Universidade de Lisboa

Faculdade de Medicina Dentária

Bacterial adhesion and biofilm formation in implant surfaces after implantoplasty

Maria Teresa Palma Calado Osório de Castro

Orientadora: Professora Doutora Helena Francisco

Co-orientadora: Professora Doutora Joana Faria Marques

Dissertação

Mestrado Integrado em Medicina Dentária

2023

Universidade de Lisboa

Faculdade de Medicina Dentária

Bacterial adhesion and biofilm formation in implant surfaces after implantoplasty

Maria Teresa Palma Calado Osório de Castro

Orientadora: Professora Doutora Helena Francisco

Co-orientadora: Professora Doutora Joana Faria Marques

Dissertação

Mestrado Integrado em Medicina Dentária

2023

“The greatest danger for most of us is not that our aim is too high and we miss it, but that it is too low and we reach it.” - Michelangelo

ACKNOWLEDGEMENTS

À minha orientadora, Professora Doutora Helena Francisco, pela orientação cuidadosa, disponibilidade e pelo conhecimento que me transmitiu na área da Implantologia. A sua dedicação ao longo deste processo foi fundamental para o meu crescimento académico, e estou grata por ter despertado em mim o gosto por esta área.

À minha co-orientadora, Professora Doutora Joana Faria Marques, por me guiar e apoiar no desafio que foi realizar um ensaio microbiológico. A sua experiência e conhecimento foram fundamentais para a realização deste trabalho.

À Neusa Silva, por tudo o que me ensinou, sempre com carinho, paciência, boa disposição e constante disponibilidade para me ajudar.

À minha família, pelo apoio incondicional nesta jornada. Em especial, aos meus pais, por todo o trabalho árduo e sacrifícios que fizeram para tornar possível estes cinco anos de faculdade.

Aos meus queridos amigos, por me motivarem e alegrarem os meus dias.

ABSTRACT

Background: Peri-implantitis is a chronic inflammatory condition caused by the presence of microorganisms. Implantoplasty is a decontamination technique that smoothens the implant surface to prevent biofilm formation. However, there remains a lack of research examining the microbiological implications of implantoplasty.

Aim: The current *in vitro* study aimed to evaluate bacterial adhesion and biofilm formation in implant surfaces after implantoplasty.

Materials and Methods: Twenty dental implants were randomly divided into two groups: non-treated (control) and treated with implantoplasty. After performing the implantoplasty on half of the samples, both groups were incubated with *Streptococcus oralis* for 1 hour and 24 hours. Colony forming units per mL were calculated. Bacterial adhesion and titanium surface topography were visualized using SEM. Descriptive statistics were performed for all variables using statistical software SPSS (version nº29). Normality was assessed using Shapiro-Wilk test. Comparisons of continuous variables mean values between groups were performed by *t*-student or Mann-Whitney statistical tests as appropriate. The significance was set at $p<0.05$.

Results: Significantly lower numbers (~60%) of viable adhered bacteria were observed in the untreated samples compared to the treated samples at both time-points. Treated implants at 1 hour: CFU/mL = $1.79 \times 10^5 \pm 1.60 \times 10^4$; non-treated implants at 1 hour: CFU/mL = $5.29 \times 10^5 \pm 1.01 \times 10^5$; treated implants at 24 hours: CFU/mL = $2.46 \times 10^5 \pm 9.75 \times 10^4$; non-treated implants at 24 hours: CFU/mL = $6.42 \times 10^5 \pm 2.89 \times 10^5$ ($p < 0.05$, *t*-student).

Conclusion: A notable decrease in surface roughness and bacterial adhesion in implant surfaces following the implantoplasty treatment was observed. This study supports the efficacy of implantoplasty in reducing bacterial adhesion. Since this was a pilot study, further studies about the effects of implantoplasty on biofilm formation are needed to confirm these results.

Keywords: Dental Implant, Implantoplasty, Peri-implantitis, Bacterial Adhesion, Surface Roughness

RESUMO

Introdução: Os avanços na Medicina Dentária têm sido significativos, especialmente com o aparecimento da Implantologia. Este campo revolucionou os tratamentos de reabilitação protética. No entanto, a gestão das complicações relacionadas com os implantes osseointegrados continua a ser um grande desafio. Uma dessas complicações são as doenças peri-implantares, que incluem a mucosite peri-implantar e a peri-implantite. Estas doenças representam riscos significativos para a longevidade dos implantes.

Em 2017, o World Workshop organizado pela American Academy of Periodontology (AAP) e pela European Federation of Periodontology (EFP), estabeleceram uma nova classificação para as doenças e condições peri-implantares. Foram definidos quatro grupos: saúde peri-implantar, mucosite peri-implantar, peri-implantite e deficiências peri-implantares dos tecidos moles e/ou duros. Os diferentes grupos têm as suas próprias características clínicas específicas que requerem uma abordagem adequada.

A mucosite peri-implantar é uma condição reversível caracterizada pela inflamação dos tecidos que rodeiam um implante. Se não for tratada, pode progredir para peri-implantite, uma condição irreversível que envolve a inflamação da mucosa peri-implantar e também perda de osso de suporte. Estima-se que a prevalência de peri-implantite em doentes com implantes seja cerca de 20%, o que sublinha a importância de estudar metodologias de tratamento eficazes.

A presença de placa bacteriana é o fator etiológico primário destas doenças. Os agentes patogénicos presentes no biofilme produzem endotoxinas que conduzem à inflamação e subsequente lesão dos tecidos peri-implantares. Existem vários fatores de risco associados às doenças peri-implantares, incluindo história prévia de periodontite, má higiene oral, hábito tabágico e diabetes.

Além disso, o desenho do implante, a presença de elementos protéticos desadaptados e o cimento submucoso podem contribuir para a formação de biofilme e aumentar a probabilidade de desenvolver doença peri-implantar. O processo de formação de biofilme nas superfícies dos implantes é complexo; envolve a adesão da microbiota oral à superfície implantar, a colonização e a formação de uma matriz extracelular. A desorganização deste biofilme é crucial para o sucesso do tratamento das doenças peri-implantares.

A escolha da estratégia de tratamento para a peri-implantite depende da extensão dos defeitos ósseos. A terapia mecânica não cirúrgica é adequada para tratar a mucosite peri-implantar e pequenos defeitos; no entanto, defeitos maiores frequentemente requerem intervenção cirúrgica. Os tratamentos não cirúrgicos, por si só, não são muitas vezes suficientes para resolver estas complicações. Existem disponíveis diferentes abordagens cirúrgicas, incluindo cirurgia de retalho acesso, cirurgia de retalho de reposicionamento apical, ou cirurgia regenerativa dependendo das circunstâncias individuais.

Em muitos casos é efetuada uma descontaminação da superfície do implante juntamente com as abordagens cirúrgicas, visto que a peri-implantite é causada por placa bacteriana. A implantoplastia é uma técnica amplamente utilizada para este fim. O seu objetivo é remover as espiras expostas do implante e alisar a superfície de modo a reduzir a formação de biofilme e facilitar a higiene oral. As propriedades físico-químicas da superfície do implante; em particular, a rugosidade de superfície e a energia livre de superfície, desempenham um papel crucial na formação do biofilme.

Embora a maioria dos investigadores acredite que uma maior rugosidade da superfície conduz a uma maior adesão bacteriana, existem estudos que contestam esta noção e sugerem que o impacto pode ser mínimo. Também existe uma falta de investigação que examine as implicações microbiológicas da implantoplastia.

Objetivo: O presente estudo teve por objetivo avaliar a eficácia da implantoplastia na redução da adesão bacteriana e da formação de biofilme nas superfícies dos implantes.

Materiais e Métodos: O estudo foi efetuado em várias fases. Inicialmente um total de 20 implantes foram divididos aleatoriamente por dois grupos: implantes não tratados (grupo de controlo) e implantes tratados com implantoplastia. A implantoplastia foi realizada em metade das amostras seguindo um protocolo de brocas pré-definido com constante refrigeração. Posteriormente foi realizado o crescimento da estirpe *Streptococcus oralis* que englobou várias etapas. Inicialmente, a estirpe foi descongelada para uma placa de Petri com ágar sangue e incubada em anaerobiose. No dia seguinte, foi realizada a subcultura e a estirpe foi novamente colocada em ambiente anaeróbico. Seguiu-se a realização do crescimento overnight em que a estirpe foi incubada em anaerobiose por 18h num meio ágar BHI-2. Por fim, procedeu-se à última fase de crescimento: o crescimento exponencial. O inóculo foi ajustado a uma densidade ótica de 0,05 e, após um período de incubação de cerca de quatro horas, foi atingida a fase exponencial (densidade ótica de 0,4). As amostras de implantes

foram adicionadas às suspensões e incubadas durante uma hora e vinte e quatro horas. Foi medida a densidade ótica das suspensões bacterianas para cada amostra para avaliar a equivalência do inóculo em cada amostra. As amostras foram lavadas e as bactérias aderidas foram desprendidas das mesmas. A partir destas suspensões foram feitas diluições seriadas que seguidamente foram semeadas em placas de Petri. Após vinte e quatro horas de incubação contaram-se as unidades formadoras de colónias. Foi realizada uma análise estatística descritiva dos dados no software SPSS (versão nº29). A normalidade foi avaliada através do teste Shapiro-Wilk. As comparações dos valores médios das variáveis entre grupos foram realizadas através dos testes estatísticos *t*-student ou Mann-Whitney, conforme apropriado. A significância foi estabelecida em $p<0,05$. Também foram obtidas imagens dos implantes, com e sem adesão bacteriana, através de microscopia eletrónica de varrimento. Foi realizada a desidratação e fixação das amostras para a microscopia.

Resultados: Após uma incubação de uma hora com *Streptococcus oralis*, as amostras de implantes não tratadas apresentaram uma média de unidades formadoras de colónias por mililitro de $5,29 \times 10^5$ CFU/mL (desvio-padrão = $1,01 \times 10^5$), enquanto as amostras de implantes submetidas a implantoplastia apresentaram uma média de $1,79 \times 10^5$ CFU/mL (desvio-padrão = $1,60 \times 10^4$). A análise estatística confirmou uma redução significativa da fixação bacteriana às superfícies dos implantes após a implantoplastia, com uma diminuição evidente de aproximadamente 66% em comparação com as amostras não tratadas (diferença média = 350,00; p = 0.003; tamanho de efeito d = 4,83).

Da mesma forma, após um período de incubação de vinte e quatro horas as amostras de implantes não tratadas demonstraram uma média de $6,42 \times 10^5$ (desvio-padrão = $2,89 \times 10^5$). Enquanto que as amostras de implantes tratadas com implantoplastia manifestaram uma quantidade média de $2,46 \times 10^5$ (desvio-padrão = $9,75 \times 10^4$). A análise estatística indicou uma diminuição significativa da fixação bacteriana com uma taxa de redução distinta de aproximadamente 62% nas amostras tratadas em comparação com as não tratadas (diferença média = 395833,33; p = 0,033; tamanho de efeito d = 4,83). As imagens de microscopia eletrónica de varrimento demonstraram uma diminuição significativa da rugosidade de superfície e adesão bacteriana à superfície dos implantes após tratados com implantoplastia.

Além disso, este estudo corroborou o facto de cada grupo ter sido carregado com uma densidade da suspensão bacteriana equivalente, evidenciando condições consistentes nos

conjuntos de amostras tratadas e não tratadas. Neste estudo piloto, a dimensão da amostra utilizada foi considerada suficiente para detetar diferenças entre os grupos de estudo.

Conclusão: Os resultados do estudo permitem rejeitar a hipótese nula, evidenciando uma diminuição significativa da adesão bacteriana nos implantes tratados com implantoplastia. Este estudo preliminar fornece informações significativas relativamente à eficácia da implantoplastia na diminuição da adesão bacteriana e formação do biofilme aos implantes dentários. Apesar de se terem observado reduções significativas, justifica-se uma análise mais aprofundada e a consideração de várias variáveis devido à persistência da adesão bacteriana mesmo após a implantoplastia. Os esforços de investigação futuros deverão alargar esta investigação a outros protocolos de implantoplastia e implantes com diferentes tratamentos de superfície para posteriormente serem confirmados em estudos *in vivo*.

Palavras-chave: Implante Dentário, Implantoplastia, Peri-implantite, Adesão Bacteriana, Rugosidade da Superfície

TABLE OF CONTENTS

TABLE OF FIGURES

LIST OF ABREVIATIONS

BHI - Brain Heart Infusion

BLT - Bone Level Tapered

CaP - Calcium Phosphate

CFU - Colony Forming Unit

Cu – Copper

DAE - Dual Acid Etch

GO - Graphene Oxide

H_0 - Null hypothesis

H_1 - Alternative hypothesis

OD - Optical Density

PBS - Phosphate-buffered saline

PEEK – Polyetheretherketone

RBM - Reabsorbable Blast Medium

SBAE - Sandblasted with Large-grit particles and Acid Etched

SD - Standard Deviation

SEM - Scanning Electron Microscope

SLA - Sandblasted, Large-grit, Acid-etched

S.o. - *Streptococcus oralis*

Ti – Titanium

1. Introduction

Dental medicine has evolved significantly with the emergence of Implantology, revolutionizing prosthetic rehabilitation treatments. Thus, modern dentistry has become increasingly concerned with finding ways to manage complications related to osseointegrated implants.[1] Major challenges involve peri-implant diseases which are inflammatory reactions to pathogens in the tissues surrounding the dental implant.[2,3] These diseases comprise the following two conditions: peri-implant mucositis and peri-implantitis.[2,4,5] Peri-implant mucositis consists of inflammation in the tissues surrounding an implant. It is reversible if taken anti-bacterial measures.[1–8] Otherwise, this pathology can progress to a more severe condition called peri-implantitis.[2,3,7,8] Peri-implantitis exhibits loss of supporting bone besides tissue inflammation. Therefore, it is an irreversible condition that may compromise implant longevity.[2–7,9] A lack of treatment can lead to a non-linear and accelerating progression, as supracrestal parallel collagen fibers are weaker mechanically, and vascularity is poorer than in natural teeth.[6–8] Studies report that peri-implantitis has an estimated prevalence of 20% in implant patients, hence the importance of studying ways to treat this disease.[2,8–10]

Non-surgical treatment alone has proven to be insufficient in the treatment of peri-implantitis.[2,3,9] Surgical intervention is required in most cases.[2,3,9] To date, many surgical approaches are available, and it is still inconclusive which approach is best.[2,9,11] Nevertheless, a surface decontamination protocol is required.[2,3,5] Implantoplasty is a decontamination technique used worldwide that has shown promising results.[2,5,9,11] One of the main goals of this method is to remove the exposed threads and smooth the implant surface to prevent biofilm formation, which is the major cause of peri-implant infections.[9,12]

After tooth brushing, the acquired pellicle covers dental abutments within seconds once exposed to the saliva in the oral cavity.[12,13] This pellicle alters the physicochemical properties of implants, allowing bacterial adhesion and co-aggregation resulting in biofilm formation.[12,13] The effect of implantoplasty on biofilm formation, mainly lies in altering two physicochemical characteristics of surfaces: surface roughness and surface free energy.[14,15] According to research, surface roughness is the primary factor affecting bacterial adhesion. Hence the importance of studying biofilm formation on implant surfaces with altered surface roughness induced by implantoplasty.[14,15] Most researchers state that higher surface

roughness increases biofilm formation.[12–16] However, this finding is not unanimous once some studies show that it has only a minimal effect on bacterial adhesion.[13]

1.1. Peri-implant diseases classification

In 2017, the World Workshop, co-presented by the American Academy of Periodontology (AAP) and the European Federation of Periodontology (EFP), created a new Classification scheme for Periodontal and Peri-Implant Diseases and Conditions.[6,7] As a result, Peri-implant Diseases and Conditions are currently divided into four groups: peri-implant health, peri-implant mucositis, peri-implantitis, and peri-implant soft and hard tissue deficiencies.[6,7] Each concept is explained in detail below.

Peri-implant health is characterized by the absence of clinical signs of inflammation, absence of bleeding or suppuration on gentle probing; no increase in probing depth compared to previous examinations; and no bone loss. Health-compatible probing depths cannot be defined.[6,7]

Peri-implant mucositis is characterized by bleeding and/or suppuration on gentle probing in the absence of bone loss, due to an inflammatory lesion in the soft tissues surrounding an implant.[6,7] Increased periodontal probing depths between 4 and 5mm are commonly present.[8] Data indicates that oral biofilm is the cause of peri-implant mucositis. Therefore, measures aimed at eliminating plaque should be used to reverse this condition.[6,7]

Hard and soft tissue implant site deficiencies refer to a reduced alveolar process, due to natural healing following tooth loss.[6,7] In this way, bone remodeling following an implant placement – and additional factors such as severe loss of periodontal support, extraction trauma, endodontic infections, root fractures, thin buccal bone plates, and poor tooth position - must be differentiated from bone resorption caused by the presence of microorganisms.[6,7]

Lastly, peri-implantitis is a pathological condition occurring in tissues around dental implants, characterized by inflammation in the peri-implant mucosa and progressive loss of supporting bone.[1–9] Clinical signs include bleeding and/or suppuration on gentle probing (~0.2N), increased probing depths (greater than 5mm) and/or recession of the mucosal

margin, and radiographic bone loss compared to previous examinations.[1,4,6,8,17] In the absence of previous data, the reference values are ≥6mm probing depths and bone levels ≥3mm apical of the most coronal portion of the intra-osseous part of the implant.[1,6] However, it should be noted that bone loss of 1mm in the first year of implant placement should be expected due to surgical trauma and loading. From then forward the annual mean vertical bone loss should be less than 0.2mm to be considered a natural biological process.[8] In most cases, bone loss around dental implants results in crater-like defects.[8]

1.2. Etiology of peri-implant diseases

As soon as an implant is placed, peri-implant hard and soft tissues are formed by a process known as osseointegration. During this wound healing process, new bone is formed in contact with the implant; while in peri-implant mucosa, junctional epithelium, and connective tissue zone are established.[6] It is well documented that, when neglecting oral hygiene, oral microbiota adheres easily to the exposed surface of dental implants. Biofilm formation occurs not only on tooth surfaces but also on implant surfaces, despite its composition – hydroxyapatites versus titanium or zirconium - and the morphologic and histologic differences between the tissues surrounding them.[12,13,16,18]

Pathogens present in the biofilm produce endotoxins such as collagenase, hyaluronidase, and chondroitin sulfates.[15] As a result, an inflammatory process may develop in the tissues surrounding dental implants, potentially leading to peri-implant mucositis or peri-implantitis in case of loss of supporting bone.[13,15] Bacterial plaque is thus the primary etiological factor for peri-implant diseases.[12,13,16,18,19] A variety of gram-negative anaerobes, such as *Treponema denticola*, *Tanarella forsythia*, *Aggregatibacter actinomycetemcomitans*, *Fretibacterium fastidiosum, Prevotella intermedia,* and *Porphyromonas gingivalis*, are commonly associated with peri-implantitis.[12,13,19] In this way, both peri-implant and periodontal diseases show a correlation to the red complex microorganisms.[15,18,19] This microbiota differs from that found in patients with caries or gingivitis, yet does not appear to differ between individuals with or without previous periodontitis.[13]

There is strong evidence that a history of periodontitis and oral biofilm control constitute risk factors for peri-implant diseases.[1,2,4,6] Meanwhile, it is inconclusive when it comes to smoking and diabetes.[2,4,6] Factors that can enhance plaque build-up include lack of keratinized mucosa, implant design inaccessible to hygiene, lack of preventive maintenance, rough implant surface exposure, and inadequate prosthetic design or adaptation.[1,5] Residual submucosal cement, residual dental floss, insufficient bone volume, inadequately performed surgical procedures, improper implant position, exposed threads and surface coatings, parafunctional habits, occlusal overload, and metal corrosion may also contribute to this negative effect.[1,3,5] These situations must be addressed when treating or even preventing peri-implant diseases.[1]

1.3. Biofilm formation

A biofilm is a community of bacteria enclosed in a self-produced matrix that adheres to a biotic or abiotic surface.[13,20] The oral microbiota serves many beneficial functions for the host when both are in a homeostatic balance. This state can be disrupted mainly by poor oral hygiene.[13] If so, pathobionts start to develop, leading to pathogenic processes in the oral environment, such as caries in natural teeth and inflammation in the tissues surrounding teeth or implants.[13,20]

The formation of biofilm is a multifaceted process.[5,12,13] Firstly, as the oral cavity is an aqueous environment, organic molecules are adsorbed within seconds to a substrate.[21] Electrostatic, van der Waals and hydrophobic forces make salivary proteins adhere to the enamel surface, leading to the formation of the acquired pellicle.[5,12,13,21] This pellicle is composed of glycoproteins (mucins), proline-rich proteins, phosphoproteins (e.g. statherin), histidine-rich proteins, enzymes (e.g. a-amylase) and other macromolecules.[21] It serves as a lubricant, protects the dental surface and supplies antibacterial components. On the downside, it also provides lectins and other adhesins that boost biofilm formation.[12,13] Early colonizers – essentially *Streptococci (Streptococcus viridens, Streptococcus mitis,* or *Streptococcus oralis)*, but also *Actinomyces*, *Neisseria*, *Rothia*, or *Veillonella* – adhere reversibly to these receptors. These microbes multiply and progressively create an anaerobic environment. Then,

secondary colonizers - *Streptococcus mutans, Streptococcus sobrinus, Treponema denticola, Prevotella intermedia, Prevotella loescheii, Porphyromonas gingivalis*, or *Aggregatibacter actinomycetemcomitans* – co-adhere irreversibly to the latter through protein and carbohydrate molecules located on the bacterial cell surfaces.[12,13,15,21] *Fusobacterium nucleatum* bacteria are responsible for binding early colonizers to late colonizers - a mechanism called co-aggregation.[12] Finally, an extracellular matrix made of proteins, lipids, extracellular DNA, exopolysaccharides (e.g., levans, dextrans), and amyloid structures embody the existing microbes.[12,13] These events lead to the formation of clusters, i.e. a well-organized intrinsic network that easily adapts to environmental changes through several mechanisms: synergistic interactions, signal transduction, gene expression modulation, quorum sensing, and secretion of substances for self-protection.[12,13,20,21]

1.4. Treatment of peri-implantitis

A treatment plan for peri-implant diseases should consider the extent of bone defects. Non-surgical methods are suitable for peri-implant mucositis and defects measuring up to 2mm, while surgical intervention may be necessary for larger defects. Removal of the implant may be necessary if no viable alternatives are available. [8]

Initially, peri-implantitis should be managed with oral hygiene instruction and non-surgical mechanical therapy.[5,8] The aim is to decrease the bacterial load by disrupting the biofilm through thorough debridement.[8] This decontamination is performed more subgingivally compared to peri-implant mucositis.[8] As a result of the implant's complex design and surface, many adjunctive peri-implant therapies could be performed, including antibiotics, antiseptics, ultrasonics, and lasers.[2,3,5,9,12,17]

After six weeks, a re-evaluation should be conducted to assess plaque, calculus, bleeding on probing, probing depth, and radiological evaluation for bone loss. If these parameters improve, recall appointments should be scheduled, according to the professional’s assessment of each patient’s needs, to start long-term supportive therapy.[3,12] Regardless of the sulcus depth, daily self-performed oral hygiene measures, as well as professional maintenance care, are essential to removing the biofilm.[13,22]

Nevertheless, non-surgical mechanical therapy has proven often insufficient to treat peri-implantitis with a tendency for disease recurrence.[1,5,8,9] Ultrasonic scalers and manual curettes are not enough in pockets greater than or equal to 5 mm. Therapies such as systemic antibiotics, air abrasion, laser, and chemotherapeutics still need further research.[19] The presence of a remaining inflammatory lesion may require a more invasive treatment once the inflammation around the implant has been controlled. Thus, when non-surgical therapy fails to control the disease, surgical therapy is indicated.[1,5,8,9]

Several surgical approaches are available, including access flap, regeneration surgery and respective surgery.[1,3,5,18,23] Access flap surgery is used when bone loss is superficial.[8,23] By lifting a full thickness mucoperiosteal flap, access is gained to decontaminate the implant surface. In the end, the flap is repositioned. The aim is to decontaminate without changing the soft tissue margin.[5,8,23] In turn, regenerative surgical techniques are employed in crater-shaped lesions with intraosseous defects, aiming not only to decontaminate but also to regenerate lost bone.[8,23] A flap is elevated, allowing the implant surface to be decontaminated. The intraosseous defect is filled with graft material, which can be covered by a resorbable or non-resorbable membrane.[8,23] Then, the tissues are repositioned. Apical flap surgery is suitable for non-aesthetic areas for the surgical treatment of peri-implantitis with intra- or supra-bone single-wall defects.[5,8] A flap is raised to allow for decontamination of the implant surface and removal of infected soft tissue. The flap is then repositioned apically, reducing pockets, and improving oral hygiene.[3,5,8,23]

Usually, a combination of these approaches is used, plus decontamination of the implant surface by using air flow, saline solution, laser, curettes, or ultrasonic cleaning.[3,5,9,18] Nevertheless, there is a procedure called implantoplasty that is often associated with a resective surgical approach or with a combined resective and regenerative surgical approach.[3,9,18] After resective surgery, the rough surface of the implant may be exposed to the oral environment. Therefore, implantoplasty aims to decontaminate the supracrestal component of the implant, reduce bacterial adhesion and ease hygiene.[24] This method was first described in 1990 by Lozada and his co-workers and is now well-known worldwide.[11]

1.5. Implantoplasty

Implantoplasty is a mechanical method for smoothing the threads of the implant surface that have been uncovered by the loss of peri-implant bone height, meaning removing an infected and exposed surface of the implant.[1,3,11,16,18,19,22,24–29] The goals of implantoplasty are to effectively remove the biofilm – to improve soft tissue adaptation - hinder bacterial adhesion and facilitate oral hygiene.[1,3,9,11,24] It is indicated when the threads of the implant are supra- or subgingivally exposed, either due to peri-implantitis or to a supracrestal positioning of the implant.[25]

Currently, there is no standardized implantoplasty protocol, but many bur sequences available to the clinician.[11,19] Initially, diamond, tungsten carbide, or multilaminar burs of decreasing coarseness can be used, followed by a polishing sequence with Arkansas burs, silicon burs (e.g. Brownie and Greenie), or finishing amalgam, and so forth. It should be done under copious irrigation by using a handpiece (e.g. turbine or contra-angle handpiece with different rotations per min depending on the burs).[11,18,19,24–29]

Finding the most optimal bur sequence is still a challenge for researchers, once several parameters such as surface roughness, overheating, and biocompatibility have to be taken into account.[11] Maal et al. concluded that adding Arkansas stone and silicon burs caused an initial increase in fibroblast growth.[11] Yildiz et al. showed that tungsten carbide burs lead to a smoother surface in comparison to the surface obtained by diamond burs.[27] Meier et al. also obtained this result, however, tungsten diamond burs required considerably more time to reach a smooth surface – which is clinically relevant.[30] Ramel et al. pointed out the potential disadvantages of using silicone polishers, once studies have demonstrated how these burs produce larger amounts of silicone particles, triggering an inflammatory process.[29] Yet, despite the current level of knowledge about this topic, further research is still needed to determine a standard implantoplasty protocol.

There are several limitations to this technique including, a demanding and time-consuming procedure, overheating of peri-implant tissues due to the use of high-speed rotary instruments, incomplete implantoplasty in implants with difficult access, potential weakening of the implant–abutment complex in narrow implants, the release of metal particles into the peri-implant tissue as a result of a drilling process, leading to potential bone loss.[3,24,25]

1.6. Implant surface and biofilm formation

Dental implants are mainly manufactured from the following materials: pure Ti, Ti alloys, zirconia, and PEEK. Zirconia can improve aesthetics and prevent Ti allergy but has a moderate rate of fracture. PEEK still needs further multicentric studies to ensure its trustworthiness. Therefore, Ti and its alloys remain the gold standard in implant dentistry with survival rates around 90%.[31,32]

Titanium implants can be treated to alter their surface properties. The main purpose is to transform a hydrophobic surface into a hydrophilic, aiming to improve osseointegration.[32] To achieve this, the surface roughness is augmented to expand the contact area and cellular attachment. Consequently, a greater concentration of bone cells is attained, facilitating strong adhesions to the implant site.[32] Surface treatment options are currently divided into additive or subtractive. Addictive procedures include calcium phosphate, hydroxyapatite, graphene oxide, plasma spray, bioglass, copper, fluoride, and phospholicate. Subtractive procedures include sandblasting, acid etching, alumina blasting, dual acid etch, electrochemical anodizing and sandblasting, and acid etching. The latter is the most widely used, but the ideal surface is still far from being established.[30–32]

Each approach possesses its unique advantages and disadvantages when compared to one another. In the case of the commonly used subtractive methods, sandblasting may carry the risk of containing residual particulates, thereby increasing the potential for fracture. Incorporating etching alongside sandblasting enhances the protein adhesion mechanism by increasing the peak height of roughness peaks. On the other hand, utilizing DAE without prior sandblasting reduces the presence of sand particles.[32] Electrochemical anodizing, another method, not only promotes osseointegration but also offers cost-effectiveness, simplicity in application, ease of control, and improved corrosion resistance.[32,33] When it comes to additive methods, the use of CaP enhances contact osteogenesis and biocompatibility. GO and Cu also exhibits osteogenic properties and possess the added advantage of exerting antibacterial effects, which are crucial in preventing peri-implantitis.[32] Therefore, the physicochemical characteristics of surfaces also play an important role in influencing bacterial adhesion, in particular surface-free energy and surface roughness. However, the influence of surface roughness appears to be of greater importance.[14,15]

The implant surface topography can be classified as smooth (Ra < 0.5 µm), minimally rough (Ra 0.5–1.0 µm), moderately rough (Ra 1.0–2.0 µm), and highly rough (Ra > 2.0 µm). To evaluate surface topography, equipment such as contact profilometers or interferometers measure the Ra, Rq, Rt, and Rz values.[27,29,30] Ra, the arithmetical mean roughness, is the arithmetical mean height that indicates the average of the absolute value along the sampling length.[27,30] Rq, root mean square deviation, indicates the root mean square along the sampling length. Rt, the total height of the profile, indicates the vertical distance between the maximum profile peak height and the maximum profile valley depth along the evaluation length.[30] Rz, maximum height of profile, indicates the absolute vertical distance between the maximum profile peak height and the maximum profile valley depth along the sampling length.[26,30] The arithmetical mean for these variables determines the surface topography.[30]

Rougher implant surfaces have been employed to promote the osseointegration process – with an ideal Ra range from 1.0 to 2.0 µm.[12,19,30,34] However, it has the disadvantage of disrupting the attachment of the connective tissue and aiding the growth of bacteria and biofilms. Thus, the concept of implantoplasty arose to overcome these difficulties.[11,12,14] In fact, previous studies have shown that human gingival fibroblasts spread more readily on smooth as compared to rough surfaces.[11,27,30]

In the same way, higher surface roughness is alleged to increase biofilm formation due to various reasons.[12–16,21] One probable explanation is that the surface area increases due to the roughness of the surface, as roughness increases the surface area available for adhesion by a factor of two or three. Other factors include difficult oral hygiene and shelter against shear forces.[12,13,16,21] As a matter of fact, many *in situ* or *in vivo* studies have demonstrated that reducing surface roughness reduces bacterial adhesion on implant surfaces.[9,12,13,16] A mean Ra<0.2 µm is considered a clinically acceptable "threshold" value in terms of preventing microbial colonization meaning it is unlikely to reduce bacterial adhesion any further.[14,21,27]

It should be noted, however, that other studies dispute this view by stating that surface roughness only has a minor effect in reducing bacterial adhesion and biofilm development.[13] That might have been the case once *in vitro* studies don't have the acquired pellicle influencing the outcomes, opposite to *in vivo* studies, as the pellicle can level out surface roughness.[13,35–39] Bacteria's capability to modulate gene expression according to the surface topography is another possible explanation.[13] In addition, surface roughness can be measured

by different parameters: Ra, Rz, Rq, and Rt and most studies only address at least the first two with different evaluation methods. Therefore, similar topographies can end up having different values and vice-versa.[13,27] The implantoplasty protocol may also influence depending on the applied pressure, the rpm of the rotatory instruments, and the absence of a predefined timeframe, to name a few.[27]

Additionally, surface-free energy is also a factor that influences the initial adhesion of oral microorganisms on dental implants.[12,14,15,20] A surface's free energy refers to the interaction between cohesion and adhesion forces due to an unfulfilled bonding potential of surface molecules that determines whether or not wetting occurs. Wettability is measured by the angle a droplet forms with a surface and the angle determines whether the material is hydrophilic or hydrophobic.[15,31,34] Implant surfaces should ideally be hydrophilic to better achieve osseointegration. A review of the evidence does show that the roughest implants are the most hydrophilic.[30,31] Moreover, *in vitro* studies show that bacteria tend to adhere better to positively than negatively charged surfaces, once they are negatively charged, and to surfaces with moderate wettability with an optimal contact angle from 40° to 130°.[13,20] In other words, hydrophobic materials or materials with low surface free energy are less likely to be adhered to by bacteria.[13–15,21] Some researchers think it is partly a matter of low bacteria-to-substratum binding strength, probably caused by a cohesive failure.[21] Therefore, hydrophobic coating materials such as hexadecyltrimethoxysilane, polytetrafluoroethylene, perfluorodecyltriethoxysilane, or a low surface free energy teflon or nanocomposite have been shown to reduce biofilm formation.[13,21] There are also superhydrophobic surfaces that can upgrade this effect by having microscaled structures on their surface that create higher contact angles resulting in a self-cleaning effect where water simply washes out the bacteria that are trying to adhere to the implant surface. Further research is still needed for these superhydrophobic surfaces.[13] Noteworthy is the fact that the wetting phenomenon varies depending on the heterogeneity and roughness of a surface.[34] For example, to achieve a superhydrophobic surface an increase in roughness is necessary and chemical modification alone is not enough to achieve this goal.[34] In addition, the wetting phenomenon on dental implants is dynamic as the contact angles may change due to protein adsorption, for example.[34]

Based on the available literature, there is no evidence on how the implantoplasty protocols influence the potential adhesion of early colonizers in the treated surface comparing to the original surface.

2. Aim

This *in vitro* study aims to evaluate bacterial adhesion and biofilm formation in implant surfaces after an established implantoplasty protocol.

The null and alternative hypotheses can be defined as follows:

H_0: There are no differences in bacterial adhesion and biofilm formation on the implant surfaces after implantoplasty.

H_1: There is a decrease in bacterial adhesion and biofilm formation on the implant surfaces after implantoplasty.

3. Materials and Methods

The present investigation is an experimental pilot study that aimed to evaluate the effect of implantoplasty in bacterial adhesion to implant surfaces. For this purpose, the adherence of the *Streptococcus oralis* strain NCTC 11427 (*S.o.*) to the surface of eight implants previously treated with implantoplasty was compared to eight implants without any prior treatment (control group). Initially, implantoplasty was performed on half of the specimens. Subsequently, the growth of the *S.o.* strain on the surface of the implants was carried out, which included several phases: thawing of the strain, subculture, overnight growth, and exponential growth. Once the desired exponential phase was achieved, the implant samples were incubated with the obtained suspensions for 1 hour and 24 hours. Subsequently, the samples were washed, and the bacteria adhered to the implants were detached using a vortex and ultrasonic bath sequence. Serial dilutions were made from these suspensions, followed by seeding onto Petri dishes. After 24 hours of incubation, the formed colonies were counted. Bacterial adhesion was also qualitatively evaluated through Scanning Electron Microscopy (SEM). Four additional samples were dehydrated and fixed for SEM analysis as a reference. These steps will be explained in detail below.

3.1. Implant specimens

This study analyzed Ti BLT implants manufactured by EBI (Evidence Implant Inc., South Korea) (Figure 1). The implant system features a cylindrical shape, a hexagonal external connection, and an SBAE surface. This RBM surface contains calcium phosphate with high biocompatibility to provide a surface roughness of Ra 1.4-1.6 μm. The implant has an insertion depth of 8 mm, a thread pitch of 0.8 mm and a diameter of 4.1 mm for both the neck and body. For ease of periodontal care, there is a 0.5 mm machine surface interval in the coronal portion. The apical portion is self-tapping in order to provide initial stability.[40] Due to this last feature, implantoplasty could not be performed in this area.

Figure 1: BTL External Implant.[40]

3.2. Implantoplasty protocol

Twenty titanium dental implants (External Implant 4.0(B)x4.1(P)xB(1.D), EBI Implant System) were randomly divided into two different groups: the control group with non-treated implants (NT) and the implantoplasty group with implants treated with implantoplasty (T).

Table 1: Table depicting study group sample size and time frame.

		Sample size (N)			Total (N)
		CFU and SEM (after incubation)		SEM (before incubation)	
		1h	24h		
Study Group	NT	4	4	2	10
	T	4	4	2	10
Total (N)		8	8	4	20

All implants from the implantoplasty group were treated *in vitro* by the same investigator using the implantoplasty protocol defined by Lozano et al. 2022. The bur sequence was as follows: a small-grained WC bur (reference H379.31. 018 followed H37UF and H379.31.023, (Brasseler, KOMET; GmbH & Co., KG, Lemgo, Germany), a coarse-grained diamond polisher (Rugbyno. 9608.314.030 KOMET; GmbH & Co., KG, Lemgo, Germany), a small-grained SiC polisher Arkansas and finishing amalgam (order no. 9618.314.553 KOMET; GmbH & Co., KG, Lemgo, Germany). [26]

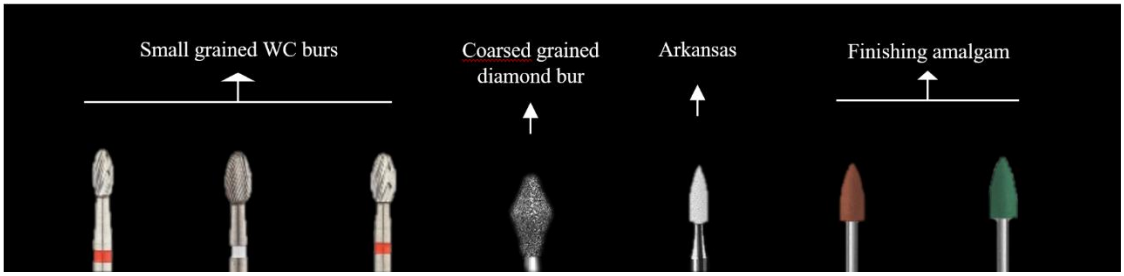

Figure 2: Bur sequence.

Visual assessment of the surface smoothness determined the protocol timeframe. An air-driven turbine (KaVo 350.000 rpm) and contra-angle handpiece (KaVo 40.000 rpm) were used under constant irrigation with a Bunsen burner to create a sterile environment around the vicinity of the open flame. All implants were rinsed with sterilized water and placed in Eppendorf tubes.

By-products in the irrigation water were collected using a sterilized container and then stored in falcon tubes. The samples were stored at -80ºC for further assays on the cytotoxicity of these compounds, which are outside the scope of the present work.

3.3. Growth of *Streptococcus oralis*

3.3.1. Thawing and subculture of the bacterial strain

The *Streptococcus oralis* NCTC 11427 strain was thawed on a blood agar medium in a Petri dish and incubated in anaerobiosis (10% CO_2, 10% H_2 in Nitrogen) at 37ºC.[41]

The following day, subculturing was carried out by transferring the specimen to another Petri dish with blood agar. The strain was incubated again under anaerobic conditions.

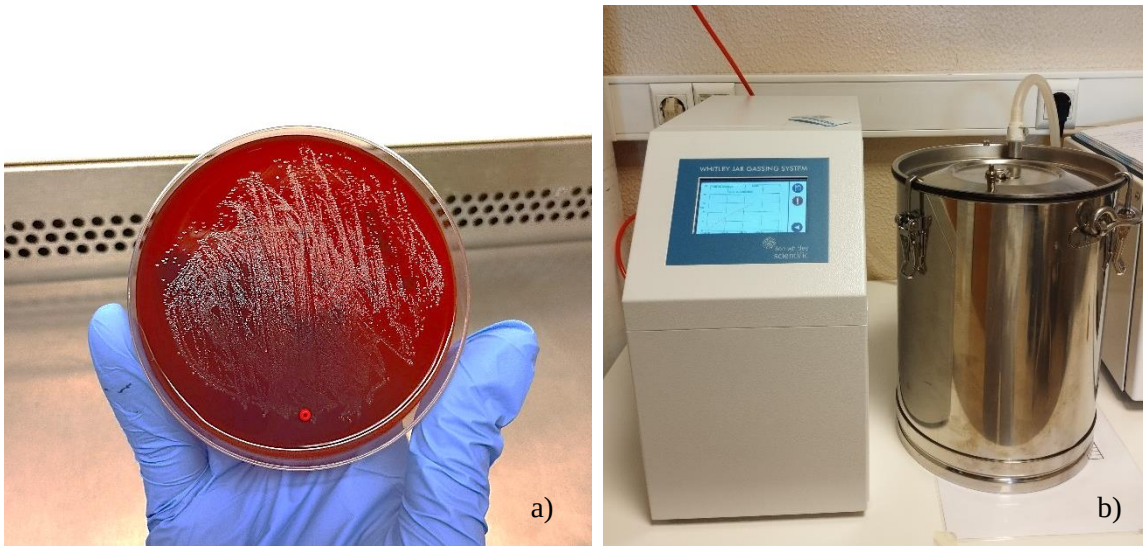

Figure 3: Subculturing: a) *S.o.* in a blood agar medium b) anaerobic jar.

3.3.2. Overnight and exponential growth

After subculture, *S. oralis* was inoculated into a falcon tube containing 15mL of a sterile BHI-2 agar medium. Then, the falcon tube was incubated in anaerobiosis (10% CO_2, 10% H_2 in Nitrogen) at 37ºC for 18h. This incubation period allowed the overnight growth of *S.o.*, resulting in the formation of colonies.

The next step was promoting the exponential growth of *S.o*. To begin, the overnight growth inoculum was transferred to a vial containing a sterile BHI-2 medium. Then, 1mL of the suspension was transferred to one cuvette, while 1mL of sterile BHI-2 medium was transferred to another cuvette. The cuvette containing sterile BHI-2 alone was used for blanking the UV-visible spectrophotometer (CamSpec M501 single beam spectrometer). After blanking, the spectrophotometer measured the optical density of the suspension at 550 nm. The inoculum was adjusted to an optical density of 0.05. Once this value was reached, the suspension was incubated in anaerobiosis (10% CO_2, 10% H_2 in Nitrogen) at 37ºC for approximately 4 hours until it reached the exponential phase (optical density of 0.4 at 550 nm).

Implant specimens from both groups were then added to the suspensions in the exponential phase and incubated in anaerobiosis for 1 hour and 24 hours. Bacterial suspensions for each sample were measured for their optical density to evaluate the equivalence of the inoculum in each sample. All procedures were performed in aseptic conditions.

3.4. Serial dilution, seeding, and counting of colonies

After 1 hour and 24 hours of incubation, samples were washed with sterile PBS and transferred to 50 mL falcon tubes with 3 ml of filtered PBS. The bacteria attached to the implants were detached using a serial step method: firstly, the falcon tubes were placed in a vortex (1600 rpm) for 1 minute; secondly, samples were incubated in an ultrasonic bath for 4 minutes (220V); finally, they were subjected again to vortex detachment (1600 rpm) for 2 minutes.[42]

The obtained suspensions were diluted in sterile PBS using a serial dilution technique (1:10). Dilutions ranging from 10^{-1} to 10^{-8} were prepared, with each Eppendorf tube containing 100 µl of sample mixed with 900 µl of sterile PBS.

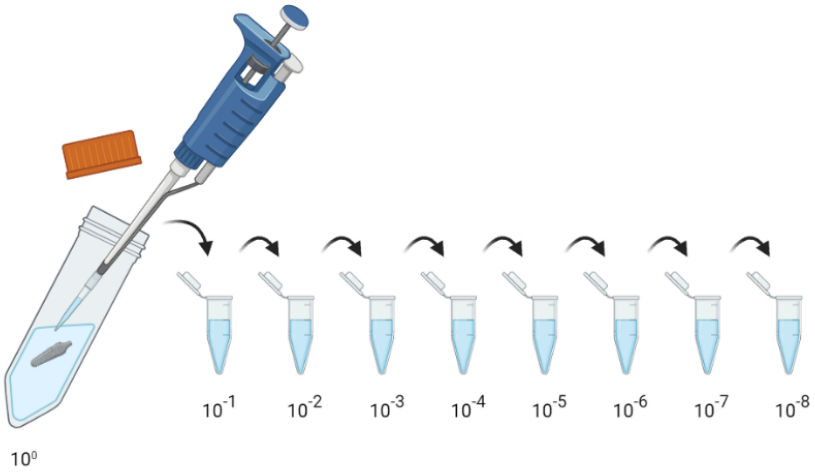

Figure 4: Serial dilution.

Each dilution, ranging from 100 to 10^{-8}, was seeded in triplicate by adding 20 µL of the suspensions onto Petri dishes containing BHI medium.

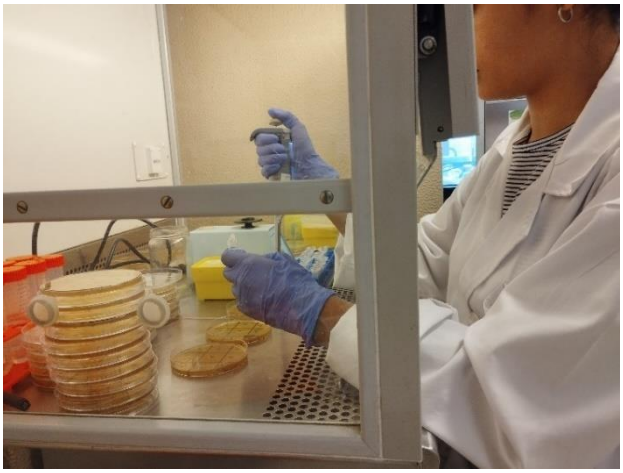

Figure 5: Seeding *S.o.* in Petri dishes with a BHI-2 medium.

After 24 hours of incubation in anaerobiosis at 37ºC, the colony count was performed to calculate the number of Colony Forming Units per milliliter (CFU/mL).

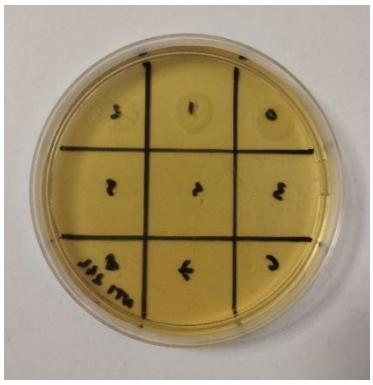

Figure 6: Counting of *S.o.* CFU.

3.5. SEM protocol

After incubation with bacteria at defined time-points, the implants were carefully washed with 500 µL of PBS to remove unattached cells. Subsequently, they were fixed with a 2.5% Glutaraldehyde solution, followed by another round of washing. The implants were then subjected to a series of alcohol solutions with increasing concentrations (300 µL of alcohol at 20%, 30%, 40% 50%, 60%, 70%, 90%, and 100%), each for 15 minutes. After this treatment, the samples were air-dried in a flow chamber under UV light. Finally, they were characterized by a scanning electron microscope (JEOL JSM-5200L). The SEM images were captured with backscattered electrons at 25 kV voltage. Obtained images were qualitatively analysed by two calibrated researchers considering a set of specific parameters. Four implants without bacteria were included in the analysis as a reference for comparison.

Figure 7: Fixing and dehydration of the implant samples for SEM.

3.6. Statistical Analysis

Descriptive statistics were performed for all variables using statistical software SPSS (Statistical Package for the Social Science, version nº29). Normality was assessed using Shapiro-Wilk test. Comparisons of continuous variables mean values between groups were performed by *t*-student or Mann-Whitney statistical tests. T-test was used when variances had a normal distribution, while the nonparametric Mann-Whitney test was used when normality was not verified. The established significance level was 5% ($p < 0.05$) for all tests. The results are presented as mean ± standard deviation.

Since this was a pilot study, no *a priori* sample size calculation was performed. However, a *post-hoc* sample size power calculation was conducted based on the results of this study using G*Power.

3.7. Study design

The study design is outlined in Figure 8.

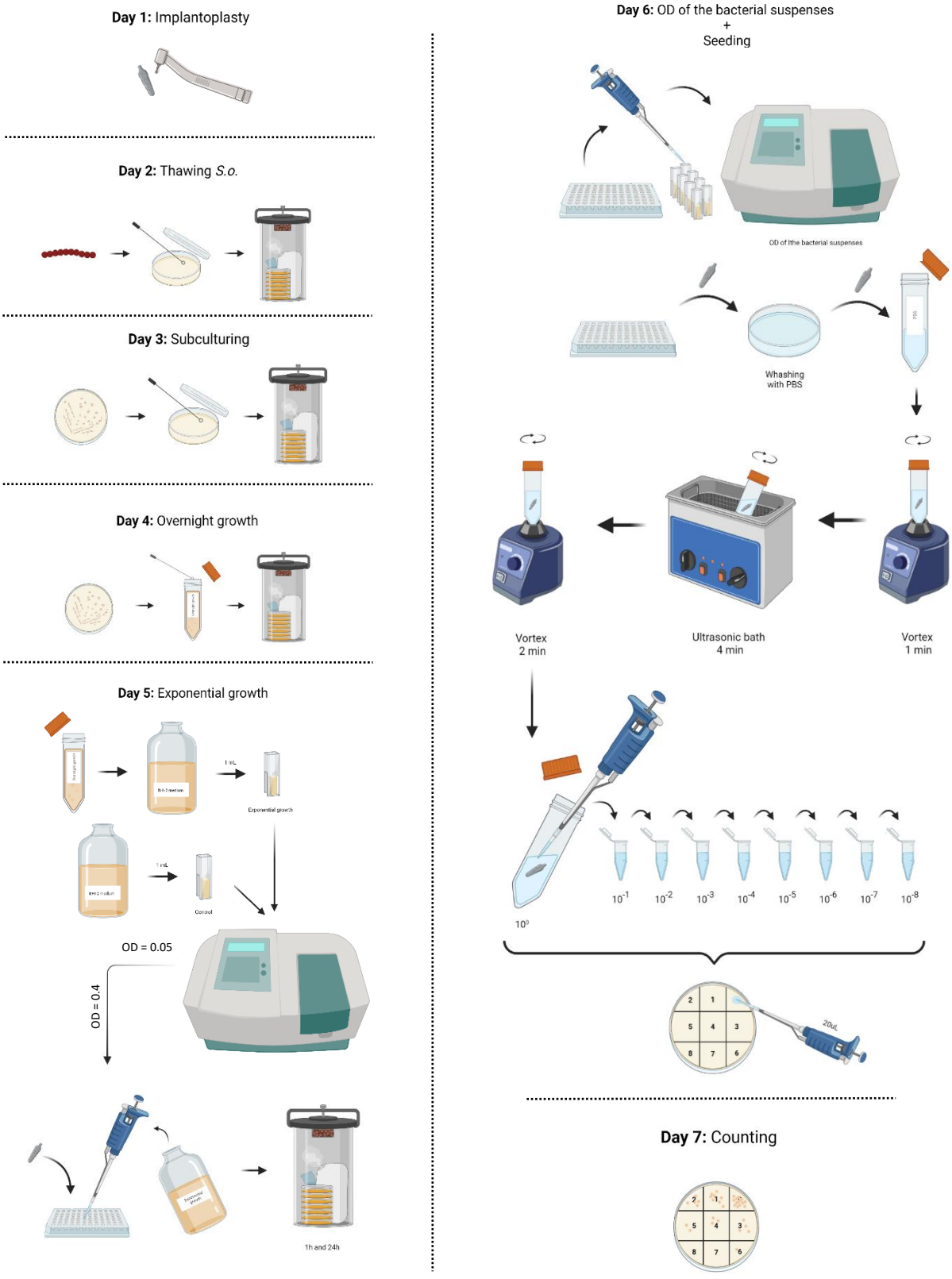

Figure 8: Study design (created with BioRender.com).

4. Results

4.1. CFU/mL of attached bacteria

After the colony count was performed, the number of Colony Forming Units per milliliter (CFU/mL) was calculated using the following equation:

*CFU/mL = (Number of colonies*dilution factor) / volume of culture plate*

The colony-forming units per milliliter (CFU/mL) for both the non-treated (NT) and treated (T) groups at 1 hour and 24 hours are displayed in Table 2 and Table 3, respectively.

Table 2: Mean CFU/mL of adherent bacteria after 1h.

Study group		Dilution Factor	UFC - 1	UFC - 2	UFC - 3	Mean CFU	CFU / mL	Mean CFU / mL	p-value
NT 1h	1	1000	13	9	11	11.00	550000.00	529000	0.003
	2	1000	10	6	7	7.67	383333.33		
	3	1000	14	9	14	12.33	616666.67		
	4	1000	12	13	9	11.33	566666.67		
T 1h	1	1000	2	6	3	3.67	183333.33	179000	
	2	1000	3	4	5	4.00	200000.00		
	3	1000	1	6	3	3.33	166666.67		
	4	1000	4	3	3	3.33	166666.67		

Table 3: Mean CFU/mL of adherent bacteria after 24h.

Study group		Dilution Factor	UFC - 1	UFC - 2	UFC - 3	Mean CFU	CFU / mL	Mean CFU / mL	p-value
NT 24h	1	1000	20	16	17	17.67	883333.33	642000	0.033
	2	1000	11	22	15	16.00	800000.00		
	3	1000	13	15	11	13.00	650000.00		
	4	1000	3	5	6	4.67	233333.33		
T 24h	1	1000	5	4	9	6.00	300000.00	246000	
	2	1000	3	2	1	2.00	100000.00		
	3	1000	7	4	6	5.67	283333.33		
	4	1000	7	6	5	6.00	300000.00		

The obtained results were subjected to descriptive statistics and the normality of the variables was evaluated using the Shapiro Wilk test. Furthermore, two graphs were generated to offer a more comprehensive analysis of the data. One graph (Figure 9) presented the results at 1 hour. While the other (Figure 10) depicted the results at 24 hours.

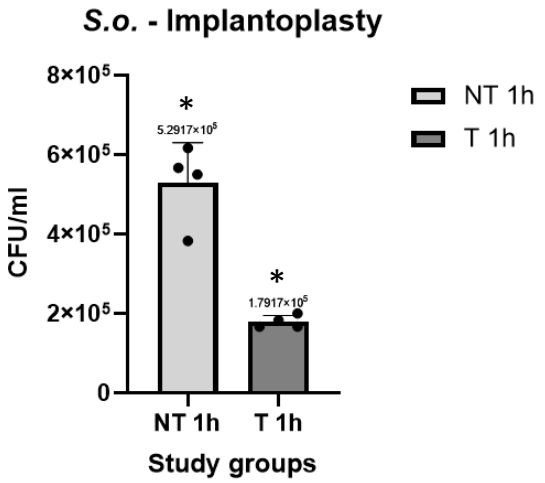

Figure 9: Barchart depicting mean values of CFU/ml of attached bacteria after 1h. Error bars represent Standard deviation. * indicates p>0.05.

After 1h of incubation with *S.o.*, the non-treated implant samples had a mean CFU/mL of 5.29 x 10^5 (SD = 1.01 x 10^5), while the implant samples treated with implantoplasty had a mean of 1.79 x 10^5 (SD = 1.60 x 10^4) CFU/mL.

Normality tests confirmed a normal distribution of data, therefore *t*-student test was applied. Significantly lower numbers of viable adhered bacteria were observed in the untreated samples after 1 hour of incubation compared to the treated samples (mean difference = 350.00; p = 0.003). These findings demonstrate a notable 66% reduction in bacterial attachment to implant surfaces following implantoplasty.

The effect size of the difference was d=4.83 indicating a very large effect size of the implantoplasty treatment protocol in reducing the number or adherent viable bacteria.[43]

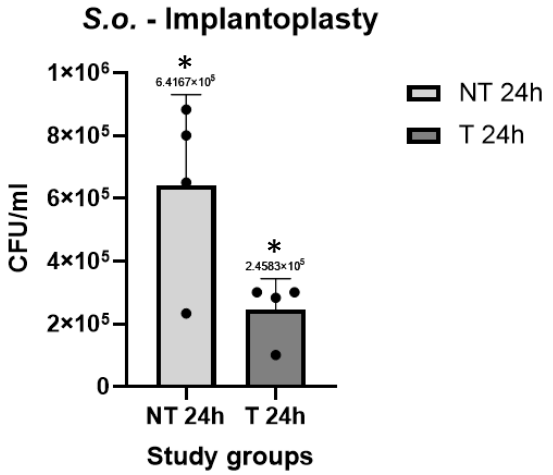

Figure 10: Barchart depicting mean values of CFU/ml of attached bacteria after 24h. Error bars represent Standard deviation. * indicates p>0.05.

After 24h of incubation with *S.o.*, the non-treated implant samples had a mean CFU/mL of 6.42 x 10^5 (SD = 2.89 x 10^5), while the implant samples treated with implantoplasty had a mean of 2.46 x 10^5 (SD = 9.75 x 10^4) CFU/mL.

Normality tests confirmed a normal distribution of data, therefore *t*-student test was applied. Significantly lower numbers of viable adhered bacteria were observed in the untreated samples after 24 hours of incubation compared to the treated samples (mean difference = 395833.33; p = 0.033). These findings also demonstrate a notable 62% reduction in bacterial attachment to implant surfaces following implantoplasty after 24h colonization.

The effect size of the difference was *d*=4.83 indicating a very large effect size of the implantoplasty treatment protocol in reducing the number or adherent viable bacteria.[43]

4.2. OD of bacterial suspensions in each group

The optical densities of the bacterial suspensions from each sample are displayed in Table 4. The table provides the mean values for each group, offering an overview of the optical density measurements conducted to assess the equivalence of the inoculum within each sample.

Table 4: Values of optical density of the suspensions after 1h and 24h in each group.

		Optical density (OD)										p-value
		NT					T					
		1	2	3	4	Mean OD	1	2	3	4	Mean OD	
Time-point	1h	0.51	0.52	0.50	0.52	0.51	0.52	0.52	0.51	0.53	0.52	0.80
	24h	0.61	0.60	0.59	0.60	0.60	0.61	0.61	0.59	0.61	0.61	0.67

Based on the obtained results, a graphical representation (Figure 11) was created to depict the optical density measurements of bacterial suspensions in contact with implants from both groups at the 1-hour and 24-hour time points.

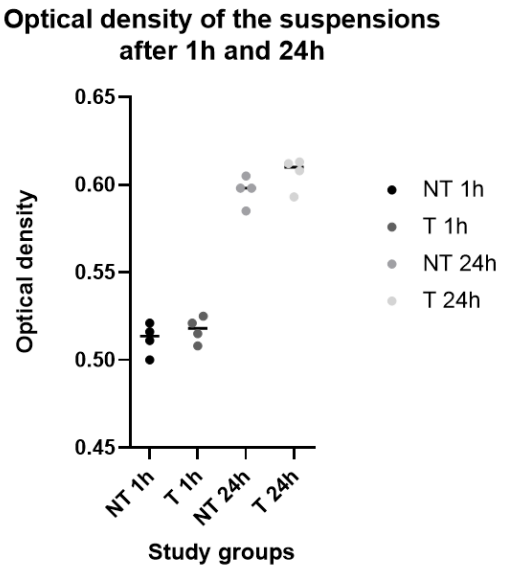

Figure 11: Scatterplot depicting the optical density mean values of the suspensions after 1h and 24h for each group.

No statistically significant differences were observed between groups, demonstrating the equivalent bacterial challenge in both groups at both timepoints (results at 1 hour: mean difference = 0.01; p = 0.80; results at 24 hours: mean difference = 0.01; p = 0.67).

4.3. Scanning electron microscopy (SEM)

SEM images were obtained from treated and untreated implant samples before and after incubating with *S.o.* bacterial suspension.

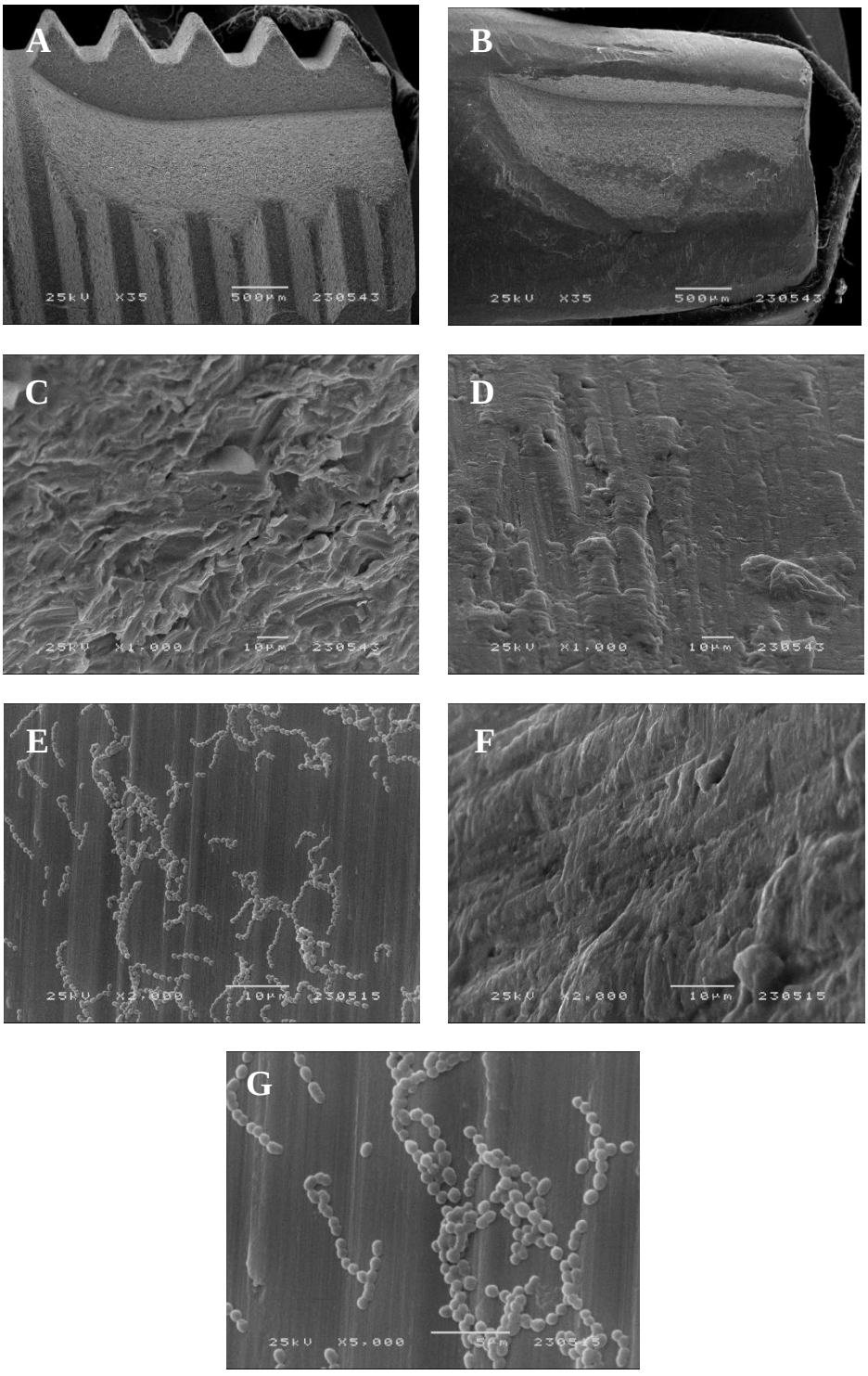

Figure 12: Composite image depicting scanning electron microscopy (SEM) micrographs of test. (A) baseline image of a non-treated implant at 35x magnifications (B) baseline image of an implant treated with implantoplasty at 35x magnifications (C) non-treated implant after 1h of incubation with *S.o*. at 1000x magnifications (D) implant treated with implantoplasty after 1h of incubation with *S.o*. at 1000x magnifications (E) non-treated implant after 24h of incubation with *S.o*. at 2000x magnifications (F) implant treated with implantoplasty after 24h of incubation with *S.o*. at 2000x magnifications. (G) *Streptococcus oralis* on untreated implant surface at 5000x magnifications.

The analysis of the base images of implants shows the loss of microroughness and a smooth surface in the T group, demonstrating the surface alterations following the implantoplasty treatment figure 12A and 12B.

In figure 12C and 12D the aspect of the NT and T surfaces after 1h of incubation in the S. oralis suspension, demonstrates the attachment of some bacteria, while at 24h (12E and F) bacterial adhesion is apparent in the NT surface. A typical morphology of *S.o.* bacteria (cocci in chains) is apparent in higher magnifications (12G and 12E).

4.4. *Post-hoc* power analysis

Since this was a pilot study, there was no data to enable an *a priori* sample size calculation. To adequately plan further studies following a similar design, a *post-hoc* power analysis was performed using G*power v3.1.9.6 for Mac using the results of this assay. According to that analysis, to achieve an alpha error probability of 0.05 (alpha) and a power of 0.95 (beta) a total of 3 samples in each group would be needed, with a critical t of 2.1318. Therefore, these results confirm that the sample size used was sufficient and even larger than the sample size needed to detect the differences between study groups.

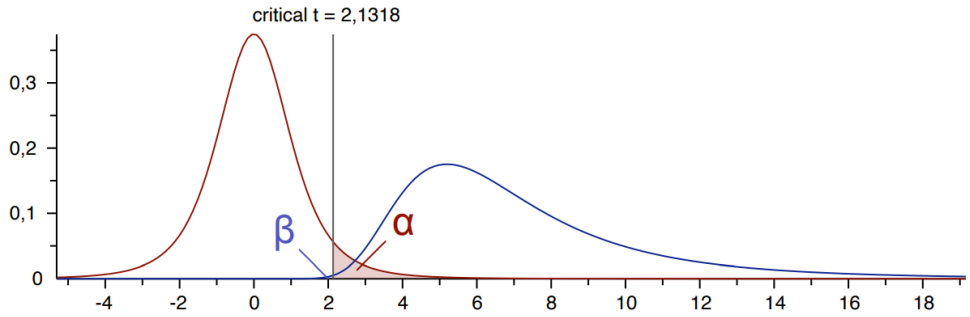

Figure 13: *Post-hoc* power analysis.

5. Discussion

Implantoplasty has been widely employed and extensively documented for its efficacy in treating peri-implantitis, either as a standalone procedure or in combination with resective or regenerative therapy.[3,9,11,18] However, despite the substantial clinical evidence supporting its efficacy, research examining the microbiological implications of implantoplasty is still scarce. As a result, there is a compelling need for additional investigations determine the influence of implantoplasty on bacterial adhesion in the context of peri-implantitis treatment.

The present study aims to assess the impact of implantoplasty on bacterial adhesion as a treatment approach for peri-implantitis. The study evaluated the adhesion of *S.oralis* to 16 BLT external dental implants with and without prior implantoplasty treatment, following 1-hour and 24-hour incubation periods. *S. oralis* was selected for having a common presence in the oral environment and being a pioneer in biofilm formation. [12,13,15,21] The implant samples utilized were composed of titanium, the most frequent material used in dental implants.[31,32] These samples had an SLA surface modification treatment, one of the most common surface treatment methods found.[30–32]

Optical density values indicated that an equivalent density of bacterial suspension was loaded to each group. A quantitative and qualitative evaluation was conducted using CFU/ml and SEM, respectively. Results revealed a significant reduction (~60%) of bacterial adhesion in treated implants at the 1-hour and 24-hour time points. In both groups, bacterial adhesion increased from 1 hour to 24 hours. SEM images revealed a notable decrease in surface roughness and bacterial adhesion in implant surfaces following the implantoplasty treatment. These results support the belief that surface roughness increases biofilm formation due to an increase in surface area. *In vivo* studies have reported even more pronounced results possibly for the sheltering effect of rough surfaces against removal forces.[12,13,16,21]

In vitro, *in situ* and *in vivo* studies have reported similar outcomes. Azzola et al. observed a 49% reduction in bacterial adhesion when comparing implants subjected to implantoplasty versus those without the procedure.[18] Similarly, Geremias et al. reported a more substantial bacterial decrease in implants treated with implantoplasty compared to those undergoing chemical decontamination and mechanical debridement.[44] Toma et al. also obtained a decreased recolonization of disc specimens after impantoplasty.[45]

Considering that the clinical manifestations of peri-implantitis are microbiologically driven, it is pertinent to acknowledge studies that investigate the impact of implantoplasty on clinical parameters. Romeo et al. found a significant (12%) increase in the implants survival rate when comparing the efficacy of resective therapy alone to the combined approach of resective therapy and implantoplasty.[46,47] Furthermore, Lasserre et al. demonstrated significant improvement in clinical parameters following implantoplasty treatment in patients with peri-implantitis.[48]

Implantoplasty has been proven to reduce implant surface roughness.[11,16,18] In this regard, it is also relevant to consider studies that evaluate implants manufactured with different roughnesses. Bermejo et al. reported a significantly lower bacterial adherence in minimal roughed titanium implants as opposed to moderaly roughed.[49] Bürgers et al. showed purely machined specimens had a significant lower surface roughness (Ra = 0.15mm versus 0.95mm) and bacterial adhesion compared to the SBAE titanium discs (3% versus 11.2% surface area covered with bacteria).[14]

It should be noted, however, that different implantoplasty protocols, implant types, and bacterial species were used in the studies mentioned above. Regarding the implantoplasty protocol used, all studies exclusively utilized diamond burs, without the inclusion of tungsten burs. The findings of certain studies support the use of tungsten and finishing burs, which is in line with the protocol adopted in the present study.[11,27,50–52] Notably, Lasserre et al. study did not include finishing burs, which might have influenced the fact that no significant differences were found in clinical parameter measurements following implantoplasty and glycine air polishing.[45,48] The absence of a bur sequence in Toma et al.'s study may explain the lack of a higher reduction in Ra values and the absence of a significant difference in bacterial adhesion reduction compared to air abrasion and titanium brush alternatives.[45] Currently, there is no standard protocol for implantoplasty, and a wide range of options are available to clinicians.[11] The lack of uniformity in study protocols makes it difficult to compare them. Therefore, it is pertinent to establish a standard implantoplasty protocol in future studies.

In addition, Lassere et al. utilized SLA implant samples, whereas the other studies employed implants treated with various methods such as acid etching, dual acid etching, and plasma spraying.[14,18,44,47,48] Neither acid etching nor dual acid etching exhibit residual sand particles, which are commonly found after SLA treatment. In turn, plasma spraying is an additive surface treatment that can potentially reduce bacterial adhesion depending on the

composition.[32,53] Considering the wide range of surface treatments available, each with its own distinctive properties, further research should examine alternative implant types beyond SLA. Toma et al. and Bürgers et al. evaluated discs rather than whole implants which are far from the clinical reality.[14,45] According to Bermejo et al., bacterial adhesion varies depending on the implant macro anatomy and topographic characteristics.[49] The inclusion of whole implant samples in the present study is a notable strength. Other advantages include being a controlled study that evaluated commercial implants and was able to detect significant differences in the results.

In terms of bacterial strains, Geremias et al. examined the growth of *Streptococcus mutans*, Toma et al. focused on *Streptococcus gordonii*, Bürgers et al. assessed a suspension of *Streptococcus sanguinis*, and the other studies mentioned investigated multi-species biofilms. [14,18,44–49] These variations in bacterial strains diverge from the focus of the present study, which specifically targeted *Streptococcus oralis*. This strain was chosen due to its role as an early colonizer in the human oral cavity. However, it is important to note that the composition of biofilms is extremely complex, and *S. oralis* represents only a small fraction of it. Additionally, it is crucial to acknowledge that conducting this study *in vitro* introduces a notable distinction between the predetermined experimental conditions and the dynamic environment experienced in clinical practice.[49,54] Many investigations have shown that it is relevant to evaluate the amount of biofilm formed, but also the type of bacteria present. Rough implants appear to have a greater tendency to accumulate pathogenic bacteria.[49,55] To address this limitation, a potential improvement would be to expose the implant samples to the natural oral microflora, akin to the *in situ* methodology employed by Azzola et al.[18]

The afore mentioned studies provide evidence of implantoplasty effectiveness in decontaminating the implant surface and providing an optimal surface for the prevention of bacterial colonization, which is further supported by the findings of the current study. However, it is crucial to recognize that other *in vivo* or *in situ* studies have suggested that reduced surface roughness plays only a modest role in preventing biofilm formation. These studies utilized different study designs, including variations in macro anatomy and topography of the samples, bacterial strains, culture conditions, time points, and evaluation methods.[35–39] Despite most studies identified a correlation between surface roughness and bacterial adhesion, it is noteworthy that there are studies that do not find such a correlation. Thus, the relevance of the present study should be acknowledged in this context.

Implantoplasty also presents drawbacks. The current study conducted the implantoplasty procedure under ideal conditions, without considering the surgical challenges commonly encountered in dental practice. However, several clinicians often describe it as a demanding and time-consuming technique. Costa-Berenguer et al., reported an average time of 10 minutes for completion.[25] Also, some find it challenging to achieve satisfactory results with implantoplasty in difficult-to-access implants.[25] There is a risk of potential overheating, which can negatively impact peri-implant tissues and the structural integrity of the implants.[25,51] However, certain studies suggest that this overheating may not have a clinically relevant effect.[52] The release of Titanium particles during implantoplasty can be detrimental to surrounding tissues due to their cytotoxic effect on human osteoblastic and fibroblastic cells, as supported by multiple studies.[3,24,25,56] To mitigate this issue, using a rubber dam during the procedure and irrigating with saline solution or employing low abrasive air-polishing powders like glycine or erythritol can be beneficial.[3,25] Furthermore, there is a possibility that implantoplasty may have a negative impact on biomechanical resistance under loading, particularly in the case of narrow implants and those with internal hexagon and conical connections.[3,25,57,58]

In addition, even after implantoplasty, bacterial adhesion could not be eliminated. Other treatment options and adjunctive measures should also be considered to address peri-implantits. For instance, Lasserre et al. revealed that implantoplasty is as effective as glycine air polishing for the surgical treatment of peri-implantitis.[48] However, the short-term follow-up (6 months) should be considered. Geremias et al., showed promising outcomes for both chemical decontamination using citric acid and implantoplasty treatment.[44] Pommer et al., in an 9-year follow-up, showed therapy via laser decontamination had a higher survival rate (92%) than implantoplasty surgery (87%).[59] Using a surgical protocol consisting of access flaps, surface decontamination with ultrasonics and glycine powder air-polishing, as well as systemic antibiotics, Luengo et al. demonstrated significant improvements in clinical parameters.[60] A systematic review emphasized the benefits of adjunctive reconstructive measures comparatively to access flap surgery.[2] Thus, since there are several treatment options available for peri-implantitis, further research should be encouraged to determine which treatment(s) and adjunctive measures are most effective.

Given the preliminary nature of this study, it is necessary to conduct further investigations involving multispecies biofilms, implant specimens with different surface treatments,

different implantoplasty protocols, and *in vivo* conditions in order to properly assess the efficacy of implantoplasty.

6. Conclusion

Within the limits of this study, it may be concluded that the implantoplasty protocol resulted in a notable decrease in early bacterial adhesion. Nonetheless, additional research is required to ascertain the optimal treatment options for peri-implantitis, considering variations in implantoplasty protocols, bacterial species, as well as variables related to the impact in peri-implant tissues.

7. References

1. Monje A, Pons R, Amerio E, Wang HL, Nart J, Trueta J. Resolution of Peri-implantitis by means of Implantoplasty as adjunct to Surgical Therapy: A Retrospective Study. J Periodontol. 2021 May 15;

2. Ramanauskaite A, Fretwurst T, Schwarz F. Efficacy of alternative or adjunctive measures to conventional non-surgical and surgical treatment of peri-implant mucositis and peri-implantitis: a systematic review and meta-analysis. Int J Implant Dent. 2021 Dec;7(1).

3. Rokaya D, Srimaneepong V, Wisitrasameewon W, Humagain M, Thunyakitpisal P. Peri-implantitis update: Risk indicators, diagnosis, and treatment. Vol. 14, European Journal of Dentistry. Georg Thieme Verlag; 2020. p. 672–82.

4. Schwarz F, Derks J, Monje A, Wang HL. Peri-implantitis. Vol. 89, Journal of periodontology. NLM (Medline); 2018. p. S267–90.

5. Prathapachandran J, Suresh N. Management of peri-implantitis. Dent Res J (Isfahan). 2012 Sep;516–21.

6. Tord Berglundh. Peri-implant health, peri-implant mucositis and peri-implantitis - Guidance for clinicians. 2019 Mar.

7. G. Caton J, Armitage G, Berglundh T, Chapple ILC, Jepsen S, S. Kornman K, et al. A new classification scheme for periodontal and peri-implant diseases and conditions – Introduction and key changes from the 1999 classification. J Clin Periodontol. 2018 Jun 1;45:S1–8.

8. Christopher C.K. Ho. Biological Complications. In: Christopher C. K. Ho, editor. Practical Procedures in Implant Dentistry. 1st ed. John Wiley & Sons Ltd.; 2021. p. 351–69.

9. Beheshti Maal M, Verket A. Implantoplasty- provoking or reducing inflammation?–a systematic scoping review. Vol. 80, Acta Odontologica Scandinavica. Taylor and Francis Ltd.; 2022. p. 105–16.

10. Rakic M, Galindo-Moreno P, Monje A, Radovanovic S, Wang HL, Cochran D, et al. How frequent does peri-implantitis occur? A systematic review and meta-analysis. Clin Oral Investig. 2018 May 1;22(4):1805–16.

11. Beheshti Maal M, Aanerød Ellingsen S, Reseland JE, Verket A. Experimental implantoplasty outcomes correlate with fibroblast growth in vitro. BMC Oral Health. 2020 Jan 30;20(1).

12. Dhir S. Biofilm and dental implant: The microbial link. Vol. 17, Journal of Indian Society of Periodontology. 2013. p. 5–11.

13. Sterzenbach T, Helbig R, Hannig C, Hannig M. Bioadhesion in the oral cavity and approaches for biofilm management by surface modifications. Clin Oral Investig. 2020;24(12):4237–60.

14. Bürgers R, Gerlach T, Hahnel S, Schwarz F, Handel G, Gosau M. In vivo and in vitro biofilm formation on two different titanium implant surfaces. Clin Oral Implants Res. 2010 Feb;21(2):156–64.

15. Subramani K, Jung RE, Molenberg A, Hämmerle CHF. Biofilm on Dental Implants: A Review of the Literature. Int J Oral Maxillofac Implants. 2009;24(4).

16. Meier RM, Pfammatter C, Zitzmann NU, Filippi A, Kühl S. Surface Quality after Implantoplasty. Schweiz Monatsschr Zahnmed. 2012 Dec 16;122(9):714–24.

17. Nguyen-Hieu T, Borghetti A, Aboudharam G. Peri-implantitis: from diagnosis to therapeutics. Vol. 3, Journal of investigative and clinical dentistry. 2012. p. 79–94.

18. Azzola F, Ionescu AC, Ottobelli M, Cavalli N, Brambilla E, Corbella S, et al. Biofilm formation on dental implant surface treated by implantoplasty: An in situ study. Dent J (Basel). 2020 Jun 1;8(2).

19. Tawse-Smith A, Kota A, Jayaweera Y, van Vuuren WJ, Ma S. The effect of standardised implantoplasty protocol on titanium surface roughness: an in-vitro study. Braz Oral Res. 2016;30(1):1–11.

20. Engel AS, Kranz HT, Schneider M, Tietze JP, Piwowarcyk A, Kuzius T, et al. Biofilm formation on different dental restorative materials in the oral cavity. BMC Oral Health. 2020 Jun 3;20(1).

21. Teughels W, Assche N Van, Sliepen I, Quirynen M, Van Assche N. Effect of material characteristics and/or surface topography on biofilm development. Vol. 17, Clin. Oral Imp. Res. 2006.

22. Schwarz F, Alcoforado G, Guerrero A, Jönsson D, Klinge B, Lang N, et al. Peri-implantitis: Summary and consensus statements of group 3. The 6th EAO Consensus Conference 2021. Clin Oral Implants Res. 2021 Oct 1;32(S21):245–53.

23. Renvert S, Polyzois I, Claffey N. Surgical therapy for the control of peri-implantitis. Clin Oral Implants Res. 2012 Oct;23(SUPPL.6):84–94.

24. Toledano-Serrabona J, Gil FJ, Camps-Font O, Valmaseda-Castellón E, Gay-Escoda C, Sánchez-Garcés MÁ. Physicochemical and biological characterization of ti6al4v particles obtained by implantoplasty: An in vitro study. part i. Materials. 2021 Nov 1;14(21).

25. Costa-Berenguer X, García-García M, Sánchez-Torres A, Sanz-Alonso M, Figueiredo R, Valmaseda-Castellón E. Effect of implantoplasty on fracture resistance and surface roughness of standard diameter dental implants. Clin Oral Implants Res. 2018 Jan 1;29(1):46–54.

26. Lozano P, Peña M, Herrero-Climent M, Rios-Santos JV, Rios-Carrasco B, Brizuela A, et al. Corrosion Behavior of Titanium Dental Implants with Implantoplasty. Materials. 2022 Feb 1;15(4).

27. Yildiz H, Bertl K, Stavropoulos A. Titanium implant surface roughness after different implantoplasty protocols: A laboratory study. Clin Exp Dent Res. 2022 Dec 1;8(6):1315–21.

28. Yildiz H, Sen E, Dalcik H, Meseli SE. Evaluation of cell morphology and adhesion capacity of human gingival fibroblasts on titanium discs with different roughened surfaces: an in vitro scanning electron microscope analysis and cell culture study. Folia Morphol (Warsz). 2023;82(1):63–71.

29. Ramel CF, Lüssi A, Özcan M, Jung RE, Hämmerle CHF, Thoma DS. Surface roughness of dental implants and treatment time using six different implantoplasty procedures. Clin Oral Implants Res. 2016 Jul 1;27(7):776–81.

30. Nicolas-Silvente AI, Velasco-Ortega E, Ortiz-Garcia I, Monsalve-Guil L, Gil J, Jimenez-Guerra A. Influence of the titanium implant surface treatment on the surface roughness and chemical composition. Materials. 2020 Jan 1;13(2).

31. Jonathan Du Toit. Implant Materials, Designs, and Surfaces. In: Christopher C.K. Ho, editor. Practical Procedures in Implant Dentistry. 1st ed. John Wiley & Sons Ltd.; 2021. p. 87–102.

32. Inchingolo AM, Malcangi G, Ferrante L, Del Vecchio G, Viapiano F, Inchingolo AD, et al. Surface Coatings of Dental Implants: A Review. J Funct Biomater. 2023 May 22;14(5):287.

33. Alipal J, Lee TC, Koshy P, Abdullah HZ, Idris MI. Evolution of anodised titanium for implant applications. Vol. 7, Heliyon. Elsevier Ltd; 2021.

34. Rupp F, Gittens RA, Scheideler L, Marmur A, Boyan BD, Schwartz Z, et al. A review on the wettability of dental implant surfaces I: Theoretical and experimental aspects. Vol. 10, Acta Biomater. Elsevier Ltd; 2014. p. 2894–906.

35. de Melo F, do Nascimento C, Souza DO, de Albuquerque RF. Identification of oral bacteria on titanium implant surfaces by 16S rDNA sequencing. Clin Oral Implants Res. 2017 Jun 1;28(6):697–703.

36. Ferreira Ribeiro C, Cogo-Müller K, Franco GC, Silva-Concílio LR, Sampaio Campos M, De Mello Rode S, et al. Initial oral biofilm formation on titanium implants with different surface treatments: An in vivo study. Arch Oral Biol. 2016 Sep 1;69:33–9.

37. Conserva E, Generali L, Bandieri A, Cavani F, Borghi F, Consolo U. Plaque accumulation on titanium disks with different surface treatments: an in vivo investigation. Odontology. 2018 Apr 1;106(2):145–53.

38. Al-Ahmad A, Karygianni L, Wartenhorst MS, Bächle M, Hellwig E, Follo M, et al. Bacterial adhesion and biofilm formation on yttriastabilized, tetragonal zirconia and titanium oral implant materials with low surface roughness - An in situ study. J Med Microbiol. 2016 Jul 1;65(7):596–604.

39. 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 Jun 1;75(6):766–72.

40. BLT External Implant system [Internet]. [cited 2023 May 5]. Available from: http://www.ebiimplant.com/eng/page/sub_03_01_04_01.html

41. Biobank C. Bacteria Collection: Streptococcus oralis. Bridge P. D. & Sneath P. H. A. 1982 Int J Syst Bact 32 410 415; Carlsson J 1965.

42. Dantas T, Padrão J, da Silva MR, Pinto P, Madeira S, Vaz P, et al. Bacteria co-culture adhesion on different texturized zirconia surfaces. J Mech Behav Biomed Mater. 2021 Nov 1;123.

43. Lakens D. Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and ANOVAs. Front Psychol. 2013 Nov 26;4:863.

44. Geremias TC, Montero JFD, Magini RDS, Schuldt Filho G, De Magalhães EB, Bianchini MA. Biofilm Analysis of Retrieved Dental Implants after Different Peri-Implantitis Treatments. Case Rep Dent. 2017 Apr 9;2017.

45. Toma S, Behets C, Brecx MC, Lasserre JF. In vitro comparison of the efficacy of peri-implantitis treatments on the removal and recolonization of streptococcus gordonii biofilm on titanium disks. Materials. 2018 Dec 6;11(12).

46. Romeo E, Lops D, Chiapasco M, Ghisolfi M, Vogel G. Therapy of peri-implantitis with resective surgery. A 3-year clinical trial on rough screw-shaped oral implants. Part II: Radiographic outcome. Clin Oral Implants Res. 2007 Apr;18(2):179–87.

47. Romeo E, Ghisolfi M, Murgolo N, Chiapasco M, Lops D, Vogel G. Therapy of peri-implantitis with resective surgery: A 3-year clinical trial on rough screw-shaped oral implants. Part I: Clinical outcome. Clin Oral Implants Res. 2005 Feb;16(1):9–18.

48. Lasserre J, Brecx M, Toma S. Implantoplasty Versus Glycine Air Abrasion for the Surgical Treatment of Peri-implantitis: A Randomized Clinical Trial. Int J Oral Maxillofac Implants. 2020 Jan;35(1):197–206.

49. Bermejo P, Sánchez MC, Llama-Palacios A, Figuero E, Herrera D, Sanz Alonso M. Biofilm formation on dental implants with different surface micro-topography: An in vitro study. Clin Oral Implants Res. 2019;30(8):725–34.

50. Sahrmann P, Luso S, Mueller C, Ender A, Attin T, Stawarczyk B, et al. Titanium Implant Characteristics After Implantoplasty: An In Vitro Study on Two Different Kinds of Instrumentation. Int J Oral Maxillofac Implants. 2019 Nov;34(6):1299–305.

51. De Souza Júnior JM, De Souza JGO, Neto ALP, Iaculli F, Piattelli A, Bianchini MA. Analysis of effectiveness of different rotational instruments in implantoplasty: An in vitro study. Implant Dent. 2016 Jun 1;25(3):341–7.

52. 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 Apr;15(2):292–6.

53. Al Mugeiren OM, Baseer MA. Dental implant bioactive surface modifiers: An update. Vol. 9, Journal of International Society of Preventive and Community Dentistry. Wolters Kluwer (UK) Ltd.; 2019. p. 5–12.

54. 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 Jun 1;75(6):766–72.

55. Bürgers R, Gerlach T, Hahnel S, Schwarz F, Handel G, Gosau M. In vivo and in vitro biofilm formation on two different titanium implant surfaces. Clin Oral Implants Res. 2010 Feb;21(2):156–64.

56. Suárez-López del Amo F, Garaicoa-Pazmiño C, Fretwurst T, Castilho RM, Squarize CH. Dental implants-associated release of titanium particles: A systematic review. Vol. 29, Clinical Oral Implants Research. Blackwell Munksgaard; 2018. p. 1085–100.

57. Camps-Font O, González-Barnadas A, Mir-Mari J, Figueiredo R, Gay-Escoda C, Valmaseda-Castellón E. Fracture resistance after implantoplasty in three implant-abutment connection designs. Med Oral Patol Oral Cir Bucal. 2020 Sep 1;25(5):691–9.

58. Chan HL, Oh WS, Ong HS, Fu JH, Steigmann M, Sierraalta M, et al. Impact of Implantoplasty on Strength of the Implant-Abutment Complex. Int J Oral Maxillofac Implants. 2013;28(6):1530–5.

59. Pommer B, Haas R, Mailath-Pokorny G, Fürhauser R, Watzek G, Busenlechner D, et al. Periimplantitis Treatment: Long-Term Comparison of Laser Decontamination and Implantoplasty Surgery. Implant Dent. 2016 Oct 1;25(5):646–9.

60. Luengo F, Solonko M, Sanz-Esporrín J, Sanz-Sánchez I, Herrera D, Sanz M. Clinical, Microbiological, and Biochemical Impact of the Surgical Treatment of Peri-Implantitis—A Prospective Case Series. J Clin Med. 2022 Aug 1;11(16).

8. Annex

8.1. Comparative analysis of implantoplasty protocols in scientific literature

SCIENTIFIC LITERATURE	IMPLANTOPLASTY PROTOCOLS
AZZOLA ET AL. 2020 **BIOFILM FORMATION ON DENTAL IMPLANT SURFACE TREATED BY IMPLANTOPLASTY: AN IN SITU STUDY.**	*diamond, 30-µm particle size, egg-shaped bur* *diamond, 15-µm particle size, egg-shaped bur (Komet, Gerb. Brasseler GmbH, Lemgo, Germany)* *Arkansas burs* *silicon polishers (Shofu Inc., Kyoto, Japan).*
MAAL ET AL. 2020 **EXPERIMENTAL IMPLANTOPLASTY OUTCOMES CORRELATE WITH FIBROBLAST GROWTH IN VITRO.**	*Carbide cutting burs, red (normal toothing) and white (fine toothing)* *Carbide cutting burs, red (normal toothing) and white (fine toothing) + Arkansas stone 661* *Carbide cutting burs red (normal) and white (fine toothing) + Silicone cup brownie 030 + Silicone cup greenie 030* *Diamond sequence of decreasing coarseness 105 µm, 40 µm, 8 µm* *Diamond sequence of decreasing coarseness 105 µm, 40 µm, 8 µm + Arkansas stone 661* *Diamond sequence of decreasing coarseness 105 µm, 40 µm, 8 µm + Silicone cups brownie 030 + Silicone cup greenie 030*
COSTA-BERENGUER ET AL. 2017 **EFFECT OF IMPLANTOPLASTY ON FRACTURE RESISTANCE AND SURFACE ROUGHNESS OF STANDARD DIAMETER DENTAL IMPLANTS.**	*oval-shape tungsten carbide bur (H379 314 023; Komet Dental, Lemgo, Germany)* *two silicon carbide polishers (9618 314 030 and 9608 314 030, Komet Dental, Lemgo, Germany)*
TAWSE-SMITH ET AL. 2016 **THE EFFECT OF STANDARDISED IMPLANTOPLASTY PROTOCOL ON TITANIUM SURFACE ROUGHNESS: AN IN-VITRO STUDY.**	*Shofu(tm) regular diamond (reference: 107RD)* *Super-fine grit diamond (reference: SF107RD)* *Brownie(tm) (reference: PN0401)* *Greenie(tm) (reference: PN040).*
LOZANO ET AL. 2022 **CORROSION BEHAVIOR OF TITANIUM DENTAL IMPLANTS WITH IMPLANTOPLASTY.**	*Small-grained WC bur (reference H37931. 018 followed H37UF and H37931023, (Brasseler, KOMET; GmbH & Co., KG, Lemgo, Germany)* *Coarse-grained diamond polisher (Rugbyno. 9608.314.030 KOMET; GmbH & Co., KG, Lemgo, Germany)* *Small-grained SiC polisher Arkansas* *Finishing amalgam (order no. 9618.314.553 KOMET; GmbH & Co., KG, Lemgo, Germany).*
YILDIZ ET AL. 2022 **TITANIUM IMPLANT SURFACE ROUGHNESS AFTER DIFFERENT IMPLANTOPLASTY PROTOCOLS: A LABORATORY STUDY.**	*2 tungsten carbide burs with standard (redring) and extra-fine (white ring) toothing (Komet Dental);* *2 tungsten carbide burs with standard (no color) and extra-fine (whitering) toothing (Hager & Meisinger GmbH)* *3 diamond burswith decreasing grit (125 [green ring], 40 [red ring], and 15µm [whitering]) (Komet Dental).* *Silicone polisher (Brownie®; Shofu Dental GmbH)*

<u>YILDIZ ET AL. 2022</u> **EVALUATION OF CELL MORPHOLOGY AND ADHESION CAPACITY OF HUMAN GINGIVAL FIBROBLASTS ON TITANIUM DISCS WITH DIFFERENT ROUGHENED SURFACES: AN IN VITRO SCANNING ELECTRON MICROSCOPE ANALYSIS AND CELL CULTURE STUDY**	*Round-tipped red diamond bur (40µm grit, Dimei Royal, China) + 4 round-tipped white diamond bur (15µm grit, Komet Dental, Germany) + Brownie silicone* *Round-tipped green diamond bur (125µm grit, Dimei Royal, China) + around-tipped white diamond bur (15µm grit, Komet Dental; Germany) + Brownie silicone*
<u>RAMEL ET AL. 2016</u> **SURFACE ROUGHNESS OF DENTAL IMPLANTS AND TREATMENT TIME USING SIX DIFFERENT IMPLANTOPLASTY PROCEDURES.**	*Diamond burs 106-, 40- and 15-lm grit + Brownie + Greenie* *Diamond burs 106-, 40- and 15-lm grit + Arkansas stone* *Diamond burs 106-, 40- and 4-lm grit* *Diamond burs 106-, 40- and 4-lm grit + Greenie* *Diamond burs 106-, 40-, 15-, 8- and 4-lm grit* *Diamond burs 106-, 40-, 15-, 8- and 4-lm grit + Greenie*