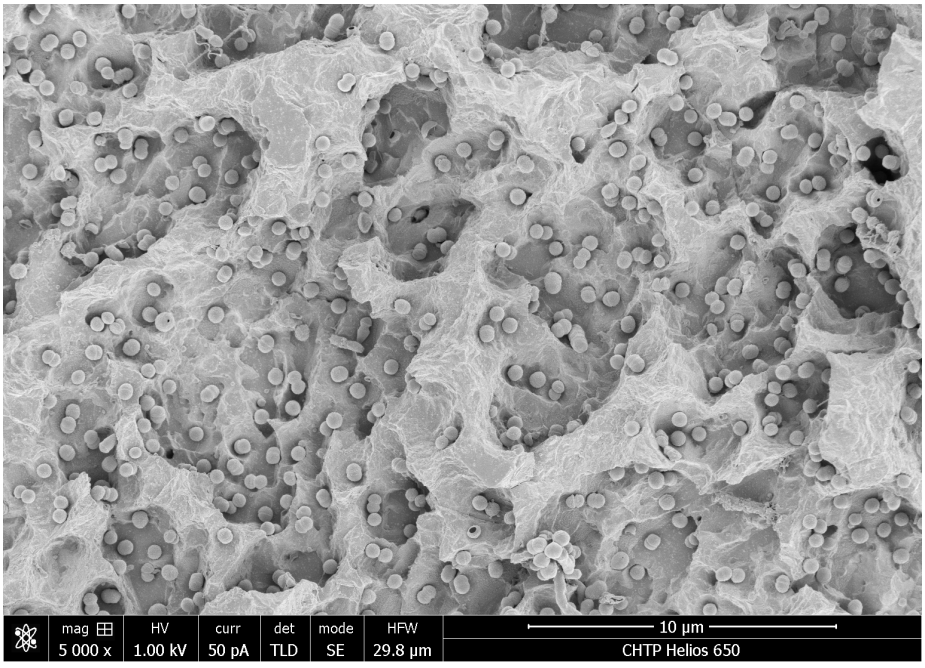

Figure 7: Single-rinse control; remnants of the bacterial biofilm following 6 mL of rinsing with sterile saline as visualized on SEM at 5000x magnification (Helios Nanolab 650 Focussed Ion Beam SEM, Oregon, USA).

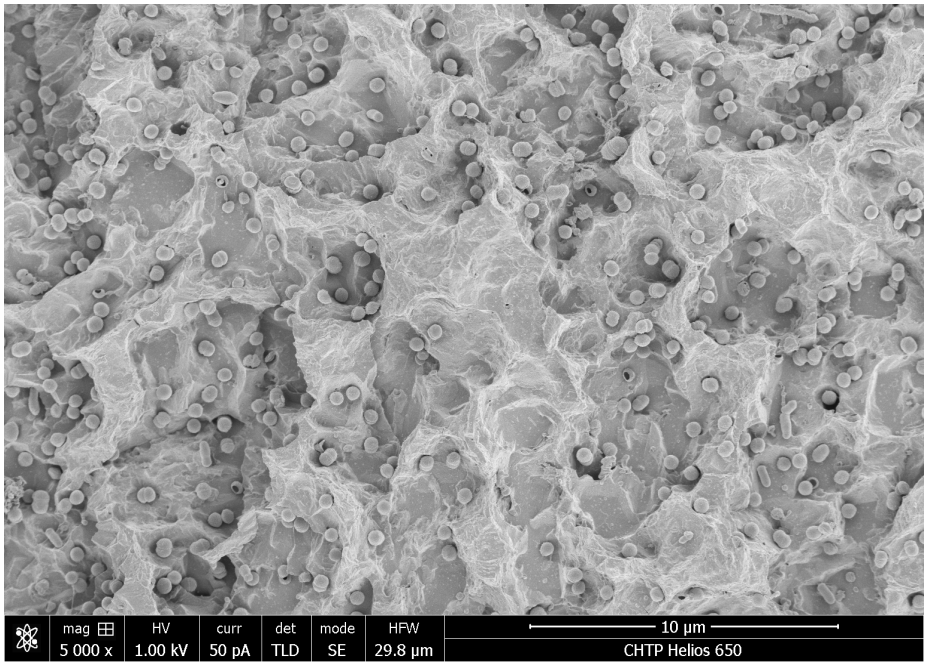

Figure 8: Double-rinsed control; remnants of the bacterial biofilm following 12 mL of rinsing with sterile saline as visualized on SEM at 5000x magnification (Helios Nanolab 650 Focussed Ion Beam SEM).

5.2 Experiment 1

The use of AFG appeared to successfully remove the majority of the biofilm, including the deeper layers of coccoid microorganisms that were not removed by saline rinsing alone. Small numbers of cocci could still be found in deeper pits and irregularities of the surfaces. The mean bacterial count after treatment with AFG and pre-rinsing was 7.7 cells per field of view. The mean bacterial count after treatment with AFG but no pre-rinse was 5.9 cells per field of view. Compared to single-rinsed and double-rinsed controls, AFG showed a statistically significant reduction of bacteria ($P < 0.01$) (Figure 13). Pre-rinsing with saline did not improve efficacy. Airflow without powder was no more effective in decontamination of the surfaces than the single-rinsed and double-rinsed controls (Figure 12). The mean bacterial count when

samples were treated with airflow alone was 169.3 cells. The result of AFG was significantly more efficacious in the decontamination of the rough surfaces than airflow without glycine powder ($P < 0.01$).

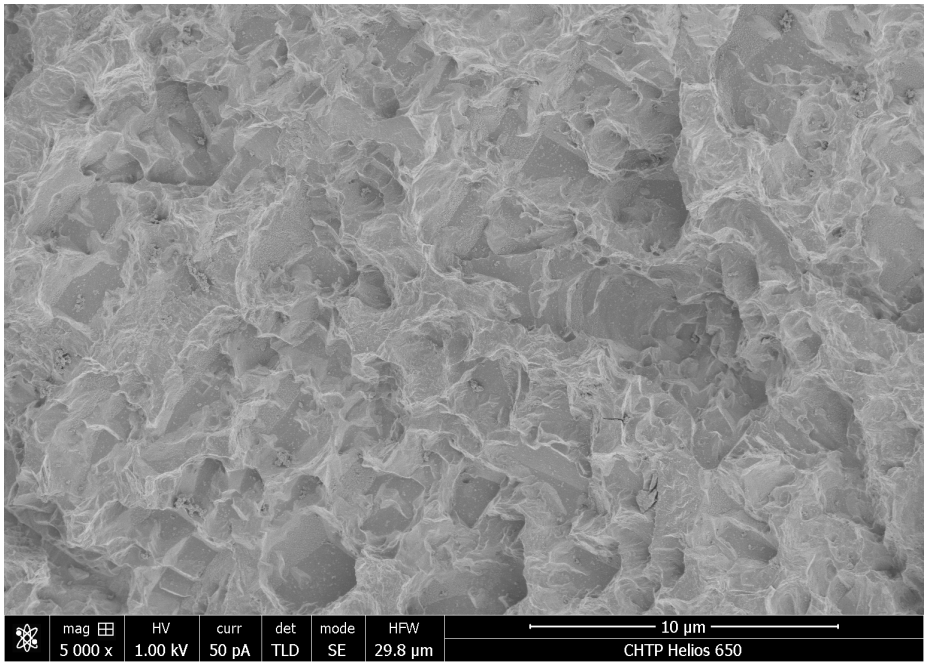

Figure 9: AFG (50% power) with saline pre-rinse (Helios Nanolab 650 Focussed Ion Beam SEM, Oregon, USA).

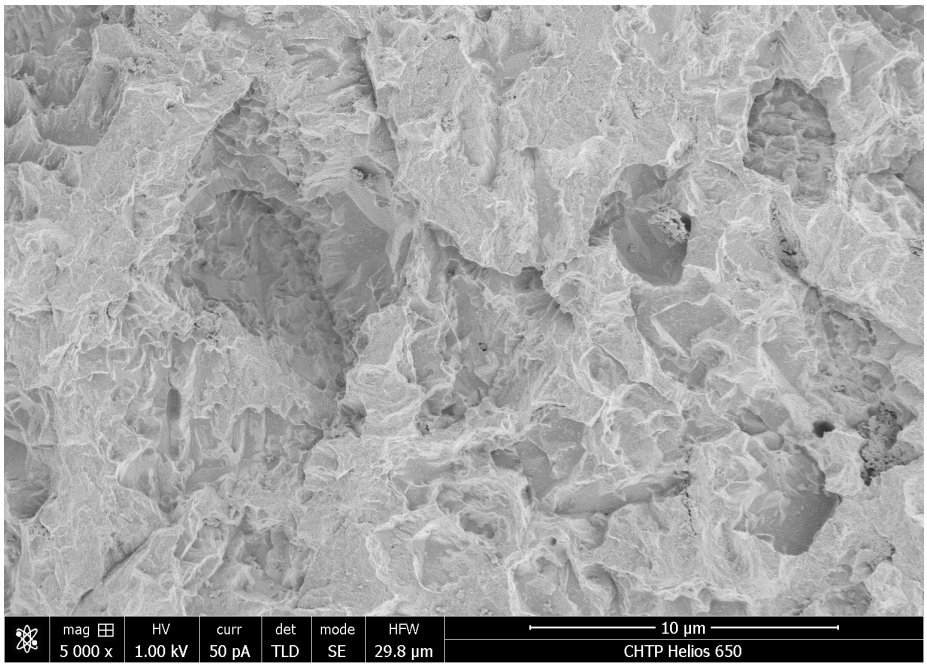

Figure 10: AFG (50% power) with no pre-rinse (Helios Nanolab 650 Focussed Ion Beam SEM, Oregon, USA).

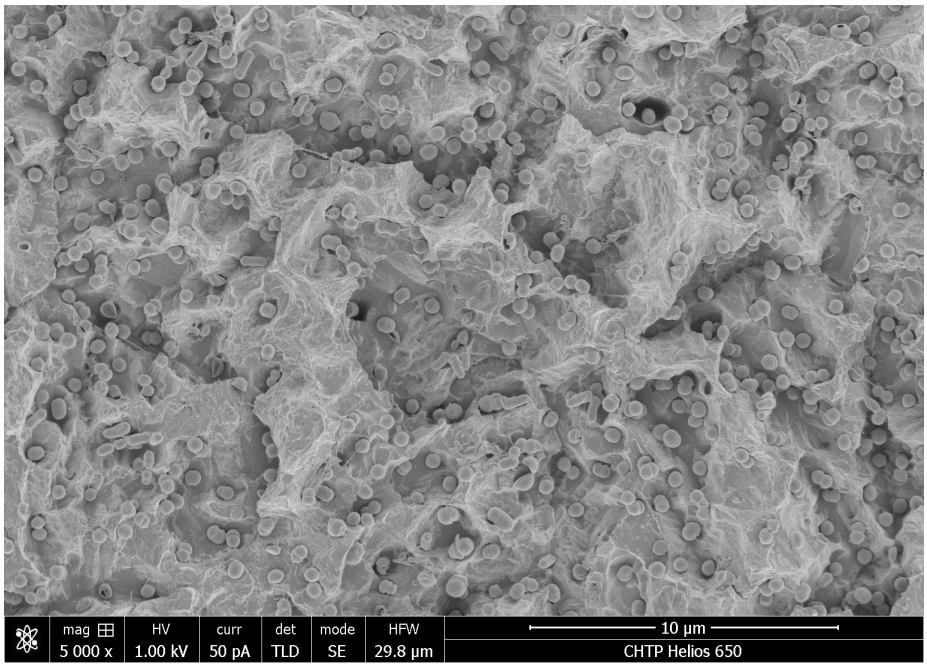

Figure 11: Airflow with no glycine powder with saline pre-rinse (Helios Nanolab 650 Focussed Ion Beam SEM, Oregon, USA).

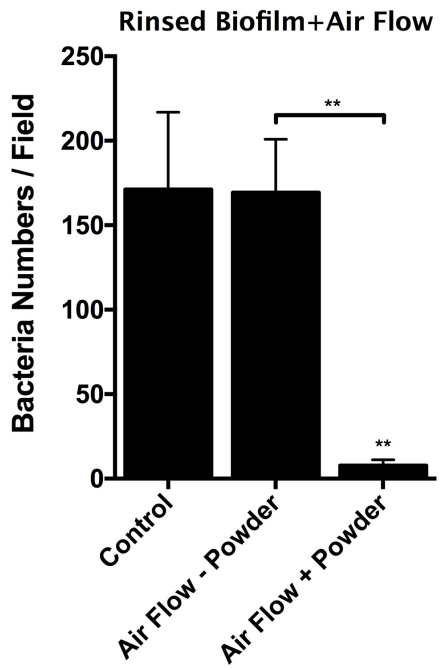

Figure 12: Compared to double rinse control and airflow without powder, AFG shows statistically significant reduction in bacterial cells ($p < 0.01$)

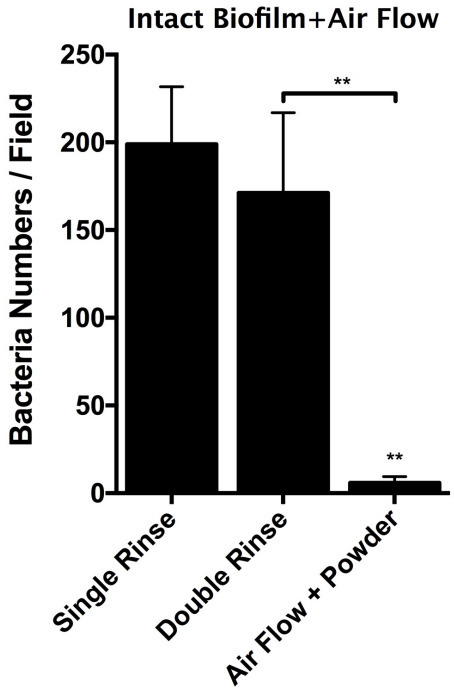

Figure 13: Compared to single rinse and double rinse, AFG shows statistically significant reduction of bacterial cells on SLA surface ($p < 0.01$).

5.3 Experiment 2

Different distances and power settings were tested in the second experiment. The variable settings for distance were 3 mm and 6 mm at 50 % power settings. A lower power setting, 25 % was selected at a distance of 3 mm.

While all test groups showed a statistically significant reduction of bacterial cells compared to the control ($P < 0.001$), there was no statistically significant difference between the results of AFG at different settings or distances (Figure 19).

At a setting of 50 % and 3 mm away from the sample, the mean remaining bacterial count per field was 31.4 cells (Figure 14). Increasing the distance to 6 mm but without changing the power setting resulted in a mean bacterial count per field of 39.0 cells (Figure 15). When the power was set to 25% at a distance of 3 mm, the remaining mean bacterial count per field was 29.4 cells (Figure 16).

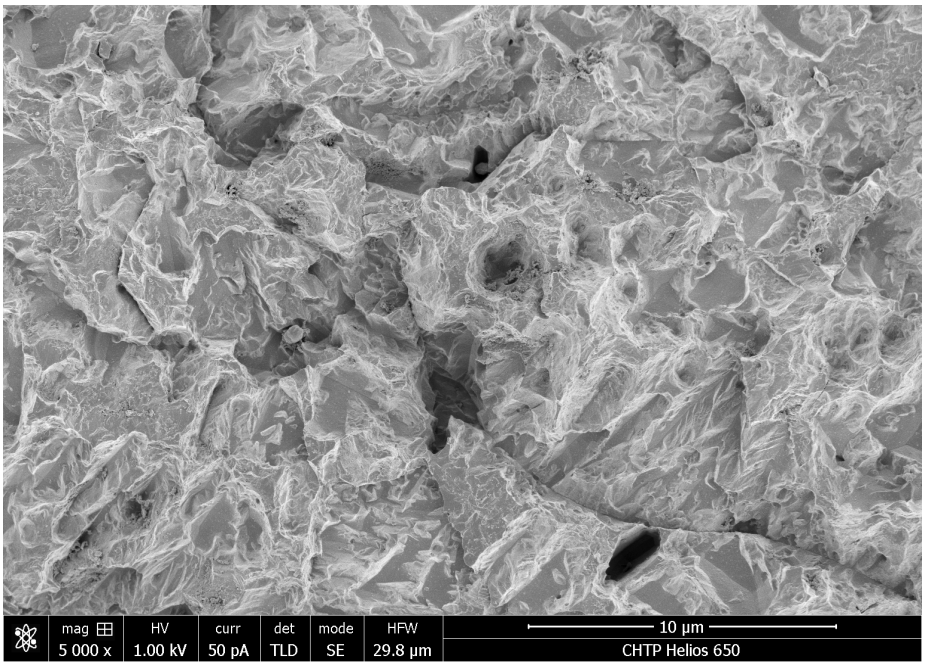

Figure 14: AFG (50% power) at distance of 3 mm from surface (Helios Nanolab 650 Focussed Ion Beam SEM, Oregon, USA).

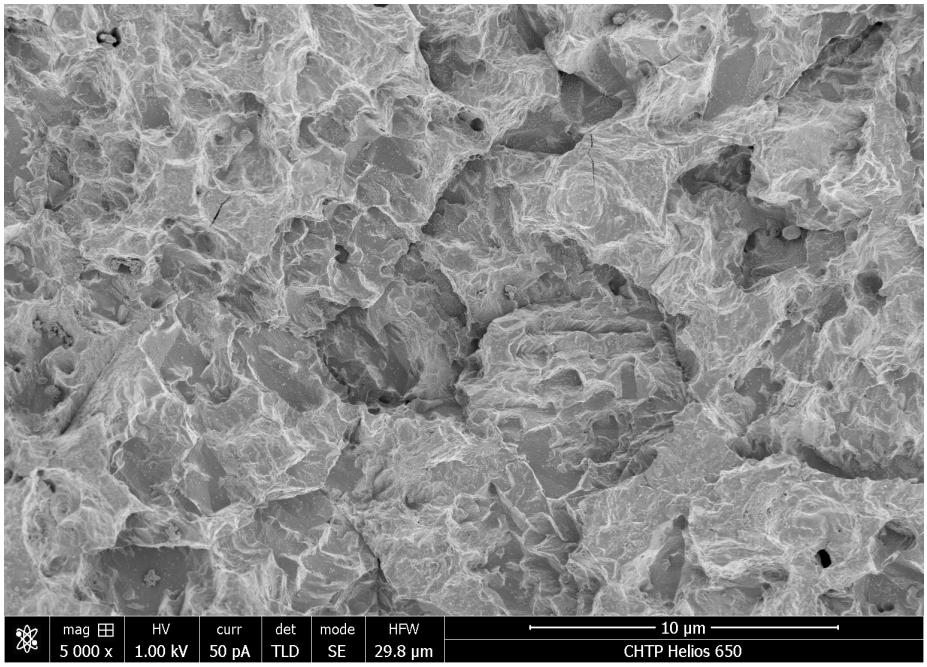

Figure 15: AFG (50% power) at a distance of 6 mm from surface (Helios Nanolab 650 Focussed Ion Beam SEM, Oregon, USA).

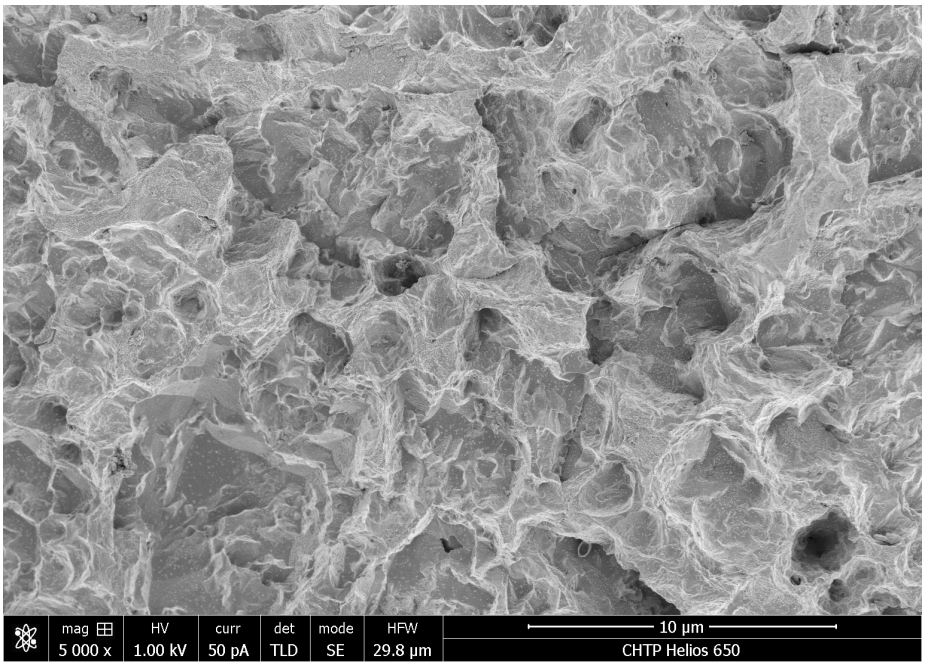

Figure 16: AFG (25% power) at distance of 3 mm from surface (Helios Nanolab 650 Focussed Ion Beam SEM, Oregon, USA).

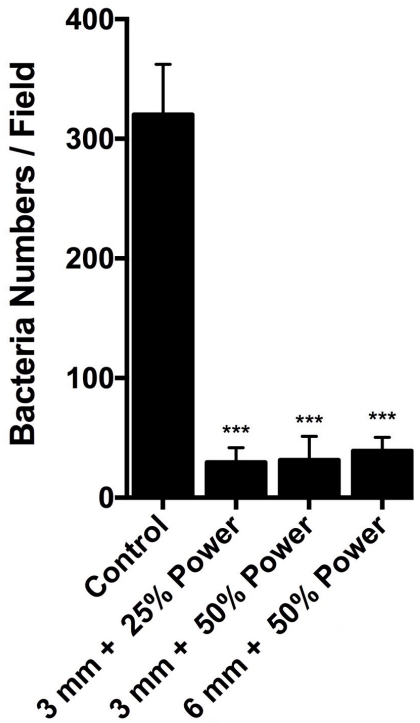

Figure 17: Different power settings and distances showed similar efficiency in bacterial removal from the SLA surface.

Chapter 6: Discussion

With the upsurge in prevalence of peri-implant diseases, their management and treatment are becoming an increasingly important topic in periodontology[24, 66]. While in a majority of cases, the most predictable course of treatment is the removal of the implant, most clinicians and patients might agree that this aggressive approach is best as the last option. Indeed, most patients wish to attempt surgical therapy to save their ailing implant(s). Treatment of peri-implantitis is particularly complicated due to the presence of bone loss and subsequent exposure of the implant micro- and macro-structure to bacterial colonization. The tenacious presence of the biofilm on the implant surface prevents the resolution of inflammation and the possibility of re-osseointegration[112]. As such, better modalities for implant decontamination are required to achieve more predictable outcomes of treatment. Modalities that have been tested thus far using the same biofilm model as presented in our study have shown limited improvements in implant surface decontamination. Commonly utilized chemical treatments such as CHX, tetracycline paste and citric acid have shown no better results than the control saline rinsing[10]. While LPRF showed excellent results *in vitro*, its use *in vivo* may be limited[141]. AFG may be an efficacious method for implant surface decontamination[13, 121].

At the time of writing this thesis, we are not aware of other studies that have assessed the efficacy of AFG on the decontamination of a mature biofilm from a rough titanium implant surface.

6.1 The mature biofilm

The biofilm model presented in this study provides a sophisticated model of a true biofilm that may be found in peri-implant mucositis or peri-implantitis lesions[10, 140]. Grown from an oral biofilm for a period of three weeks at body temperature and in anaerobic conditions, a subgingival multispecies microbial composition is favoured thereby mimicking the situation *in vivo*. Further studies using different biofilm models may be warranted, however. Indeed, it may be beneficial to culture a biofilm on a moving surface and / or using variable temperature settings to better replicate the oral environment *in vivo*.

Previous studies have detailed the morphology and thickness of the mature, multispecies biofilm grown[10, 142]. In this study, a single healthy donor provided the sample from which the oral biofilm was grown. Whether or not a biofilm collected from a single donor is just as representative as that collected from multiple donors was assessed in an earlier study[140]. It was found that following the incubation period of three weeks, mature biofilms grown from various sources were similar to that which was taken from a sample of oral biofilm from the single healthy donor[140]. While this method provides us with a mature, multispecies, subgingival biofilm, it may be beneficial to consider sampling bacteria from a peri-implant mucositis or peri-implantitis site to better replicate the peri-implant diseased biofilm.

6.2 Results and limitations of study

The use of AFG in biofilm decontamination proved to be extremely effective. The mean bacterial count after treatment with AFG and pre-rinsing was 7.7 cells per field of view. Rinsing prior to the AFG made little to no difference in the effectiveness of AFG in decontamination of

the implant surface. The mean bacterial count after treatment with AFG but no pre-rinse was 5.9 cells per field of view. By comparison, the double-rinsed control resulted in a mean bacterial count per field of 171.1 cells.

A question that arose during experimentation was if the glycine powder was a requirement for the removal of the biofilm, and whether or not the velocity at which the airflow forced sterile water in the direction of the biofilm was sufficient to completely disrupt the adhesion between the bacteria and the implant surface. The airflow device was therefore set so that the powder was turned off and the device itself was purged prior to application of airflow only to the biofilm. Airflow without glycine proved to be ineffective with respect to removing the biofilm compared to AFG. The mean bacterial count when samples were treated with airflow alone was 169.3 cells. Therefore, effectiveness between the control treatments with sterile saline rinsing and airflow without glycine was not statistically significant. Indeed, it was evident that glycine powder is required to remove the biofilm from the implant surface. It is difficult to reconcile the average size of glycine particle (45 μm) and that of the roughened lacunae and grooves on the surface which measured approximately 10 μm. It may be considered that glycine, being water soluble, may begin to dissolve when added to the water within the airflow device. As such, it is possible that variable, smaller sizes of glycine particles exist in the pressurized flow of air, water and powder which may access the smaller grooves of the rough surface implant. Imaging of glycine particles on SEM may have been beneficial to illustrate the size of the particles against that of the implant surface.

The number of microbes in the untreated biofilm was far too great to accurately enumerate. In all the treatment groups, the majority of the biofilm was removed. With the control groups involving single or double rinsing with sterile saline, the biofilm was disrupted, but a large number of microbial cells persisted in the roughened surface of the implant. In the control groups, these persisting bacteria appeared to coexist in aggregations as opposed to in the samples that had been treated with AFG. The continued presence of microorganisms in the pits and grooves of the implant surface following treatment was a common finding. This issue persists with respect to decontamination of rough-surface implants as the microscopic pits of the titanium surfaces are inaccessible by methods used to disinfect and thereby serve as refuges for remaining bacterial cells. However, in comparison to similar studies[10, 141] using the same biofilm model as ours, AFG proved to be much more effective in removing bacteria even from the rough pits and grooves. As stated above, compared to control groups, bacterial cells that persisted following treatment with AFG did so not in clusters, but planktonically. This showed that AFG is able to disrupt and remove the biofilm to such a degree that only single cells may persist in the usually unreachable craters of the rough-surface implant. Indeed, it would be expected that in a healthy patient the few remaining planktonic bacterial cells, once removed from the sessile state by AFG, should be readily eradicated by host immune cells. In addition, because glycine has an inhibitory effect on bacterial growth[135, 137], its presence following treatment may result in a sustained antibacterial effect on the implant surface[133].

The morphology of the persisting bacterial cells is also of interest. In the untreated biofilm, it is evident that a multispecies, stratified biofilm is present, representative of what might be expected in peri-implant disease[51]. Morphologies that are visible include filamentous

rods, spirochetes and coccoid cells organized in layers in a thick, 3-dimensional structure. Following the removal of a majority of the biofilm with sterile saline rinsing as was done in the single- and double-rinsed control groups, only rods and cocci remain. In AFG-treated samples, only cocci bacteria remain. These bacteria appear to have the same morphology as the early colonizers, suggesting that AFG is successful in removing the majority of the biofilm, including many of the base layer of microbes which directly attach to the implant substratum[46].

A limitation of the study is that the level at which survival bacterial cells persisted was not quantified. In SEM, only the presence of living or dead cells can be qualitatively assessed. This means that while cells can be visualized as persisting on the samples following treatment, we cannot ascertain whether or not they are still viable. As such, there is a possibility that the coccoid cells which persist may actually be nonviable cells. Methods to determine whether or not AFG was effective in killing all cells of the biofilm might be considered in future studies. Techniques such as confocal laser scanning microscopy or culturing of the discs might provide additional information regarding the full potential of AFG.

In this study, it was apparent that regardless of treatment provided, persisting cells remained and could be visible via SEM. AFG appears to have been the most effective modality in our group of studies, being capable of almost completely decontaminating the titanium surfaces. The small number of cocci cells which remaining do so in a planktonic form. Future studies may assess the viability of these persisting cells. Theoretically, in clinical situations the host immune system would be capable of and expected to eradicated any remaining planktonic cells. Especially if etiological factors were also managed prior to surgical therapy, the formation

of a new biofilm would likely be prevented. As well, there is no known threshold for biofilm removal nor does a study exist wherein a minimum number of residual bacterial cells required to re-establish the biofilm has been identified.

According to recommendations for use, our particular AFG device was directed at a 45° angle to the tooth or implant surface at a distance of 3 mm from the surface. It was also recommended that the power settings be set to approximately 50 %, although the manufacturer recommendations do instruct to begin with powder settings at a minimum before increasing steadily as required when used clinically. According to the manufacturer, the maximum dynamic pressure produced by the AirFlow Master Piezon when set to "PERIO" mode ranges from 42 – 50.8 psi. Therefore, at a power setting of 25 %, the pressure generated by the unit would range from 10.5 – 12.7 psi, and at a power setting of 50 %, the pressure generated would range from 21 – 25.4 psi. In our study, we selected distances of 3 or 6 mm to assess the effect of distance on the efficacy of biofilm removal. The ability of AFG to remove the biofilm from the implant surface was unchanged regardless of power setting or distance from the surface to the nozzle. Indeed, there was no statistically significant difference between the results of AFG under different settings. These results contradict those of Wei et al[143] who found that increasing pressure of airflow with abrasive powders would increase the efficacy of cleaning. In this particular study, ink was used to stain the surface and cleaning efficacy was measured by ink removal alone[143]. AFG was found to be relatively ineffective at removing ink at a 25 psi, but increased in efficacy at 35 psi[143]. Effects for all test groups appeared to plateau at a certain air pressure and while higher pressures appeared to result in better cleaning efficacy, more surface

modification was noted, even in the glycine – treated groups[143], an observation that was not made in our particular study.

It is not feasible *in vivo* that a distance of 3 mm can always been maintained between the AFG source and the implant surface, especially when one considers the high likelihood of obstruction of the device by adjacent teeth, restorations, implants, and/or the suprastructure of the implant. Indeed, even the depth of the peri-implant pocket would serve as a factor in increasing distance between the airflow source and the implant surface. As such, 6 mm was selected as the upper range of distance between the device and the biofilm and therefore might best replicate the distance between the implant surface and AFG *in vivo*.

In order to standardize the distance between the AFG nozzle and the implant surface, a probe was attached to the side of the nozzle and extended from its tip. Therefore, as the AFG was activated, some of the periphery of the glycine and water slurry may have been somewhat blocked and therefore would not have contacted the sample at the same velocity as it would if unobstructed. As such, in the second experiment, the effectiveness of AFG might have even been underestimated due to the interruption of AFG to the implant surface. Indeed, to have more accurately test the variable distances between AFG source and the titanium surface, a mount could be used to situate the AFG nozzle at variable heights. Instead of being handheld, the AFG could therefore be operated from a fixed position and the biofilm-contaminated implant surface could be held below the nozzle and moved back and forth. This may have allowed for an uninhibited treatment of the biofilm.

Another limitation of this study was that the biocompatibility of the surface and the healing properties, if any, of glycine were not assessed. Studies have assessed the use of biocompatible, osteoconductive powders with air-polishing on contaminated titanium surfaces and have found promising results with respect to cell differentiation on treated surfaces[144]. Future directions in our study might be to include the assessment of fibroblast and / or osteoblast cell attachment, growth and differentiation when seeded onto AFG-treated implant surfaces.

The intrasurgical use of AFG is not yet been approved by the Federal Food and Drug Administration or Health Canada. Indeed, the risk of air emphysema if used surgically exists[121, 139]. However, based on the premise of this study, in order to adequately remove the biofilm from the implant surface using AFG, the clinician may be required to gain direct access to the implant surface to avoid obstruction of the implant surface. Indeed, in order for AFG to have much effect, it appears that surgical access to subgingival, suprabony implant surfaces would be required. The macrostructure of the implant also presents a challenge with respect to access. Due to the threading on dental implants, the pitches of the implants may remain inaccessible for the most part should the AFG be directed from coronal to apical as would be the case in a nonsurgical approach. However, it would be reasonable to assume that in order to achieve effective decontamination of the implant *in vivo*, the clinician may be required to angulate the AFG nozzle above and under the threads in order to access the entirety of the pitches. As such, for purposes of managing and treating peri-implantitis, it may be required that AFG be used intrasurgically.

With respect to the manner by which AFG actually decontaminates the implant surface, it is unlikely that the antimicrobial properties of glycine, which a number of studies have alluded to[133, 135, 137], play much of a role in eradicating the biofilm. Based on the short duration of the AFG, it is unlikely that there is the exposure time of the biofilm to the glycine is long enough for the antimicrobial effects to take place. Instead, it is more likely that the abrasive qualities of the powder itself serve to physically remove the biofilm as opposed to chemically.

Chapter 7: Conclusions and Future Directions

7.1 Conclusions

The use of airflow with glycine is significantly more effective in the removal of a mature, multispecies biofilm from a roughened titanium surface than control treatments with sterile saline rinsing alone.

The use of AFG on a biofilm-contaminated implant disk was significantly more effective in the removal of the biofilm than airflow alone, without glycine. Therefore, the addition of glycine powder is required to remove the biofilm.

Variable power settings of the AFG unit and distances between the AFG nozzle and the biofilm made no significant difference in the effectiveness of AFG.

7.2 Future directions

In this study, SLA® implant surfaces were assessed, but it would be of interest to investigate the potential of AFG in the decontamination of other commonly used titanium implant surfaces which have been roughened or treated in alternate ways. As previously discussed, an investigation of the viability of remaining bacteria could also be completed. In future studies of the post-treatment effects of AFG, research should also be done to assess if the presence of residual glycine on the implant surfaces may have a sustained bacteriostatic effect. The application of a locally delivered antibiotic following AFG may also be assessed due to a putative additive effect between glycine and antibiotics. As well, the biocompatibility and

healing potential of glycine should be evaluated by assessing the degree of cell attachment and differentiation when seeded onto AFG-treated implant surfaces.

The use of AFG in implant decontamination should be assessed *in vivo* to understand how well the results of this *in vitro* study may translate into a clinical setting. If the disinfection of implant surfaces *in vivo* is as effective as the results of this *in vitro* study, the risks of potential complications, such as air-emphysema, should be further investigated. As well, the potential for re-osseointegration following complete decontamination after AFG should be assessed in more randomized controlled clinical trials.

Bibliography

1. Daubert DM, Weinstein BF, Bordin S, Leroux BG, Flemming TF. Prevalence and predictive factors for peri-implant disease and implant failure: a cross-sectional analysis. *J Periodontol* 2015;86:337-347.
2. Moraschini V, Poubel LA, Ferreira VF, Barboza Edos S. Evaluation of survival and success rates of dental implants reported in longitudinal studies with a follow-up period of at least 10 years: a systematic review. *Int J Oral Maxillofac Surg* 2015;44:377-388.
3. Carr AB, Revuru VS, Lohse CM. Association of Systemic Conditions with Dental Implant Failures in 6,384 Patients During a 31-Year Follow-up Period. *The International Journal of Oral & Maxillofacial Implants* 2017;32:1153-1161.
4. Heitz-Mayfield LJ. Peri-implant diseases: diagnosis and risk indicators. *J Clin Periodontol* 2008;35:292-304.
5. John G, Becker J, Schwarz F. Modified implant surface with slower and less initial biofilm formation. *Clin Implant Dent Relat Res* 2015;17:461-468.
6. De Bruyn H, Christiaens V, Doornewaard R, et al. Implant surface roughness and patient factors on long-term peri-implant bone loss. *Periodontology 2000* 2017;73:218-227.
7. Subramani K, Jung RE, Molenberg A, Hammerle CH. Biofilm on dental implants: a review of the literature. *The International journal of oral & maxillofacial implants* 2009;24:616-626.
8. Teughels W, Van Assche N, Sliepen I, Quirynen M. Effect of material characteristics and/or surface topography on biofilm development. *Clin Oral Implants Res* 2006;17 Suppl 2:68-81.
9. Busscher HJ, Rinastiti M, Siswomihardjo W, van der Mei HC. Biofilm formation on dental restorative and implant materials. *J Dent Res* 2010;89:657-665.
10. Dostie S, Alkadi LT, Owen G, et al. Chemotherapeutic decontamination of dental implants colonized by mature multispecies oral biofilm. *J Clin Periodontol* 2017;44:403-409.
11. Graumann SJ, Sensat ML, Stoltenberg JL. Air polishing: a review of current literature. *J Dent Hyg* 2013;87:173-180.
12. Petersilka GJ. Subgingival air-polishing in the treatment of periodontal biofilm infections. *Periodontology 2000* 2011;55:124-142.
13. Schwarz F, Becker K, Renvert S. Efficacy of air polishing for the non-surgical treatment of peri-implant diseases: a systematic review. *J Clin Periodontol* 2015;42:951-959.
14. Tastepe CS, van Waas R, Liu Y, Wismeijer D. Air powder abrasive treatment as an implant surface cleaning method: a literature review. *The International Journal of Oral & Maxillofacial Implants* 2012;27:1461-1473.
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. *The International Journal of Oral & Maxillofacial Implants* 1986;1:11-25.
16. Manor Y, Oubaid S, Mardinger O, Chaushu G, Nissan J. Characteristics of early versus late implant failure: a retrospective study. *J Oral Maxillofac Surg* 2009;67:2649-2652.
17. Lindhe J, Meyle J, Group DoEWoP. Peri-implant diseases: Consensus Report of the Sixth European Workshop on Periodontology. *J Clin Periodontol* 2008;35:282-285.

18. Mombelli A, Lang NP. The diagnosis and treatment of peri-implantitis. *Periodontology 2000* 1998;17:63-76.
19. Peri-implant mucositis and peri-implantitis: a current understanding of their diagnoses and clinical implications. *J Periodontol* 2013;84:436-443.
20. Lee CT, Huang YW, Zhu L, Weltman R. Prevalences of peri-implantitis and peri-implant mucositis: systematic review and meta-analysis. *J Dent* 2017;62:1-12.
21. Zitzmann NU, Berglundh T. Definition and prevalence of peri-implant diseases. *J Clin Periodontol* 2008;35:286-291.
22. Renvert S, Lindahl C, Persson GR. Occurrence of cases with peri-implant mucositis or peri-implantitis in a 21-26 years follow-up study. *J Clin Periodontol* 2018;45:233-240.
23. Sanz M, Chapple IL, Working Group 4 of the VEWoP. Clinical research on peri-implant diseases: consensus report of Working Group 4. *J Clin Periodontol* 2012;39 Suppl 12:202-206.
24. Derks J, Tomasi C. Peri-implant health and disease. A systematic review of current epidemiology. *J Clin Periodontol* 2015;42 Suppl 16:S158-171.
25. Papathanasiou E, Finkelman M, Hanley J, Parashis AO. Prevalence, Etiology and Treatment of Peri-Implant Mucositis and Peri-Implantitis: A Survey of Periodontists in the United States. *J Periodontol* 2016;87:493-501.
26. Lang NP, Berglundh T, Working Group 4 of Seventh European Workshop on P. Periimplant diseases: where are we now?--Consensus of the Seventh European Workshop on Periodontology. *J Clin Periodontol* 2011;38 Suppl 11:178-181.
27. Stacchi C, Berton F, Perinetti G, et al. Risk Factors for Peri-Implantitis: Effect of History of Periodontal Disease and Smoking Habits. A Systematic Review and Meta-Analysis. *J Oral Maxillofac Res* 2016;7:e3.
28. Bevilacqua L, Milan A, Del Lupo V, Maglione M, Dolzani L. Biofilms Developed on Dental Implant Titanium Surfaces with Different Roughness: Comparison Between In Vitro and In Vivo Studies. *Curr Microbiol* 2018; 75:766-772.
29. Jepsen S, Berglundh T, Genco R, et al. Primary prevention of peri-implantitis: managing peri-implant mucositis. *J Clin Periodontol* 2015;42 Suppl 16:S152-157.
30. Schwarz F, Sculean A, Engebretson SP, Becker J, Sager M. Animal models for peri-implant mucositis and peri-implantitis. *Periodontology 2000* 2015;68:168-181.
31. Abrahamsson I, Berglundh T, Lindhe J. Soft tissue response to plaque formation at different implant systems. A comparative study in the dog. *Clin Oral Implants Res* 1998;9:73-79.
32. Ericsson I, Berglundh T, Marinello C, Liljenberg B, Lindhe J. Long-standing plaque and gingivitis at implants and teeth in the dog. *Clin Oral Implants Res* 1992;3:99-103.
33. Loe H, Theilade E, Jensen SB. Experimental Gingivitis in Man. *J Periodontol* 1965;36:177-187.
34. Pontoriero R, Tonelli MP, Carnevale G, Mombelli A, Nyman SR, Lang NP. Experimentally induced peri-implant mucositis. A clinical study in humans. *Clin Oral Implants Res* 1994;5:254-259.
35. Meyer S, Giannopoulou C, Courvoisier D, Schimmel M, Muller F, Mombelli A. Experimental mucositis and experimental gingivitis in persons aged 70 or over. Clinical and biological responses. *Clin Oral Implants Res* 2017;28:1005-1012.

36. Salvi GE, Aglietta M, Eick S, Sculean A, Lang NP, Ramseier CA. Reversibility of experimental peri-implant mucositis compared with experimental gingivitis in humans. *Clin Oral Implants Res* 2012;23:182-190.
37. Haffajee AD, Socransky SS. Introduction to microbial aspects of periodontal biofilm communities, development and treatment. *Periodontology 2000* 2006;42:7-12.
38. Hojo K, Nagaoka S, Ohshima T, Maeda N. Bacterial interactions in dental biofilm development. *J Dent Res* 2009;88:982-990.
39. Marsh PD. Are dental diseases examples of ecological catastrophes? *Microbiology* 2003;149:279-294.
40. Marsh PD. Dental plaque as a biofilm and a microbial community - implications for health and disease. *BMC Oral Health* 2006;6 Suppl 1:S14.
41. Kolenbrander PE, Palmer RJ, Jr., Rickard AH, Jakubovics NS, Chalmers NI, Diaz PI. Bacterial interactions and successions during plaque development. *Periodontology 2000* 2006;42:47-79.
42. Pokrowiecki R, Mielczarek A, Zareba T, Tyski S. Oral microbiome and peri-implant diseases: where are we now? *Ther Clin Risk Manag* 2017;13:1529-1542.
43. Gibbins HL, Yakubov GE, Proctor GB, Wilson S, Carpenter GH. What interactions drive the salivary mucosal pellicle formation? *Colloids Surf B Biointerfaces* 2014;120:184-192.
44. Maruyama N, Maruyama F, Takeuchi Y, Aikawa C, Izumi Y, Nakagawa I. Intraindividual variation in core microbiota in peri-implantitis and periodontitis. *Sci Rep* 2014;4:6602.
45. Lima EM, Koo H, Vacca Smith AM, Rosalen PL, Del Bel Cury AA. Adsorption of salivary and serum proteins, and bacterial adherence on titanium and zirconia ceramic surfaces. *Clin Oral Implants Res* 2008;19:780-785.
46. Teles FR, Teles RP, Uzel NG, et al. Early microbial succession in redeveloping dental biofilms in periodontal health and disease. *J Periodontal Res* 2012;47:95-104.
47. Charalampakis G, Belibasakis GN. Microbiome of peri-implant infections: lessons from conventional, molecular and metagenomic analyses. *Virulence* 2015;6:183-187.
48. Quirynen M, Vogels R, Peeters W, van Steenberghe D, Naert I, Haffajee A. Dynamics of initial subgingival colonization of 'pristine' peri-implant pockets. *Clin Oral Implants Res* 2006;17:25-37.
49. Diaz PI, Chalmers NI, Rickard AH, et al. Molecular characterization of subject-specific oral microflora during initial colonization of enamel. *Appl Environ Microbiol* 2006;72:2837-2848.
50. Mombelli A, Marxer M, Gaberthuel T, Grunder U, Lang NP. The microbiota of osseointegrated implants in patients with a history of periodontal disease. *J Clin Periodontol* 1995;22:124-130.
51. Sanz-Martin I, Doolittle-Hall J, Teles RP, et al. Exploring the microbiome of healthy and diseased peri-implant sites using Illumina sequencing. *J Clin Periodontol* 2017;44:1274-1284.
52. Quirynen M, Vogels R, Pauwels M, et al. Initial subgingival colonization of 'pristine' pockets. *J Dent Res* 2005;84:340-344.
53. Elter C, Heuer W, Demling A, et al. Supra- and subgingival biofilm formation on implant abutments with different surface characteristics. *The International Journal of Oral & Maxillofacial Implants* 2008;23:327-334.

54. Darveau RP, Hajishengallis G, Curtis MA. Porphyromonas gingivalis as a potential community activist for disease. *J Dent Res* 2012;91:816-820.
55. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL, Jr. Microbial complexes in subgingival plaque. *J Clin Periodontol* 1998;25:134-144.
56. Socransky SS, Haffajee AD. Periodontal microbial ecology. *Periodontology 2000* 2005;38:135-187.
57. Hajishengallis G, Darveau RP, Curtis MA. The keystone-pathogen hypothesis. *Nat Rev Microbiol* 2012;10:717-725.
58. Mombelli A, Decaillet F. The characteristics of biofilms in peri-implant disease. *J Clin Periodontol* 2011;38 Suppl 11:203-213.
59. Lafaurie GI, Sabogal MA, Castillo DM, et al. Microbiome and Microbial Biofilm Profiles of Peri-Implantitis: A Systematic Review. *J Periodontol* 2017;88:1066-1089.
60. Tabanella G, Nowzari H, Slots J. Clinical and microbiological determinants of ailing dental implants. *Clin Implant Dent Relat Res* 2009;11:24-36.
61. Zhuang LF, Watt RM, Mattheos N, Si MS, Lai HC, Lang NP. Periodontal and peri-implant microbiota in patients with healthy and inflamed periodontal and peri-implant tissues. *Clin Oral Implants Res* 2016;27:13-21.
62. Thurnheer T, Belibasakis GN. Incorporation of staphylococci into titanium-grown biofilms: an in vitro "submucosal" biofilm model for peri-implantitis. *Clin Oral Implants Res* 2016;27:890-895.
63. Renvert S, Aghazadeh A, Hallstrom H, Persson GR. Factors related to peri-implantitis - a retrospective study. *Clin Oral Implants Res* 2014;25:522-529.
64. Genco RJ. Current View of Risk Factors for Periodontal Diseases. *J Periodontol* 1996;67 Suppl 10S:1041-1049.
65. Sanz M, Chapple IL. Clinical research on peri-implant diseases: consensus report of Working Group 4. *J Clin Periodontol* 2012;39 Suppl 12:202-206.
66. Schwarz F, Derks J, Monje A, Wang HL. Peri-implantitis. *J Clin Periodontol* 2018;45 Suppl 20:S246-S266.
67. Roos-Jansaker AM, Renvert H, Lindahl C, Renvert S. Nine- to fourteen-year follow-up of implant treatment. Part III: factors associated with peri-implant lesions. *J Clin Periodontol* 2006;33:296-301.
68. Rinke S, Ohl S, Ziebolz D, Lange K, Eickholz P. Prevalence of periimplant disease in partially edentulous patients: a practice-based cross-sectional study. *Clin Oral Implants Res* 2011;22:826-833.
69. Berglundh T, Armitage G, Araujo MG, et al. Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Clin Periodontol* 2018;45 Suppl 20:S286-S291.
70. Schwarz F, Becker K, Sahm N, Horstkemper T, Rousi K, Becker J. The prevalence of peri-implant diseases for two-piece implants with an internal tube-in-tube connection: a cross-sectional analysis of 512 implants. *Clin Oral Implants Res* 2017;28:24-28.
71. Costa FO, Takenaka-Martinez S, Cota LO, Ferreira SD, Silva GL, Costa JE. Peri-implant disease in subjects with and without preventive maintenance: a 5-year follow-up. *J Clin Periodontol* 2012;39:173-181.

72. Matarasso S, Rasperini G, Iorio Siciliano V, Salvi GE, Lang NP, Aglietta M. A 10-year retrospective analysis of radiographic bone-level changes of implants supporting single-unit crowns in periodontally compromised vs. periodontally healthy patients. *Clin Oral Implants Res* 2010;21:898-903.
73. Roccuzzo M, De Angelis N, Bonino L, Aglietta M. Ten-year results of a three-arm prospective cohort study on implants in periodontally compromised patients. Part 1: implant loss and radiographic bone loss. *Clin Oral Implants Res* 2010;21:490-496.
74. Swierkot K, Lottholz P, Flores-de-Jacoby L, Mengel R. Mucositis, peri-implantitis, implant success, and survival of implants in patients with treated generalized aggressive periodontitis: 3- to 16-year results of a prospective long-term cohort study. *J Periodontol* 2012;83:1213-1225.
75. Berglundh T, Gotfredsen K, Zitzmann NU, Lang NP, Lindhe J. Spontaneous progression of ligature induced peri-implantitis at implants with different surface roughness: an experimental study in dogs. *Clin Oral Implants Res* 2007;18:655-661.
76. Albouy JP, Abrahamsson I, Berglundh T. Spontaneous progression of experimental peri-implantitis at implants with different surface characteristics: an experimental study in dogs. *J Clin Periodontol* 2012;39:182-187.
77. Tomar SL, Asma S. Smoking-Attributable Periodontitis in the United States: Findings From NHANES III. *J Periodontol* 2000;71:743-751.
78. Tomar SL, Asma S. Smoking-attributable periodontitis in the United States: findings from NHANES III. National Health and Nutrition Examination Survey. *J Periodontol* 2000;71:743-751.
79. Mirbod SM, Ahing SI, Pruthi VK. Immunohistochemical study of vestibular gingival blood vessel density and internal circumference in smokers and non-smokers. *J Periodontol* 2001;72:1318-1323.
80. Numabe Y, Ogawa T, Kamoi H, et al. Phagocytic function of salivary PMN after smoking or secondary smoking. *Ann Periodontol* 1998;3:102-107.
81. Ryder MI, Fujitaki R, Johnson G, Hyun W. Alterations of neutrophil oxidative burst by in vitro smoke exposure: implications for oral and systemic diseases. *Ann Periodontol* 1998;3:76-87.
82. Benatti BB, Cesar-Neto JB, Goncalves PF, Sallum EA, Nociti FH, Jr. Smoking affects the self-healing capacity of periodontal tissues. A histological study in the rat. *Eur J Oral Sci* 2005;113:400-403.
83. Esfahrood ZR, Zamanian A, Torshabi M, Abrishami M. The effect of nicotine and cotinine on human gingival fibroblasts attachment to root surfaces. *J Basic Clin Physiol Pharmacol* 2015;26:517-522.
84. Cesar-Neto JB, Benatti BB, Sallum EA, Casati MZ, Nociti FH, Jr. The influence of cigarette smoke inhalation and its cessation on the tooth-supporting alveolar bone: a histometric study in rats. *J Periodontal Res* 2006;41:118-123.
85. Gurlek O, Gumus P, Buduneli N. Smokers have a higher risk of inflammatory peri-implant disease than non-smokers. *Oral Dis* 2018;24:30-32.
86. SS AL, BinShabaib MS, Ahmed HB, Mehmood A, Khan J, Javed F. Comparison of Peri-implant Clinical and Radiographic Inflammatory Parameters Among Cigarette and Waterpipe (Narghile) Smokers and Never-smokers. *J Periodontol* 2017:1-10.

87. Pimentel SP, Shiota R, Cirano FR, et al. Occurrence of peri-implant diseases and risk indicators at the patient and implant levels: A multi-level cross-sectional study. *J Periodontol* 2018;15 [epub ahead of print].
88. Renvert S, Lindahl C, Persson GR. Occurrence of cases with peri-implant mucositis or peri-implantitis in a 21-26 years follow-up study. *J Clin Periodontol* 2017;45:233-240.
89. Grossi SG, Genco RJ. Periodontal disease and diabetes mellitus: a two-way relationship. *Ann Periodontol* 1998;3:51-61.
90. Chapple IL, Genco R, working group 2 of the joint EFPAAPw. Diabetes and periodontal diseases: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. *J Periodontol* 2013;84:S106-112.
91. Ferreira SD, Silva GL, Cortelli JR, Costa JE, Costa FO. Prevalence and risk variables for peri-implant disease in Brazilian subjects. *J Clin Periodontol* 2006;33:929-935.
92. Gould TR, Westbury L, Brunette DM. Ultrastructural study of the attachment of human gingiva to titanium in vivo. *J Prosthet Dent* 1984;52:418-420.
93. Larjava H, Koivisto L, Hakkinen L, Heino J. Epithelial integrins with special reference to oral epithelia. *J Dent Res* 2011;90:1367-1376.
94. Fujiseki M, Matsuzaka K, Yoshinari M, Shimono M, Inoue T. An experimental study on the features of peri-implant epithelium: immunohistochemical and electron-microscopic observations. *Bull Tokyo Dent Coll* 2003;44:185-199.
95. Berglundh T, Lindhe J, Ericsson I, Marinello CP, Liljenberg B, Thomsen P. The soft tissue barrier at implants and teeth. *Clin Oral Implants Res* 1991;2:81-90.
96. Bartold PM, Van Dyke TE. Periodontitis: a host-mediated disruption of microbial homeostasis. Unlearning learned concepts. *Periodontology 2000* 2013;62:203-217.
97. Page RC, Schroeder HE. Pathogenesis of inflammatory periodontal disease. A summary of current work. *Lab Invest* 1976;34:235-249.
98. Ohlrich EJ, Cullinan MP, Seymour GJ. The immunopathogenesis of periodontal disease. *Australian dental journal* 2009;54 Suppl 1:S2-10.
99. Thorbert-Mros S, Larsson L, Berglundh T. Cellular composition of long-standing gingivitis and periodontitis lesions. *J Periodontal Res* 2015;50:535-543.
100. Garlet GP. Destructive and protective roles of cytokines in periodontitis: a re-appraisal from host defense and tissue destruction viewpoints. *J Dent Res* 2010;89:1349-1363.
101. Zitzmann NU, Berglundh T, Marinello CP, Lindhe J. Experimental peri-implant mucositis in man. *J Clin Periodontol* 2001;28:517-523.
102. Lang NP, Bosshardt DD, Lulic M. Do mucositis lesions around implants differ from gingivitis lesions around teeth? *J Clin Periodontol* 2011;38 Suppl 11:182-187.
103. Ata-Ali J, Flichy-Fernandez AJ, Ata-Ali F, Penarrocha-Diago M, Penarrocha-Diago M. Clinical, microbiologic, and host response characteristics in patients with peri-implant mucositis. *The International Journal of Oral & Maxillofacial Implants* 2013;28:883-890.
104. Tonetti MS, Gerber L, Lang NP. Vascular adhesion molecules and initial development of inflammation in clinically healthy human keratinized mucosa around teeth and osseointegrated implants. *J Periodontal Res* 1994;29:386-392.
105. Berglundh T, Zitzmann NU, Donati M. Are peri-implantitis lesions different from periodontitis lesions? *J Clin Periodontol* 2011;38 Suppl 11:188-202.
106. Hiyari S, Wong RL, Yaghsezian A, et al. Ligature-induced peri-implantitis and periodontitis in mice. *J Clin Periodontol* 2018;45:89-99.

107. Salvi GE, Cosgarea R, Sculean A. Prevalence and Mechanisms of Peri-implant Diseases. *J Dent Res* 2017;96:31-37.
108. Renvert S, Polyzois IN. Clinical approaches to treat peri-implant mucositis and peri-implantitis. *Periodontology 2000* 2015;68:369-404.
109. Renvert S, Samuelsson E, Lindahl C, Persson GR. Mechanical non-surgical treatment of peri-implantitis: a double-blind randomized longitudinal clinical study. I: clinical results. *J Clin Periodontol* 2009;36:604-609.
110. Renvert S, Lessem J, Dahlen G, Renvert H, Lindahl C. Mechanical and repeated antimicrobial therapy using a local drug delivery system in the treatment of peri-implantitis: a randomized clinical trial. *J Periodontol* 2008;79:836-844.
111. Persson LG, Mouhyi J, Berglundh T, Sennerby L, Lindhe J. Carbon dioxide laser and hydrogen peroxide conditioning in the treatment of periimplantitis: an experimental study in the dog. *Clin Implant Dent Relat Res* 2004;6:230-238.
112. Renvert S, Polyzois I, Maguire R. Re-osseointegration on previously contaminated surfaces: a systematic review. *Clin Oral Implants Res* 2009;20 Suppl 4:216-227.
113. Froum SJ, Froum SH, Rosen PS. A Regenerative Approach to the Successful Treatment of Peri-implantitis: A Consecutive Series of 170 Implants in 100 Patients with 2- to 10-Year Follow-up. *The International Journal of Periodontics & Restorative Dentistry* 2015;35:857-863.
114. Schwarz F, Sahm N, Becker J. Combined surgical therapy of advanced peri-implantitis lesions with concomitant soft tissue volume augmentation. A case series. *Clin Oral Implants Res* 2014;25:132-136.
115. Schwarz F, Herten M, Sager M, Bieling K, Sculean A, Becker J. Comparison of naturally occurring and ligature-induced peri-implantitis bone defects in humans and dogs. *Clin Oral Implants Res* 2007;18:161-170.
116. Schwarz F, Sahm N, Iglhaut G, Becker J. Impact of the method of surface debridement and decontamination on the clinical outcome following combined surgical therapy of peri-implantitis: a randomized controlled clinical study. *J Clin Periodontol* 2011;38:276-284.
117. Sharon E, Shapira L, Wilensky A, Abu-Hatoum R, Smidt A. Efficiency and thermal changes during implantoplasty in relation to bur type. *Clin Implant Dent Relat Res* 2013;15:292-296.
118. Matarasso S, Iorio Siciliano V, Aglietta M, Andreuccetti G, Salvi GE. Clinical and radiographic outcomes of a combined resective and regenerative approach in the treatment of peri-implantitis: a prospective case series. *Clin Oral Implants Res* 2014;25:761-767.
119. Riben-Grundstrom C, Norderyd O, Andre U, Renvert S. Treatment of peri-implant mucositis using a glycine powder air-polishing or ultrasonic device: a randomized clinical trial. *J Clin Periodontol* 2015;42:462-469.
120. Gutmann ME. Air polishing: a comprehensive review of the literature. *J Dent Hyg* 1998;72:47-56.
121. Schwarz F, Becker K, Bastendorf KD, et al. Recommendations on the clinical application of air polishing for the management of peri-implant mucositis and peri-implantitis. *Quintessence Int* 2016;47:293-296.

122. Waerhaug J. Healing of the dento-epithelial junction following subgingival plaque control. II: As observed on extracted teeth. *J Periodontol* 1978;49:119-134.
123. Caffesse RG, Sweeney PL, Smith BA. Scaling and root planing with and without periodontal flap surgery. *J Clin Periodontol* 1986;13:205-210.
124. Flemmig TF, Hetzel M, Topoll H, Gerss J, Haeberlein I, Petersilka G. Subgingival debridement efficacy of glycine powder air polishing. *J Periodontol* 2007;78:1002-1010.
125. Petersilka GJ, Tunkel J, Barakos K, Heinecke A, Haberlein I, Flemmig TF. Subgingival plaque removal at interdental sites using a low-abrasive air polishing powder. *J Periodontol* 2003;74:307-311.
126. Flemmig TF, Arushanov D, Daubert D, Rothen M, Mueller G, Leroux BG. Randomized controlled trial assessing efficacy and safety of glycine powder air polishing in moderate-to-deep periodontal pockets. *J Periodontol* 2012;83:444-452.
127. Dennison DK, Huerzeler MB, Quinones C, Caffesse RG. Contaminated implant surfaces: an in vitro comparison of implant surface coating and treatment modalities for decontamination. *J Periodontol* 1994;65:942-948.
128. Zablotsky MH, Diedrich DL, Meffert RM. Detoxification of endotoxin-contaminated titanium and hydroxyapatite-coated surfaces utilizing various chemotherapeutic and mechanical modalities. *Implant Dent* 1992;1:154-158.
129. Augthun M, Tinschert J, Huber A. In vitro studies on the effect of cleaning methods on different implant surfaces. *J Periodontol* 1998;69:857-864.
130. Persson GR, Roos-Jansaker AM, Lindahl C, Renvert S. Microbiologic results after non-surgical erbium-doped:yttrium, aluminum, and garnet laser or air-abrasive treatment of peri-implantitis: a randomized clinical trial. *J Periodontol* 2011;82:1267-1278.
131. Buhler J, Amato M, Weiger R, Walter C. A systematic review on the effects of air polishing devices on oral tissues. *Int J Dent Hyg* 2016;14:15-28.
132. Petersilka G, Faggion CM, Jr., Stratmann U, et al. Effect of glycine powder air-polishing on the gingiva. *J Clin Periodontol* 2008;35:324-332.
133. Cochis A, Fini M, Carrassi A, Migliario M, Visai L, Rimondini L. Effect of air polishing with glycine powder on titanium abutment surfaces. *Clin Oral Implants Res* 2013;24:904-909.
134. Bulet P, Hetru C, Dimarcq JL, Hoffmann D. Antimicrobial peptides in insects; structure and function. *Dev Comp Immunol* 1999;23:329-344.
135. Holo H, Nes IF. High-Frequency Transformation, by Electroporation, of Lactococcus lactis subsp. cremoris Grown with Glycine in Osmotically Stabilized Media. *Appl Environ Microbiol* 1989;55:3119-3123.
136. Dunny GM, Lee LN, LeBlanc DJ. Improved electroporation and cloning vector system for gram-positive bacteria. *Appl Environ Microbiol* 1991;57:1194-1201.
137. Cosquer A, Ficamos M, Jebbar M, et al. Antibacterial activity of glycine betaine analogues: involvement of osmoporters. *Bioorg Med Chem Lett* 2004;14:2061-2065.
138. Cobb CM, Daubert DM, Davis K, et al. Consensus Conference Findings on Supragingival and Subgingival Air Polishing. *Compend Contin Educ Dent* 2017;38:e1-e4.
139. Bassetti M, Bassetti R, Sculean A, Salvi GE. [Subcutaneous emphysema following non-surgical peri-implantitis therapy using an air abrasive device: a case report]. *Swiss Dent J* 2014;124:807-817.

140. Bi J, Koivisto L, Pang A, et al. Suppression of alphavbeta6 Integrin Expression by Polymicrobial Oral Biofilms in Gingival Epithelial Cells. *Sci Rep* 2017;7:4411.
141. Schuldt LA. In vitro evaluation of the effects of leukocyte-platelet rich fibrin on disinfection of a rough implant surface (T) [10.14288/1.0354260]. Vancouver, British Columbia, Canada: University of British Columbia; 2017.
142. Shen Y, Qian W, Chung C, Olsen I, Haapasalo M. Evaluation of the effect of two chlorhexidine preparations on biofilm bacteria in vitro: a three-dimensional quantitative analysis. *J Endod* 2009;35:981-985.
143. Wei MCT, Tran C, Meredith N, Walsh LJ. Effectiveness of implant surface debridement using particle beams at differing air pressures. *Clin Exp Dent Res* 2017;3:148-153.
144. Tastepe CS, Lin X, Donnet M, Doulabi BZ, Wismeijer D, Liu Y. Re-establishment of Biocompatibility of the In Vitro Contaminated Titanium Surface Using Osteoconductive Powders With Air-Abrasive Treatment. *J Oral Implantol* 2018;44:94-101.