TREATMENT OF PERI-IMPLANTITIS

NON-SURGICAL AND RESECTIVE SURGICAL APPROACHES

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non-surgical and resective surgical approaches

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Printing: Ridderprint, www.ridderprint.nl
Layout and design: Marilou Maes, persoonlijkproefschrift.nl

rijksuniversiteit groningen

Treatment of peri-implantitis

non-surgical and resective surgical approaches

Proefschrift

ter verkrijging van de graad van doctor aan de
Rijksuniversiteit Groningen
op gezag van de
rector magnificus prof. dr. C. Wijmenga
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

woensdag 26 januari 2022 om 12.45 uur

door

Diederik Floris Maurits Hentenaar

geboren op 26 maart 1988
te Geldrop

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CHAPTER 1

GENERAL INTRODUCTION

Over the past decades, dental implantology has been successfully integrated into the field of modern dentistry (Buser et al. 2017). Dental implants have shown good long term results with survival rates of 93% and 84% after 20 and 25 years (Bakker et al. 2019, Jemt 2018). In addition, dental implant placement is considered a feasible treatment in almost any medically compromised patient, when the required preventive measures are taken and follow-up care is at a high level (Doll et al. 2015, Vissink et al. 2018). Although accurate data about the number of implants placed worldwide is lacking, a yearly increase in the U.S. between 1999 and 2016 of 14% is reported (Elani et al. 2018). Moreover, an increasing trend of implant placement ranging from 6% to 23% up to 2026, has been estimated (Elani et al. 2018). Despite the increased popularity of dental implant placement, challenges in regard to preservation of hard and soft tissue health around the implant are seen (Lang et al. 2019). Characterized by inflammatory processes affecting these tissues, one of the biggest challenges clinicians are facing today are peri-implant diseases, specifically known as peri-implant mucositis and peri-implantitis (Lang & Berglundh 2011).

Anatomical comparisons and differences between implants and teeth

Following implant placement, peri-implant hard and soft tissues are formed as a result of a wound-healing process (Eggert & Levin 2018). The formation of new bone (hard tissue) in contact with the implant is recognised as osseointegration, while the establishment of peri-implant mucosa (soft tissue) includes the build-up of a junctional epithelium and a connective-tissue zone in contact with components of the implant/suprastructure (Albrektsson et al. 1981). Osseointegrated implants are directly anchored to the bone and hence, compared to the presence around natural teeth, lack periodontal ligament (PDL). As a result, blood supply is limited to the supraperiostal vessels, thereby restricting the amount of nutrients and immune cells that may extravasate to tackle bacterial infection. Both the natural tooth and the implant show equivalence in the form of presence of junctional epithelial attachment, however, a major difference between teeth and implants is the absence around implants of organized groups of collagenous connective tissue fibers that insert into root cementum, bone, and soft gingival tissues (Eggert & Levin 2018). Instead, connective tissue fibers around implants/suprastructures are organized in a vertical manner (see figure 1).

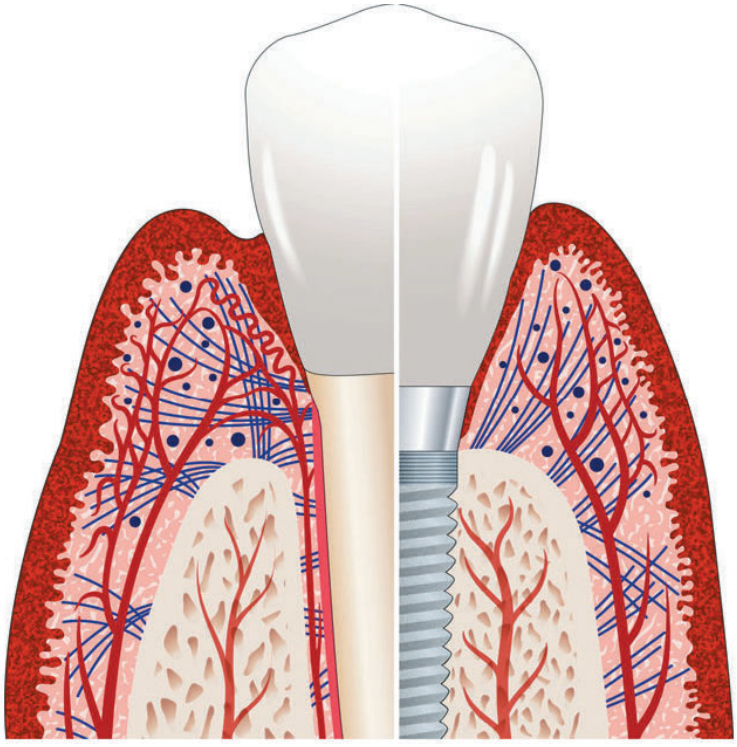

Figure 1. Schematic illustration of hard and soft tissue around a tooth and an implant. Copyright Renvert-Giovannoli Periimplantitis, Quintessence International, 2012

It is thought that the absence of the perpendicular attached connective tissue fibers cause the implant to have a weaker defence mechanism i.e., weaker peri-implant soft tissue seal/cuff to bacterial invasion and places peri-implant tissues in an 'open wound' conformation (Kim et al. 2019). Histologically, it has been shown that inflammatory reactions of peri-implant lesions extend apical of the junctional epithelium and become larger around implant sites than those around natural teeth (Berglundh et al. 2011). In addition, a faster progression of disease is observed with the occurrence in a non-linear and accelerating pattern (Fransson et al. 2010).

Classification and definition of peri-implant diseases and conditions

A classification of peri-implant diseases and conditions was for the first time addressed in the 2017 World Workshop (Berglundh et al. 2018). Although definitions of peri-implant diseases had been previously presented, the term 'definition' often provoked misunderstanding. Therefore the aim was to distinguish disease definition from case definition. Disease definitions are descriptive and present the typical characteristics of the disease, whereas case definitions should provide the clinical guidelines for diagnosis,

i.e. how to assess the condition. Hence, the current classification is described with the following disease definitions: *peri-implant health* is characterised by the absence of clinical signs of inflammation, such as swelling, redness, and bleeding on probing (BoP) (Araujo & Lindhe 2018). *Peri-implant mucositis* is characterised by an inflammatory lesion in the soft tissues surrounding an implant in the absence of loss of supporting bone (Heitz-Mayfield & Salvi 2018) and *peri-implantitis* is a plaque-associated pathological condition that occurs in tissues around dental implants. Peri-implantitis is characterised by inflammation in the peri-implant mucosa and loss of supporting bone (Schwarz et al. 2018). Subsequently, the currently used case definitions in day-to-day clinical practice are:

Peri-implant health

- absence of clinical signs of inflammation;
- absence of bleeding/suppuration on gentle probing;
- no increase in probing depth compared to previous examinations;
- no bone loss.

Peri-implant mucositis

- bleeding and/or suppuration on gentle probing;
- no bone loss.

Peri-implantitis

- bleeding and/or suppuration on gentle probing;
- increased probing depth compared to previous examinations;
- bone loss.

Importantly, if previous diagnostic data are not available, the diagnosis of peri-implantitis can be made based on the presence of bleeding and/or suppuration on gentle probing, probing depths ≥6 mm, and bone levels ≥3 mm apical to the most coronal portion of the intraosseous part of the implant.

Etiopathogenesis

Peri-implant soft tissue inflammatory reactions can be initiated by several factors such as excess cement remnants, inadequate/loose restoration-abutment connections, implant malposition, and fracture of implant components (Atieh et al. 2020). However, a major etiological role is reserved for bacteria as trigger of the host defence mechanism (Belibasakis et al. 2015, Belibasakis & Manoil 2021). Embedded in a self-produced matrix, these microbial communities form a biofilm which adheres to the surface of the implant and implant supported restoration (Lee & Wang 2010). These biofilms represent a

significant health risk because of their resistance to the host defence mechanisms and their decreased susceptibility to conventional antimicrobial agents (Koo et al. 2017, Gebreyohannes et al. 2019). Experimental studies in humans showed similar patterns of disease initiation around dental implants and natural teeth and confirmed the direct cause and effect relationship between biofilm accumulation and peri-implant mucositis (Pontoriero et al. 1994, Zitzmann et al. 2001). Animal studies confirmed the alteration of peri-implant mucosa following biofilm accumulation with migration of leukocytes through the sulcular and junctional epithelium, formation of inflammatory infiltrate, and increased proportions of T and B cells in the adjacent connective tissue around dental implants and natural teeth (Berglundh et al. 1992, Ericsson et al. 1992). On the other hand, the progression from peri-implant mucositis to peri-implantitis and correspondingly the etiology of loss of marginal bone around the implant is subject of debate in the current literature. In recent years, studies appeared which questioned the 'old Scandinavian concept' of marginal bone loss around dental implants, which concept is based on the original notion that peri-implantitis is the same bacterial disease as periodontitis and primarily related to bacterial infection (Fransson et al. 2005). A new concept was introduced in which marginal bone loss around an initially osseointegrated implant is generally thought to be an example of an imbalance in the foreign body reaction due to non-optimal implant components, surgery, prosthodontics and/or compromised patient factors (Albrektsson et al. 2014, Albrektsson et al. 2016, Albrektsson et al. 2017, Coli & Jemt 2021). In addition, such marginal bone loss, which is thought to be an example of aseptic loosening, might occur as a result of sudden overloading of a previously successful implant or as a reaction to cement residues from the prosthodontics treatment. Secondary to such a situation an infection may develop, with clinical symptoms such as suppuration, swelling, pain and further bone loss – i.e. peri-implantitis.

Prevalence of peri-implantitis

To this day, difficulty remains in getting a true picture of the current prevalence of peri-implant diseases since studies performed on the prevalence of disease yield a high heterogeneity (Cosgarea et al. 2019). Generally, subject-based weighted mean prevalences and ranges are estimated around 43% (range: 19%-65%) for peri-implant mucositis and to 22% (range: 1%-47%) for peri-implantitis (Derks & Tomasi 2015, Atieh et al. 2013, Lee et al. 2017). Differences between studies in terms of definitions criteria (i.e., using different cut-off thresholds of marginal bone loss), patient sampling, clinical scenarios (with different time of follow-up) and the levels of reporting (i.e., implant vs. patient) complicate comparisons and precise evaluation of the global burden of the disease. A systematic review by Derks & Tomasi (2015) found eight different definitions for how much radiographic bone loss was required to diagnose peri-implantitis.

For example, studies defining a threshold of marginal bone loss >5 mm yielded a prevalence of peri-implantitis of 1% (Zetterqvist et al. 2010) whereas a threshold of 0.4 mm marginal bone loss increased the prevalence of peri-implantitis to 47% (Koldsland et al. 2010). Studies in which the newly presented diagnostic 2017 World Workshop criteria were applied remain rare. However, interestingly, a research group of Shimchuk and coworkers re-evaluated their own previous used data set with the new 2017 criteria, concluded a drop in peri-implantitis at both patient and implant level of nearly 50% compared to the prior analysis (Shimchuk et al. 2020). This might suggest that prevalence rates reported in previous published literature present an overestimation of disease prevalence. However, more studies using these new criteria are needed to confirm this finding. Nevertheless, despite a lack of consensus on the prevalence, a PubMed search (up to August 2021) using the words 'Peri-implantitis' AND 'Treatment', shows an exploding number of studies over the last 30 years, indicating an increase of interest within the scientific field of research on this topic (Marcantonio Junior et al. 2019). Moreover, a general consensus under clinical experts on an expected increase of peri-implanitis prevalence up to 2030 is reported (Sanz et al. 2019).

Risk factor

Peri-implant diseases have been linked to a large number of potential patient and implant-related risk factors/indicators (Monje et al. 2019, Maney et al. 2020). Risk factors are causal agents of a disease which are usually confirmed by longitudinal studies, whereas risk indicators are based on cross-sectional data (Beck, 1998). There is strong evidence that the presence and history of periodontitis increases the risk for peri-implantitis and that poor oral hygiene and lack of compliance with regular maintenance therapy may play an important role (Dreyer et al. 2018). The evidence for an association with peri-implantitis remains equivocal regarding cigarette smoking and diabetes mellitus, these modifying factors are considered as potential risk indicators or emerging risk factors (Schwarz et al. 2018). In addition, bone quality, obesity, metabolic syndrome, implant surface characteristics and placement depth have also been reported to be predisposing factors for the development of peri-implantitis. Other factors that may play a role but currently not well-understood are certain medications, age, gender, low vitamin D, autoimmune diseases, amount of keratinized mucosa and peri-implant tissue-bound titanium particles (Araujo & Lindhe 2018, Delgado-Ruiz & Romanos 2018, Mombelli et al. 2018, Safioti et al. 2017). The role of genetics is still unclear, but studies show that certain polymorphisms may be associated with peri-implantitis (Laine et al. 2006).

Prosthetic risk factors, such as improper restorative design, occlusal overload, microgap, and residual cement are considered significant as well (Misch et al. 2005, Dixon &

London 2019). In terms of proper restorative prosthetic design, it is recommended that implant suprastructures should be designed in such a way that oral hygiene measures can be performed effectively, plaque accumulation is prevented and implants are accessible for probing (Serino & Ström 2009). An over-contoured prosthetic design may limit the effect of peri-implantitis treatment modalities. To date, prosthetically focused studies mainly concern aesthetic outcome (Barwacz et al. 2018, Esposito et al. 2018, Linkevicius et al. 2015). However, the ideal crown contour in terms of emergence angle and emergence profile with respect to preserving marginal bone level and peri-implant soft tissue health remains unclear.

Biomarkers in per-implant disease diagnosis

The host inflammatory response to the microbial challenge may induce specific inflammatory responses/signatures around dental implants. During the active state of disease, inflammatory markers such as cytokines, proteinases and local tissue-degradation products are released into the peri-implant crevicular fluid (PICF) (Carinci et al. 2019). It is thought that these biomarkers could serve as adjunctive parameters to ameliorate the diagnosis and management of peri-implant disease and help to differentiate between different conditions of peri-implant health (Ramseier et al. 2009, Sexton et al. 2011, Syndergaard et al. 2014, Kinney et al. 2014, Carinci et al. 2019). Since traditional clinical diagnostic methods, such as bleeding on probing, pocket depth measurement, and radiographic assessment seem to exert a weak sensitivity/specificity to diagnose peri-implant disease, identifying potential markers seems necessary (Heitz-Mayfield 2008, Hashim et al. 2018, Monje et al. 2018, Monje et al. 2020). To date, most studies have focused on a limited number of biomarkers, including pro-inflammatory cytokines such as interleukin-1β, tumor necrosis factor-α and/or interleukin-6 (Alassy et al. 2019). Studies on potential important anti-inflammatory biomarkers, collagen degradation enzymes, osteoclastogenesis-related cytokines and chemokines to diagnose peri-implant health, have however never been properly performed (Duarte et al. 2016). Hence, whether biomarkers in PICF could potentially flank clinical and radiographical examination in monitoring peri-implant tissue health remains to be established.

THERAPY

The primary goal of peri-implantitis treatment is to re-establish and maintain a long-term state of peri-implant health. Ideally, complete resolution of disease should be attained in combination with bone and soft tissue regeneration and re-integration on the implant surface. An important step believed to successfully resolve peri-implantitis is to efficiently decontaminate the implant surface and/or debride the peri-implant area

(removal of necrotic/granulation tissue) and treat periodontitis if present. Hence, the implant surface should be decontaminated from the adhered biofilm, calculus and/or necrotic bone and the bacterial colonization should be reduced to an extent that is compatible with peri-implant health. Many of the existing therapies used to maintain and treat infected implants have been developed from the treatment of natural teeth. Therapies range from mechanical approaches to chemical agents and light mediated therapy (Ntrouka et al. 2011, Louropoulou 2014, Abrahammi 2019, Jungbauer et al. 2021).

Traditionally, mechanical debridement is performed using curettes and/or ultrasonic devices. However, compared to the smooth natural tooth surface the implant surface has areas which are protected, with inaccessible parts to the conventional professional instruments. At the macro-level most implants have threads, which impede with the action of hand scalers and ultrasonic scalers. In other words, although they might touch the outer parts of the threads, difficulty remains in reaching areas between the threads. Moreover, on the microscopic level, crevices on the roughened surfaces can harbor bacteria that defy effective debridement. Hence, the clinical effect of various types of curettes ranging from stainless steel to carbon-fiber and teflon, in the treatment of peri-implant disease, is limited. In general, these studies show a reduction in bleeding on probing in the short term (up to 3-months) but a resolution of disease is rarely achieved.

Ultrasonic devices are also proposed for debridement of the implant surface. Modern ultrasonic scalers, which mainly fall into two categories: piezoelectric and magneto-strictive devices, exert a cleaning action which is principally based on vibrational energy. Effective debridement is however limited by how much contact the oscillating tip has with the surface area to shatter surface deposits. In addition, ultrasonic scalers are also able to create cavitation, which is characterized by formation and rapid collapse of gas or vapour bubbles in a fluid (Brennen 1995, Vyas et al. 2020). The forces generated from cavitation bubbles and acoustic streaming (fluid flow) are thought to remove the bacterial biofilm. While traditional (metal) ultrasonic tips are available, dedicated ultrasonic tips made of implant compatible materials (i.e., carbon fiber, silicone or poly ether ether ketone (PEEK) /plastic) have been proposed to treat the implant surface to minimize scratches on the implant surfaces. Ultrasonic therapy seems able to reduce clinical signs of inflammation to a greater extent than carbon fiber/titanium curettes in the non-surgical treatment of peri-implantitis (Karring et al. 2005; Renvert et al. 2009). However, resolution of disease has not been reported following the use of ultrasonic therapy.

To date, most promising in cleansing the implant surface seems the use of particle beam devices *i.e.* air-polishing / air abrasion (Louropoulou et al. 2014). The superiority of air-polishing methods over the use of rigid instruments with regard to cleaning efficacy and surface damage in non-surgical/covered and surgical/open *in vitro* models, has been repeatedly proven (Keim et al. 2019, Ronay et al. 2017, Sahrmann et al. 2015). As described by Petersilka (2011), the powder-water ejection of air-polishing is subject to the additional so-called ricochet effect. For particles that hit a hard surface, this effect describes an uncontrolled rebound, bounce, or skip off a surface which may have an influence on the cleaning efficacy. According to the mode of clinical application (supra- or subgingival) tip designs vary since these require different angulations for applying the particle beam at the appropriate working distance from the surface being cleaned. Previous clinical non-surgical peri-implantitis treatment studies using air-polishing reported small sample sizes, different peri-implantitis case definitions and primarily the use of a single type of investigative powder (i.e., glycine) (John et al. 2015, Karring et al. 2005, Renvert et al.2011). From these studies it was concluded that non-surgical therapy shows modest improvements and limited predictability in the resolution of mucosal inflammation (Heitz-Mayfield & Mombelli 2014, Suárez-López del Amo et al. 2016, Schwarz et al. 2016).

One of the challenges clinicians are facing when trying to effectively detoxify the implant surface, in order for the implant tissues to re-integrate, is to preserve the implant topographical and chemical composition. In the search for an air-polishing powder that does not or hardly alters the implant surface and maintains the biocompatibility, a new low-abrasive powder, i.e. erythritol, was introduced to the dental field. This powder, which is a sugar alcohol similar to xylitol and used as sugar substitute, is non-caloric, has a high gastrointestinal tolerance and does not increase blood glucose or insulin levels (de Cock 1999, de Cock 2018). *In vitro* studies report that erythritol seems to be more effective in terms of cleaning efficacy compared to previously used powders (e.g., glycine and natriumbicarbonate) (Drago et al. 2014, Moharrami et al. 2018). Moreover, studies describe a more effective reduction in the bacterial biofilm and inhibition of post-treatment biofilm re-growth, improved cell attachment, cell viability, and proliferation of osteoblasts, anticipating promising effects of this powder in a clinical setting (Drago et al., 2017, Matthes et al. 2017, Mensi et al. 2018, Tastepe et al. 2018). Clinical periodontal maintenance studies on subgingival air-polishing with erythritol powder, report comparable clinical and microbiological effects to ultrasonic therapy (Müller et al. 2014). However, thus far, studies on the treatment of peri-implantitis with eythritol air-polishing are lacking.

In addition to mechanical approaches, chemical agents to disinfect the implant surface such as chlorhexidine digluconate 0.2%/0.12%, sodium hypochlorite and hydrogen peroxide 3% have also previously been studied in different clinical trials (Gosau et al. 2010, Heitz-Mayfield et al. 2012, De Waal et al 2013, De Waal et al. 2015). However, no superior clinical effectiveness has been shown in a single study for a specific chemical decontamination protocol (Ntrouka et al. 2011, Subramani & Wismeijer 2012, Meyle 2012). Studies using acids at low pH (< 2) have shown potentially beneficial antiseptic effects (Zablotsky et al. 1992, Dennison et al. 1994, Strooker et al. 1998, Wohlfahrt et al. 2012, Wiltfang et al. 2012, Htet et al. 2016). For example, animal studies showed re-osseointegration and direct bone-to-implant contact when acids were used (Kolonidis et al. 2003, Alhag et al. 2008). Also, acid-etching might positively influence the epithelial seal around dental implants (An et al. 2012). Clinical studies that evaluated the use of phosphoric acid (pH 1) showed an instant greater reduction of colony forming units (Wiltfang et al. 2012, Strooker et al. 1998). However, use of phosphoric acid etching gel as decontaminating agent has not been evaluated in a randomized controlled trial.

Conclusively, a number of mechanical intervention methods, solely or in combination with chemical agents, have been described over the years in *in vivo* and *in vitro* studies, as well as in a non-surgical and surgical setting (Leonhardt et al. 2003, Máximo et al. 2009, Serino & Turri 2011, Heitz-Mayfield et al. 2012, De Waal et al. 2013, Bassetti et al. 2014, De Waal et al. 2016, Riben-Grundstrom et al. 2015, Ramanauskaite et al. 2016). Due to the frequently combined different regimens, evaluation of the true impact of the single implant surface decontaminating agent on clinical outcomes remained difficult to identify. Hence, a gold standard debridement regimen in the treatment of peri-implantitis did not exist before the start of this thesis (Esposito 2012, Subramani & Wismeijer 2012, Louropoulou et al. 2014, Schwarz et al. 2015). Therefore, an ongoing search to find potential alternative therapies or effective combination of therapies seemed imperative. Moreover, research on previously not well researched therapies was needed.

GENERAL AIM AND OUTLINE OF THE THESIS

The general aim of this thesis was to assess non-surgical and surgical resective approaches in the treatment of peri-implantitis evaluating clinical, radiographical and microbiological parameters. Additionally, the role of immunological biomarkers in the diagnosis of peri-implantitis was investigated as well as the effect of the non-surgical treatment of peri-implantitis on these biomarkers. At last, the relation of the cervical crown contour with peri-implant bone loss and peri-implant soft tissue inflammation was evaluated.

The specific aims were:

- To assess immunological biomarkers in peri-implant crevicular fluid of healthy implants and implants with peri-implantitis (before and after *non-surgical treatment*) (**chapter 2**);
- To evaluate the effect of erythritol air-polishing in the *non-surgical treatment* of peri-implantitis in terms of clinical, radiographical and microbiological parameters in a randomized controlled study design (**chapter 3**);
- To evaluate the effectiveness of erythritol air-polishing as implant debridement method during the *surgical treatment* of peri-implantitis in a randomized controlled study design (**chapter 4**);
- To evaluate the microbiological and clinical effectiveness of 35% phosphoric etching gel as a decontaminating agent of the implant surface during resective *surgical treatment* of peri-implantitis in a randomized controlled study design (**chapter 5**);
- To evaluate the cervical crown contour on dental implants in relation to the peri-implant marginal bone level and peri-implant soft-tissue health (**chapter 6**).

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CHAPTER 2

BIOMARKER LEVELS IN PERI-IMPLANT CREVICULAR FLUID OF HEALTHY IMPLANTS, UNTREATED AND NON-SURGICALLY TREATED IMPLANTS WITH PERI-IMPLANTITIS

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This chapter is an edited version of the accepted article: *Journal of Clinical Periodontology* 2021, 48, 590-601. doi: 10.1111/jcpe.13423.

ABSTRACT

Aim

To compare biomarker levels in peri-implant crevicular fluid (PICF) of healthy implants with levels in PICF of implants with peri-implantitis (before and after non-surgical treatment).

Materials and methods

Samples were taken from 20 healthy implants (n=17 patients) and from 20 implants with peri-implantitis (n=19 patients) before and 3 months after non-surgical treatment using the Airflow Master Piezon® (EMS). A Luminex™ assay was used to evaluate pro-inflammatory and anti-inflammatory cytokines IL-1β, TNF-α, IL-6 & G-CSF, collagen degradation enzyme MMP-8, chemokines MCP-1 & MIP-1α/CCL3, bone markers OPG & sRANKL and interferon-γ. Clinical and radiographical characteristics were assessed. A Mann-Witney U and Wilcoxon signed-rank test analysed between and within group differences.

Results

IL-1β and MMP-8 levels were found significantly elevated in implants with peri-implantitis (p= .007; p=< .001, respectively). No difference in levels of TNF-α, IL-6, MCP-1 and MIP-1α/CCL3, OPG & G-CSF between healthy and diseased implants were found. Levels of sRANKL and INF-γ were under the level of detection. None of the biomarker levels improved after non-surgical therapy, levels of IL-1β and MMP-8 remained high.

Conclusion

Implants diagnosed with peri-implantitis have higher levels of IL-1β and MMP-8 in PICF compared to healthy implants. Non-surgical therapy did not influence the inflammatory immune response.

Clinical relevance

Scientific rationale for study

To assess whether peri-implant health and disease is accompanied by different biomarker levels in PICF as well as to evaluate the effect of non-surgical peri-implantitis treatment on these levels.

Principal findings

Peri-implantitis implants showed higher levels of IL-1β and MMP-8 in PICF compared to healthy implants. Immune response seemed not to change by a single non-surgical peri-implantitis intervention. sRANKL and INF-γ appeared under level of detection using a customized Luminex™ assay.

Practical implications

PICF collection, in addition to clinical and radiographical examination, helps to understand peri-implant health. Evaluation of non-surgical peri-implantitis therapy outcome using PICF diagnostics did not show to be helpful.

INTRODUCTION AND RATIONAL

Peri-implant infections are characterized by an inflammatory response to a bacterial imbalance leading to soft tissue inflammation with or without progressive loss of supporting bone (i.e., peri-implantitis or peri-implant mucositis, respectively) (Schwarz et al. 2018). Objective methods to differentiate between peri-implant health and disease, and to evaluate the effect of therapeutic intervention are lacking since traditional clinical diagnostic methods, such as pocket probing, bleeding on probing and radiographic assessment exert a weak sensitivity/specificity (Heitz-Mayfield 2008, Hashim et al. 2018). It is thought that immunological host-derived molecules, such as cytokines, chemokines, proteolytic and tissue breakdown enzymes, could serve as adjunctive parameters to ameliorate the diagnosis, prediction and management of peri-implant disease (Ramseier et al. 2009, Sexton et al. 2011, Syndergaard et al. 2014, Kinney et al. 2014, Carinci et al. 2019).

Since 1989, when Apse et al. (1989) demonstrated the presence of fluid in the osseointegrated implant pocket (i.e., peri-implant crevicular fluid (PICF)), a broad research effort has been spent to analyse host-derived immunological biomarkers present in this fluid (Aspe et al. 1989, Armitage 2004, Lamster & Ahlo 2007). Ideally, with the idea that this could lead to PICF diagnostics (e.g., point-of-care testing), to flank clinical and radiographical examination in the monitoring of peri-implant tissue health (Carinci et al. 2019). To date, most studies have focused only on a limited number of

biomarkers, mainly including only 1 or 2 classical pro-inflammatory cytokines (i.e., IL-1β, TNF-α and/or IL-6). These markers are commonly chosen since they exert synergistic properties in the initiation of inflammatory marker cascade and are produced at local sites with inflammation. In addition, they probably play an important role in osteoclast formation and hence, resorption of bone structures (Hirano et al. 1990, Dinarello 2000, Tanaka et al. 2014). Studies on several important anti-inflammatory biomarkers, collagen degradation enzymes, osteoclastogenis-related cytokines, and chemokines (e.g., granulocyte-colony stimulating factor (G-CSF), matrix metalloproteinase-8 (MMP-8), monocyte chemoattractant protein (MCP-1), macrophage inflammatory protein (MIP-1α/CCL3), bonemarkers (OPG & sRANKL) and interferon-γ) have, however, never been performed properly (Duarte et al. 2016). Also, whether these biomarker levels in PICF could be helpful to evaluate the outcome of peri-implantitis therapy, has scarcely been evaluated in previous studies (Bassetti et al. 2014, Renvert et al. 2016). Considering that cytokines are involved in broad networks, which to a large extent orchestrate the immuno-inflammatory process, an expanded approach of biomarker evaluation is necessary to increase the chance to find biomarkers that could help to distinguish between healthy and diseased implants (Feghali & Wright 1997, Duarte et al. 2016).

Therefore, the aim of the present study was to assess scarcely investigated or priorly not investigated biomarkers next to the commonly studied classical pro-inflammatory biomarkers in PICF of healthy implants and implants with peri-implantitis (before and after non-surgical treatment).

MATERIALS AND METHODS

Study design

A combined cross-sectional and intervention study was conducted. The SPIRIT guidelines for reporting a clinical trial were followed (Chan et al. 2013).

Study population

Patients with implants with peri-implantitis from the Center of Dentistry and Oral Hygiene and the Department of Oral and Maxillofacial Surgery of the University Medical Center Groningen (UMCG), Groningen, The Netherlands were consecutively recruited to participate in the study, according to specific in- and exclusion criteria (see Table 1). The study was executed at the Department of Oral and Maxillofacial Surgery of the University Medical Center Groningen between September 2017 and November 2019. The study was conducted in accordance with the Medical Research Involving Human Subjects Act (WMO) and in full accordance with the principles of the Declaration of Helsinki as stated in 64th WMA General Assembly, Fortaleza, Brazil,

October 2013. Before the start of the study all patients signed an informed consent. All patients were scheduled for a single non-surgical intervention using the Airflow Master Piezon® (Electro Medical Systems, EMS). This therapy was applied on 6 sites around the implant (mesial/mid/distal buccal, mesio/mid/disto lingual) for 5 seconds per site. All treatments were performed by one experienced dental hygienist (SS). Patients received oral hygiene instructions with emphasis on the daily use of interdental brushes with application of 0.12% chlorhexidine gel (Dentaid Benelux).

Table 1. In- and exclusion criteria

Inclusion criteria

- The patient was ≥ 18 years of age;
- The patient had at least one endosseous implant in the oral cavity with clinical and radiographical signs of peri-implantitis. Peri-implantitis was defined as progressive loss of marginal bone ≥ 2mm, as compared to the baseline radiograph (after placement of the definitive restoration) in combination with bleeding and/or suppuration on probing (de Waal et al. 2014);
- The implants had been in function for at least two years;
- The patient was capable of understanding and giving informed consent.

Exclusion criteria

- Medical and general contraindications for intervention;
- A history of local radiotherapy to the head and neck region;
- Pregnancy and lactation;
- Uncontrolled diabetes mellitus (HbA1c > 7% or > 53 mmol/mol);
- Use of antibiotics during the last 3 months;
- Known allergy to chlorhexidine;
- Long-term use of anti-inflammatory drugs;
- Incapability of performing basal oral hygiene measures as a result of physical or mental disorders;
- Implants with bone loss exceeding 2/3 of the length of the implant or implants with bone loss beyond the transverse openings in hollow implants;
- Implant mobility;
- Implants at which no position can be identified where proper probing measurements can be performed;
- Previous surgical treatment of the peri-implantitis lesions;
- Previous non-surgical treatment of the peri-implantitis lesions during the last 3 months (scaling or curettage)
- Chronic bronchitis and asthma

Seventeen adult patients scheduled for routine dental/implant check-up at the Center for Dentistry and Oral Hygiene and the Department of Oral and Maxillofacial Surgery

of the University Medical Center Groningen, with only healthy implants and free from periodontal inflammation (probing pocket depth (PPD) ≤ 3mm, no bleeding on probing (BoP) and no anatomical loss of periodontal structures), were consecutively asked to participate as control subjects. Healthy implants (HI) were defined as: PPD <5mm, no bleeding/suppuration on probing (BoP/SoP), and no marginal bone loss (MBL). Control group patients did not underwent a therapeutic intervention. The Medical Ethics Review Board of the University Medical Center Groningen (METc UMCG) has discussed and considered whether or not the sampling the study protocol falls within the scope of the Medical Research Involving Human Subjects Act (WMO) and decided that no ethics committee approval was needed for assessment of these patients (METc 2018.537). In both groups, all eligible implants present were included until 20 implants per group (according to our sample size calculation) were sampled.

Peri-implant crevicular fluid (PICF)

Biomarker sampling and volume quantification

A 30-second sampling protocol following Wassall & Preshaw (2016) was applied. In brief, before sampling the sample site was isolated with cotton rolls and dried with a gentle stream of air. Two PICF samples were collected from the same implant pocket per included implant in the healthy and diseased group using Periopaper® strips (Oraflow Inc. Smithtown, NY, USA) (Stewart et al. 1993). These presterilized filter paper strips were placed 1-2mm into the sulcus/pocket and absorbed fluids up to 1.2µl. To minimize evaporation, volume quantification was performed immediately after sampling, using a Periotron 8000 device (Oraflow Inc. Smithtown, NY, USA). Volumes were used to calculate modified concentration levels. The Periotron 8000 was calibrated before commencing the study and recalibrated periodically, following the manufacturer's recommendations. A calibration curve was generated accordingly. Directly after quantification, the two Periopaper® strips per implant were pooled in a dry Eppendorf tube® (Eppendorf AG, Hamburg, Germany). The tubes were placed on ice for transport to the laboratory and stored at -80°C until antibody array quantification took place. Implants with peri-implantitis (PI) were sampled at baseline (T0) and additionally at 3 months after therapy (T3). Health implants were only sampled at baseline.

Biomarkers of interest, determination and analysis (using Luminex™ xMAP multi-analyte profiling technology)

Periopaper® strips were thawed at the day of analysis after being stored dry at -80°C. To extract PICF from the strips, Luminex™ assay buffer (23µl) was added to each vial after which all the samples were vortexed for 30 minutes. Before centrifugation, the Periopaper® strips were fixed in the tube's cap. The samples were then centrifuged for 60 min at 300 rpm (8.7 ***g***) at 4°C, followed by another 2 minutes of centrifugation

at 12000 rpm (13.800 ***g***) at 4°C. All sampled were washed 3 times to yield a total elution volume of 70µl. The samples were processed according to the manufacturer's recommendations (ThermoFisher Scientific Inc., Bleiswijk, The Netherlands), in duplicate on a 96-well plate, including a standard line on all runs. The results were analysed using the MAGPIX® (with xPONENT® software) fluorescent detection system. Total biomarker concentration levels (Luminex™ output) were determined in the elution buffer as pg/mL.

A customized, highly sensitive bead-based multiplex immunoassay (Invitrogen ProcartaPlex Human 10-plex Luminex™ panel) was used to simultaneous analyse the following 10 biomarkers: interleukin 1β (IL-1β), interleukin 6 (IL-6), tumour necrosis factor alpha (TNF-α), monocyte chemoattractant protein 1 (MCP-1/CCL2), macrophage inflammatory protein 1 alpha (MIP-1α/CCL3), interferon gamma (IFN-γ), matrix metalloproteinase 8 (MMP-8), soluble receptor activator of nuclear factor kappa-B ligand (sRANKL), osteoprotegerin (OPG) and granulocyte-colony stimulating factor (G-CSF). According to the manufacturer's instructions, the selected biomarkers' lower limits of detection were: 1.62 pg/mL for IL-1β; 8.01 pg/mL for IL-6; 9.96 pg/mL for TNF-α; 3.56 pg/mL for MCP-1; 1.87 pg/mL for MIP-1α/CCL3; 14.40 pg/mL for INF-γ; 35.91 pg/mL for MMP-8; 9.11 pg/mL for OPG; 7.40 pg/mL for sRANKL; 12.72 pg/mL for G-CSF.

Clinical and radiographic examination

Peri-implant and full mouth clinical parameters were assessed, including bleeding on probing (BoP), suppuration on probing (SoP), probing pocket depth (PPD) and plaque index (PI). Peri-implantitis implants were assessed at baseline (T0) and 3 months (T3) after therapy. Healthy implants were only assessed at baseline (T0). All examinations were undertaken by the same researcher (DFMH).

Peri-apical radiographs were taken (Planmeca Intra X-ray unit; Planmeca, Helsiniki, Finland) using a paralleling technique and an individualized X-ray holder (Meijndert et al. 2004). Peri-implant bone loss was assessed at the mesial and distal implant site using DICOM software (DicomWorks 1.5, UMCG, Groningen) by two examiners (DFMH and HJAM) showing an almost perfect observer agreement (Viera et al. 2005). Within group (peri-implantitis before and after treatment) bone loss differences were examined.

Sample size calculation and statistical analysis

The findings of priorly performed recoverability experiments were used to perform a sample size calculation. A group size of 18 implants per group was determined with an average effect size (Cohen's D) of 0.9 and a power of 80%. To correct for a possible 10% drop-out (of implants), a total of 20 implants per group were required as sufficient amount to reach a reliable statistical significant difference using a significant (α) level

of 0.05. Data analysis was performed using IBM SPSS Statistics (Version 23.0 for Windows, Armonk, NY: IBM Corp) and GraphPad software (GraphPad Prism version 7.02 for Windows). The outcomes were tested for normality using the Shapiro-Wilk test. A Chi-square and Fishers exact test were used to analyse the categorical baseline characteristics between the healthy control subjects and peri-implantitis patients. A Mann-Whitney U test was used to evaluate group differences between healthy and diseased implants. A Wilcoxon signed-rank test was applied to analyse within group differences (untreated versus treated peri-implantitis implants). A p value of <0.05 was considered to be statistically significant for all the parameters.

RESULTS

In this study, a total of 60 samples (healthy implants at baseline (n=20), peri-implantitis implants at baseline (n=20) and 3 months after treatment (n=20)) was collected for further evaluation. The mean age in the peri-implantitis group [10 males, 9 females] and control group [12 males, 5 females] was 56.5 (±11.5) and 63.9 (±17.6) years, respectively (Table 2). No significant differences in the patient and implant characteristics were found between healthy and diseased implants at baseline. However, a greater variety of implant brands was seen in the peri-implantitis group (see Table 2).

Table 2. Characteristics of the healthy implants in the control group and the peri-implantitis implants in the test group.

	Healthy	Peri-implantitis
Total implants / total patients	20/17	20/19
Mean age (± SD)	63.9 (± 17.6)	56.5 (± 11.5)
Sex [M/F] (n)	12/5	10/9
Smokers	3 (17%)	5 (26%)
Implants brand, implant surface; n implants		
• Nobel	10 (50%)	6 (30%)
◦ Porous anoidized surface, TiUnite®		
• Straumann	10 (50%)	8 (40%)
◦ Sandblasted large grit acid-etched, SLAactive®		
• Other	0	6 (30%)

Luminex™ concentration biomarker levels (in *pg/ml per 30 seconds* and *pg per 30-seconds*) as well as modified concentration levels [in *pg/µl*] are presented using the equation described by Wassall & Preshaw (2016) [(Luminex™ x 0.2)/ PICF volume)] to show that correction of biomarker concentration levels for low crevicular fluid volumes creates artificial elevated biomarker levels and therefore a potential source of error for analysis (see tables 3a and 3b). Especially in healthy implants correction shows unreliable

significantly different outcomes (see Table 3a and 3b; the modConc column). Therefore, Luminex™ concentration outcomes in pg/ml per 30 seconds (see figure 1) were used for the assessment of biomarker levels in this study. At last, quantitative analysis of PICF showed a significant higher amount of PICF in diseased implants compared to healthy implants at baseline (Table 4).

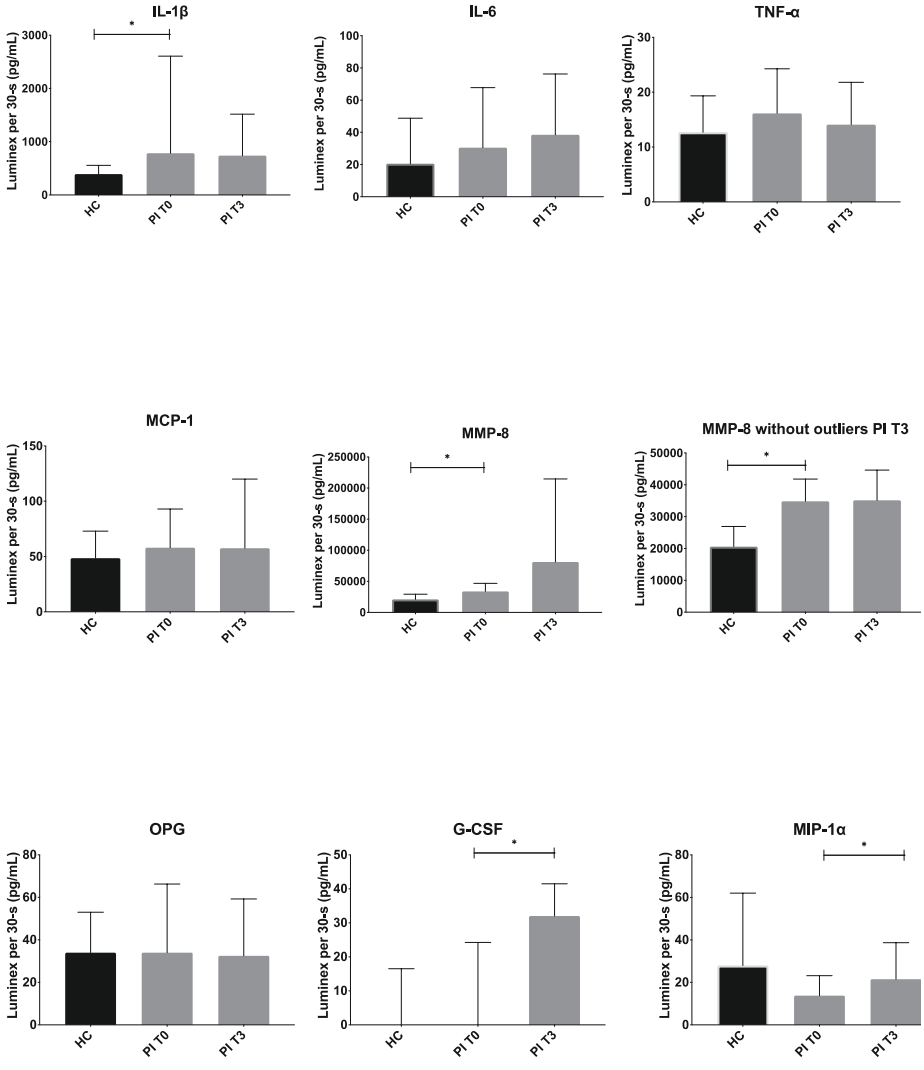

Figure 1. Indicating changes in biomarker levels per 30-sec in pg/ml of IL-1β, IL6, TNF-α, MCP-1, MIP-1α/CCL3, MMP-8, OPG and G-CSF between healthy control implants and diseased implants before (PI T0) and after (PI T3) non-surgical peri-implantitis therapy. Significant differences between groups are represented as * (p-value ≤ 0.05).

Regarding the clinical parameters, no significant change in mean peri-implant BoP, PPD and PI was seen in peri-implantitis patients after treatment (Table 4). However, a significant reduction in mean periodontal full mouth plaque scores (%) was observed (17.3 (±22.0) versus 6.6 (±9.9) (p = .021)).

Table 4. Mean (SD) clinical peri-implant parameters in the healthy implant group (HI), peri-implantitis group at baseline (PI T0) and peri-implantitis group 3 months after non-surgical treatment (PI T3).

Clinical parameters [mean ±SD]	Healthy	PI T0	*p* value (healthy vs PI T0)	PI T3	*p* value (PI T0 vs PI T3)
PPD (mm)	1.9 (±0.6)	5.0 (±1.1)	<.001	4.7 (±1.3)	0.140
BoP (%)	3.4 (±7.0)	58.4 (±27.8)	<.001	47.5 (±32.5)	0.108
SoP (%)	0.0 (±0.0)	19.2 (±23.7)	<.001	20.8 (±28.0)	0.944
PI (%)	0.0 (±0.0)	15.0 (±23.4)	0.002	8.4 (±16.7)	0.256
Full mouth PPD (mm)	NA	1.48 (±1.01)	NA	1.42 (±1.08)	0.844
Full mouth BoP (%)	NA	9.7 (1±4.4)	NA	7.2 (±8.2)	0.875
Full mouth SoP (%)	NA	0.0 (±0.0)	NA	0.0 (±0.0)	1.000
Full mouth PI (%)	NA	17.3 (±22.0)	NA	6.6 (±9.9)	*0.021*
MBL (mm)	0.56 (±0.5)	4.17 (±1.75)	<.001	4.24 (±1.84)	0.737
mean PICF volume (µl)	0.14 (±0.11)	0.42 (±0.25)	<.001	0.39 ± (0.28)	0.588
Mean Periotron value	52.93 (±24.05)	101.3 (±34.88)	<.001	96.2 (±33.3)	0.467

PPD, probing pocket depth; BoP, bleeding on probing; SoP, suppuration on probing; PI, Plaque index; MBL, marginal bone loss; PICF, peri-implant crevicular fluid; NA, not applicable

Biomarker levels in healthy versus diseased

Significant higher median levels of classical pro-inflammatory enzyme IL-1β (390.5 [87.0;555.5 pg/ml per 30 seconds] versus 783.5 [414.0;2607.3 pg/ml per 30 seconds]) and extracellular matrix (ECM) degradation enzyme MMP-8 (20590.2 [13512.4;26929.4] pg/ml per 30 seconds versus 34829.5 [24145.0;41791.5]) pg/ml per 30 seconds (p = .007; p < .001, respectively) were found in the PICF of peri-implantitis implants compared to healthy implants (Table 3a). Pro-inflammatory levels of TNF-α and IL-6, anti-inflammatory levels of G-CSF, chemokine levels of MIP-1α/CCL3 and MCP-1 and bone remodelling levels of OPG showed comparable amounts in the PICF of healthy and diseased implants (p = .402, p = .680, p = .109, p = .829, respectively). Levels of sRANKL and INF-γ were under the limit of detection (levels under 7.40 pg/mL for sRANKL and under 14.40 pg/mL for INF-γ).

Table 3a. Median (IQR) biomarker levels in the healthy implants (HI) and diseased implants at baseline (PI T0)

Biomaker (median; [IQR])	Implant status		Lum-30sec (pg/ml) HI vs PI T0		Lum-30sec (pg) HI vs PI T0		modConc [pg/µl] HI vs PI T0
		Luminex-30sec (pg/ml)	*p* value	Luminex-30sec (pg)	*p* value	modConc [pg/µl]	*p* value
IL-1β	HI	390.5 [87.0;555.5]	**0.007**	29.29 [6.5;41.7]	**0.007**	45.1 [26.3;121.2]	0.267
	PI T0	783.5 [414.0;2607.3]		58.8 [31.1;195.5]		57.9 [38.0;158.9]	
IL-6	HI	20.3 [10.3;48.9]	0.402	1.54 [0.8;3.7]	0.394	2.2 [1.1;3.0]	**0.002**
	PI T0	30.6 [10.6;67.4]		2.3 [0.8;5.1]		1.7 [.8;1.3]	
TNF-α	HI	11.3 [7.8;16.6]	0.133	0.86 [0.6;1.2]	0.162	2.15[1.1;2.96]	**0.002**
	PI T0	13.0 [10.5;20.4]		1.0 [0.8;1.5]		1.1 [0.8;1.33]	
MCP-1	HI	48.13[26.9;72.9]	0.136	3.64 [2.0;5.5]	0.133	7.4 [1.9;12.0]	0.136
	PI T0	58.2 [40.3;92.8]		4.4 [3.0;7.0]		5.2 [2.8;6.6]	
MIP-1α	HI	15.63 [8.8;31.8]	0.109	1.2 [0.7;2.5]	0.081	3.0 [1.8;5.2]	**0.001**
	PI T0	10.8 [7.2;17.9]		0.8 [0.5;1.4]		0.8 [0.5;1.5]	
MMP-8	HI	20590.2 [13512.4;26929.4]	**0.001**	1544.25 [1013.4;2019.7]	**0.001**	3224.6 [1773.3;5627.2]	0.094
	PI T0	34829.5 [24145.0;41791.5]		2612.2 [1810.8;3134.4]		1974.7 [1292.1;3895.6]	
OPG	HI	34.3 [19.3;53.0]	0.829	2.55 [1.4;4.0]	0.839	5.5[3.3;9.2]	**0.001**
	PI T0	33.9 [20.3;66.2]		2.5 [1.6;5.0]		2.2[1.3;4.1]	
G-CSF	HI	0.0 [0.0;16.7]	0.680	0.0 [0.0;1.2]	0.667	0 [0.0;1.7]	0.989
	PI T0	0.0 [0.0;24.0]		0.0 [0.0;1.8]		0.0 [0.0;1.33]	

Note: Median (IQR) biomarker levels in the healthy implants (HI) and diseased implants at baseline (PI T0). Luminex™ total amounts per 30-seconds in pg/mL, Luminex™ total amounts in pg per 30-seconds (corrected for 75µl elution) and as modified concentration in [pg/µL] corrected for 75µl elution and PICF volume.
Mann–Whitney U test significant outcome $p < 0.05$.
Abbreviation: IQR, interquartile range.

Table 3b. Median (IQR) biomarker levels in the peri-implantitis group at baseline (PI T0) and 3 months after treatment (PI T3)

Biomaker (median; [IQR])	**Implant status**		**Lum-30sec (pg/ml) PI T0 vs PI T3**		**Lum-30sec (pg) PI T0 vs PI T3**		**modConc [pg/µl] PI T0 vs PI T3**
		Luminex-30sec (pg/ml)	*p* value	Luminex-30sec (pg)	*p* value	modConc [pg/µl]	*p* value
IL-1β	PI T0	783.5 [414.0;2607.3]	0.156	58.8 [31.1;195.5]	0.156	57.9 [38.0;158.9]	0.145
	PI T3	738.5 [358.3;1516.8]		55.4[26.9;113.8]		47.1 [21.5;123.4]	
IL-6	PI T0	30.6 [10.6;67.4]	0.247	2.3 [0.8;5.1]	0.227	1.7 [0.8;1.3]	**0.003**
	PI T3	38.5 [11.3;76.4]		2.9 [0.9;5.7]		2.1 [1.1;6.2]	
TNF-α	PI T0	13.0 [10.5;20.4]	0.247	1.0 [0.8;1.5]	0.219	1.1 [0.8;1.3]	1.000
	PI T3	11.5 [9.0;16.8]		0.9 [0.7;1.3]		0.9 [0.7;1.4]	
MCP-1	PI T0	58.2 [40.3;92.8]	0.526	4.4 [3.0;7.0]	0.514	5.2 [2.8;6.6]	0.709
	PI T3	57.6 [40.0;120.3]		4.31[3.2;9.0]		4.1 [2.4;6.6]	
MIP-1α	PI T0	10.8 [7.2;17.9]	**0.001**	0.8 [0.5;1.4]	**0.001**	0.8 [0.5;1.5]	**0.012**
	PI T3	14.9 [9.4;30.0]		1.13[0.7;2.2]		0.9 [0.8;2.7]	
MMP-8	PI T0	34829.5 [24145.0;41791.5]	0.167	2612.2 [1810.8;3134.4]	0.167	1974.7 [1292.1;3895.6]	**0.028**
	PI T3	36775.0 [23351.5;65973.3]		2758.1[1751.4;4947.9]		3288.0 [1582.9;5359.8]	
OPG	PI T0	33.9 [20.3;66.2]	0.526	2.5 [1.6;5.0]	0.588	2.2[1.3;4.1]	0.411
	PI T3	32.4 [25.0;59.0]		2.4 [1.9;4.4]		2.89 [1.6;4.2]	
G-CSF	PI T0	0.0 [0.0;24.0]	**< .001**	0.0 [0.0;1.8]	**< .001**	0.0 [0.0;1.3]	**< .001**
	PI T3	32.3 [26.8;41.6]		2.4 [2.0;3.1]		2.39[1.8;3.6]	

Note: Outcomes presented in Luminex™ concentration per 30-seconds in pg/mL, Luminex™ concentration in pg per 30-seconds (corrected for 75µl elution) and as modified concentration [in pg/µL]; corrected for 75µl elution and PICF volume.
Wilcoxon signed-rank test significant outcome $p < 0.05$.
Abbreviation: IQR, interquartile range.

Biomarker levels in diseased implants before and after non-surgical therapy

Biomarker levels of untreated and treated peri-implantitis implants are presented in table 3b. The majority of biomarkers did not change at 3 months after therapy; levels of IL-1β and MMP-8 remained high. Moreover, a significant increase in median levels of chemokine MIP-1α/CCL3 (10.8 [7.2;17.9] pg/ml per 30 seconds versus 14.9 [9.4;30.0] pg/ml per 30 seconds, $p < .001$) and anti-inflammatory growth factor G-CSF (0.0 [0.0;24.0] pg/ml per 30 seconds versus 32.3 [26.8;41.6] pg/ml per 30 seconds, $p < .001$) was seen at 3 months after treatment.

DISCUSSION

In this study, 10 host-derived biomarkers were assessed in PICF of healthy implants and compared with biomarkers in PICF of implants with peri-implantitis using a customized Luminex™ multiplex panel. Additionally, the effect of non-surgical peri-implantitis therapy on the 10 host-derived biomarkers was evaluated. Outcomes showed that implants with peri-implantitis had significantly higher levels of IL-1β and MMP-8 compared to healthy implants whereas no difference in levels of IL-6, TNF-α, MIP1-a/CCL3, MCP-1, OPG and G-CSF were found between both groups. Levels of sRANKL and INF-y appeared to be under using the customized Luminex™ panel in this study. The effect of therapy on these biomarkers, as well as on peri-implant clinical and radiographical outcomes, appeared low.

Healthy versus diseased biomarker levels in PICF

Classical pro-inflammatory cytokines (IL-1β, TNF-α and IL-6), alone or in combination, belong to the most frequent investigated immunological markers in relation to peri-implant disease. Recent systematic reviews and meta-analyses have indicated moderate evidence in the literature to support that pro-inflammatory cytokines could differentiate between peri-implant health and peri-implant disease, especially regarding levels of IL-1β and TNF-α (Faot et al. 2015, Duarte et al. 2016, Ghassib et al. 2019). This study seems to be in accordance with this finding for levels of IL-1β, underlining the potential adjunctive role for this marker in the diagnosis of peri-implantitis. In contrast to IL-1β, we were not able to find a difference between health and disease for levels of TNF-α and IL-6. Although it is hypothesized in the literature that TNF-α or IL-6, next to IL-1β, are potential diagnostic markers, a recent meta-analysis by Ghassib et al. (2019) has shown that the literature on these markers is still scarce and with a high level of heterogeneity. Especially for levels for IL-6 limited evidence has been found to discriminate between peri-implantitis and healthy implants. In accordance with our study, two previous studies did not find a difference between implants with peri-implantitis and healthy

implants regarding levels of IL-6 (Melo et al. 2010 and Severino et al. 2016). Therefore, if IL-6 and TNF-α exert the same diagnostic potential as IL-1β remains inconclusive.

In addition to the classical pro-inflammatory markers, the extracellular matrix degradation enzyme major matrix metalloproteinase 8 (i.e., MMP-8) is another frequently evaluated marker in PICF (Thierbach et al. 2016. Teixera et al. 2017). Comparable to what is found in patients with periodontal disease, there seems moderate evidence in the literature showing upregulated levels of MMP-8 in PICF of implants with peri-implant disease (Salvi et al. 2012 Ghassib et al. 2019, Alassy et al. 2019). However, a true comparison between peri-implant health and peri-implantitis for this marker was only sparsely studied (Arakawa et al. 2012, Wang et al. 2016, Janska et al. 2016). In line with the studies by Janska et al. (2016) and Arakawa et al. (2012), our study is one of the few studies who reported elevated levels for peri-implantitis implants compared to healthy implants. Therefore, the present study seems to enhance the moderate evidence of upregulated levels of MMP-8 in PICF of implants with peri-implantitis. It therefore might be hypothesized that MMP-8 may serve a promising role, in addition to IL-1β, to differentiate between peri-implant health and disease (Tierbach et al. 2016, Al-Majid et al. 2018, Alassy et al. 2019). Alongside with pro-inflammatory markers and MMP-8, MIP-1α/CCL3 is a protein with chemotactic (stimulation of cell migration) properties which plays an important role in inflammation and increased activation of bone resorption cells (osteoclasts). In a study by Petković et al. (2010) increased levels of MIP-1α/CCL3 in PICF of diseased implants were found when compared to healthy implants, whereas no difference between healthy and diseased sites was found for levels of MIP-1α/CCL3 in a more recent study by Bhavsar et al. (2019). In addition to this latter study, our findings also indicate that this marker does not seem to differentiate between peri-implant health and disease. To date, it therefore does not seem likely to expect a diagnostic potential role for MIP-1α/CCL3 in peri-implant disease, however more studies evaluating this marker are needed to confirm this finding.

Another important chemotactic protein is MCP-1. This protein is considered the first discovered human chemokine and is a well-known chemoattractant for monocytes (Deshmane et al. 2009, Rollins 1996, Mulholland et. al. 2019). To the best of our knowledge, MCP-1 has not been previously evaluated in PICF of peri-implantitis patients. So far, we have only found two in-vitro studies on MCP-1 in the current literature reporting inconsistent outcomes (Bordin et al. 2009, Irshad et al. 2013). Our study seems the first to report on this marker in a clinical setting with no difference in the concentration levels of MCP-1 in the PICF between healthy and diseased implants. However there was a trend towards increased levels in diseased implants (p = .136). To what extend this marker plays a role in peri-implant disease remains to be found.

As part of the RANK/sRANKL/OPG system, the markers OPG and sRANKL play a pivotal role in bone biology (i.e., regulation of osteoblast and osteoclast activities). OPG protects the bone from excessive resorption by binding to sRANKL. Hereby, sRANKL is prevented from binding to RANK (a receptor bound to osteoclasts) which in turn prevents activation of osteoclast cells (Rakic et al. 2013). It therefore seems likely that both markers are involved in alveolar bone destruction in peri-implantitis (Arikan et al. 2011). However, to date, conflicting results regarding both markers have been reported in the literature. In a study Monov et al. (2006), subjects with increased peri-implant bone loss and clinical signs of inflammation did not show increased levels of sRANKL. Additionally, significantly lower sRANKL concentrations, OPG total amounts and OPG concentrations in peri-implantitis implants were reported by Arikan et al. (2011) when compared to healthy implants. Our study seems in line with the results on OPG reporting no difference in levels between both groups. On the other hand, significantly higher levels were found in peri-implantitis sites by Rakic et al. (2013), but without a difference in OPG/RANKL ratio. Therefore, although it seems reasonable to believe that both markers are important in peri-implant sites with bone loss, as of yet, the literature does not seem to support this thought.

At last, a biomarker which previously not seemed to be evaluated in peri-implant fluid is G-CSF (Panopoulos & Watowich 2008). This cytokine is known as a type of growth factor that stimulates bone marrow to produce white blood cells (e.g., neutrophil granulocytes). Although relatively few of the samples in this study (in both groups) had G-CSF levels above the level of detection, we believe with our study to be the first to show no difference in G-CSF between healthy and diseased implants. Considering that a recent study, which focused on a close relative of G-CSF (i.e., macrophage-CSF) in the PICF of peri-implantitis patients, found higher levels of macrophage-CSF when compared to peri-implant mucositis patients (Lira-Junior et al. 2020) interpretation of our outcomes should be done cautiously. However, one might suggest that colony stimulating factors might play a role in the pathogenesis of peri-implant disease.

Altogether, the research effort spent thus far on markers around implants with and without signs of inflammation has identified one potential biomarker (IL-1β) which could reliably be used in PICF diagnostics, to flank clinical and radiographical examination in differentiating between both groups. In addition, our study shows a promising role for the association between the expression of MMP-8 and the pathophysiology of peri-implantitis. However, this needs to be rigorously confirmed in future studies together with data on other potential markers (e.g., TNF-α, MCP-1 and G-CSF).

Biomarker levels in PICF before and after therapy

As far as we know, only two studies assessed the influence of non-surgical peri-implantitis therapy on markers in the PICF of peri-implantitis sites (Basetti et al. 2014, Renvert et al. 2016). Our study seems in accordance with study by Renvert et al. (2016) who neither found any differences in the majority of the studied cytokines (6 out of 9). A clinically stable treatment outcome was found in their study in only 22% of the cases at 6 months after therapy, using a single intervention with either an air-abrasive device or Er:YAG laser. Our study noticed a similar limited clinical effect, with persisting signs of inflammation (±50% of patients showing BoP and unchanged levels of SoP) 3 months after non-surgical peri-implantitis treatment and unchanged levels for the majority of biomarkers. In contrast, Basetti et al. (2014) found lower levels of IL-1β and MMP-8 at 3 months after therapy. However, additional delivery of local minocycline microspheres to the mechanical debridement with titanium curettes and glycine powder air-polishing was applied in their study. Hence, this could have led to a suppressed immune response with subsequently lower biomarker levels after therapy. Considering that the non-surgical therapy seemed unsuccessful, it might be speculated that clinical and radiographical parameters after non-surgical therapy are immunologically underlined. However, with our study, it seems not possible to truly support or deny the potential use of a change in biomarker as a monitor to assess the effectiveness of a peri-implantitis treatment with PICF analysis.

Limitations of the study and future recommendations

Interpreting the findings of this study, the following limitations should be kept in mind. Due to the limited sample size, no sub-analyses could be performed for several possible confounding factors (e.g., smoking, age, sex). Therefore, interpretation of our results with previous studies should be done with caution. Although an association between elevated inflammatory biomarkers levels (such as interleukin-1β, interleukin-6, interleukin-10, and tumor necrosis factor-α) in the PICF and smoking is described, (Tatli et al. 2013, Ata-Ali et al. 2016), no differences in smoking prevalence between the healthy control and peri-implantitis subjects in this study was seen. Therefore, interference of smoking with our analysis was not assumed.

A minor drawback of the study might be the difference in therapies applied. We used the Airflow Master Piezon® to either apply air-polishing or ultrasonic therapy. Considering the limited effect of non-surgical peri-implantitis interventions in general, as well as the limited effect observed in our study, the influence of therapy difference on immunological markers was considered rather low.

At last, for future research groups with interest in sRANKL and INF-γ, recoverability experiments seem recommended when using a ThermoFisher Luminex assay plate, in order to obtain accurate and reliable outcomes.

CONCLUSION

PICF diagnostics of implants diagnosed with peri-implantitis showed higher levels of IL-1β and MMP-8 compared to healthy implants. Non-surgical therapy did not seem to influence the inflammatory immune response. Hence, evaluation of non-surgical peri-implantitis therapy outcome using PICF diagnostics does not seem helpful.

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3

CHAPTER 3

ERYTHRITOL AIR-POLISHING IN THE NON-SURGICAL TREATMENT OF PERI- IMPLANTITIS; A RANDOMIZED CONTROLLED TRIAL

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This chapter is an edited version of the accepted article: *Clinical Oral Implants Research* 2021, 32, 840-852. doi: 10.1111/clr.13757.

ABSTRACT

Objectives

To compare erythritol air-polishing with piezoelectric ultrasonic scaling in the non-surgical treatment of peri-implantitis.

Material and Methods

Eighty patients (n=139 implants) with peri-implantitis (probing pocket depth (PPD) ≥5mm, marginal bone loss (MBL) ≥2mm as compared to bone level at implant placement, bleeding and/or suppuration on probing (BoP/SoP)) were randomly allocated to air-polishing or ultrasonic treatment. The primary outcome was mean BoP (%) at 3 months after therapy (T3). Secondary outcomes were mean SoP (%), plaque score (Plq) (%), PPD (mm), MBL (mm), full mouth periodontal scores (FMPS) (%), levels of 8 classical periodontal pathogens and treatment pain/discomfort (Visual Analog Scale, VAS). Patients who were considered successful at T3 were additionally assessed at 6, 9 and 12 months. Differences between both groups were analysed using multilevel statistics.

Results

Three months after therapy, no significant difference in mean BoP (%) between the air-polishing and ultrasonic therapy was found (crude analysis β (95% CI) -0.037 (-0.147; 0.073), p = 0.380). Neither secondary outcomes SoP (%), Plq (%), PPD (mm), MBL (mm), FMPS (%) and periodontal pathogens showed significant differences. Treatment pain/discomfort was low in both groups (VAS score air-polishing group 2.1 (±1.9), ultrasonic 2.6 (±1.9); p = 0.222). All successfully treated patients at T3 (18.4%) were still considered successful at 12 months follow-up.

Conclusions

Erythritol air-polishing seems as effective as piezoelectric ultrasonic scaling in the non-surgical treatment of peri-implantitis, in terms of clinical, radiographical and microbiological parameters. However, neither of the proposed therapies effectively resolved peri-implantits. Hence, the majority of patients required further surgical treatment.

Trial registry: www.trialregister.nl; identifier: NL8339

A brief summary of clinical and research implications

Non-surgical peri-implantitis treatment using either air-polishing or piezoelectric ultrasonic scaling seems to result in a reduction in clinical inflammatory outcomes up to the 3 month follow-up, however without effectively arresting disease progression in the majority of cases. Therefore, our findings underline the limited effect of a single non-surgical intervention in the treatment of peri-implantitis.

Interestingly, in patients which show a positive outcome at 3 months after therapy, stable peri-implant health could be expected up to 12 months after therapy. A priori identification of potentially successful patients characteristics (i.e., specific clinical, implant and patient characteristics) need to be further assessed in future studies.

Although the overall effect for non-surgical therapies seems limited, a non-surgical treatment phase per se seems imperative in the overall treatment approach since a small number of patients may benefit from a non-surgical treatment in such a way that no further surgical treatment is required. Additionally, the clinician can evaluate patient motivation and use this phase to educate patients about the disease process and modifying factors.

INTRODUCTION

Over the past decades a variety of interventions, alone or in combination, have been investigated for the non-surgical treatment of peri-implantitis including, mechanical (e.g., carbon fiber/titanium curettes, glycine air-polishing, ultrasonic therapy), chemical (i.e., local or systemic antibiotics, chlorhexidine irrigation) and light-mediated therapies (e.g., Er:YAG laser or photodynamic therapy) (Renvert, Roos-Jansåker, Claffey, 2008; Renvert, Lindahl, Roos Jansåker, Persson, 2011; Bassetti et al. 2014; Schwarz et al. 2015, Renvert 2015, Mettreux et al. 2016). Despite these various treatment strategies, the most effective treatment option for treating peri-implantitis lesions in a non-surgical way remains unclear (Faggion, Listl, Frühauf, Chang, Tu, 2014; Renvert et al. 2019, Wang et al. 2019).

However, among the previously investigated interventions the use of air-polishing is considered a promising treatment method (Schwarz, Becker, Renvert, 2015, Schwarz 2016). A myriad of *in-vitro* studies on air-polishing have appeared in the recent literature showing positive results on implant surface cleaning efficacy and surface damage (Tastepe et al. 2012, Iouropoulou 2014, Moharrami 2018). Clinically, air-polishing has been scarcely investigated in the treatment of peri-implantitis (Renvert et al. 2011, John et al. 2015). Previous studies reported small sample sizes, different peri-implantitis case

definitions and the use of a single type of investigative powder (i.e., glycine). Although beneficial clinical results (i.e., reduction of BoP and PPD) were found, complete disease resolution (e.g., no pockets with a PPD > 5mm, with concomitant bleeding and/or suppuration on probing and absence of progressive marginal bone loss > 0.5mm) seemed difficult to achieve. Glycine air-polishing could therefore not be appointed as favourable treatment method over others (i.e., plastic/titanium curettes, ultrasonic or laser therapy).

Recently, a new air-polishing powder, i.e. erythritol, which is considered a sugar alcohol (similar to xylitol) and used as sugar substitute, has been introduced to the dental field. This powder is non-caloric, has a high gastrointestinal tolerance and does not increase blood glucose or insulin levels (de Cock 1999, de Cock 2018). *In vitro* studies report that erythritol seems to be more effective in terms of cleaning efficacy compared to previously used powders (e.g., glycine and sodium bicarbonate) (Drago et al. 2014, Moharrami et al. 2018). Moreover, studies describe a more effective reduction in the bacterial biofilm and inhibition of post-treatment biofilm re-growth, improved cell attachment, cell viability, and proliferation of osteoblasts (Drago et al., 2017, Matthes et al. 2017, Mensi, Cochis, Sordillo, Uberti, & Rimondini, 2018,).

On the other hand, clinical periodontal maintenance studies on ultrasonic therapy, report comparable clinical and microbiological effects to subgingival air-polishing with erythritol powder (Müller, Moëne, Cancela, Mombelli, 2014). Ultrasonic therapy seems therefore another efficacious way to achieve infection control (Suvan et al., 2020). Compared to hand instrumentation, an ultrasonic device requires less effort and is less time consuming which makes it a preferable cleaning method in day-to-day clinical practice. Ultrasonic therapy seemed able to reduce clinical signs of inflammation (i.e., BoP) to a greater extent than carbon fiber/titanium curettes in the non-surgical treatment of peri-implantitis (Karring, Stavropoulos, Ellegaard, 2005; Renvert, Samuelsson, Lindahl, Persson, 2009). Yet, the effectiveness of both therapies (eryhtritol air-polishing and ultrasonic scaling) in the non-surgical treatment of peri-implantitis has not been investigated in a randomized controlled trial.

Therefore, the current study was set up to test the hypothesis that air-polishing with erythritol powder has the same effect as ultrasonic therapy on clinical, radiographical and microbiological parameters in the non-surgical treatment of peri-implantitis. In addition, the aim was to evaluate the pain/discomfort of both therapies.

MATERIALS AND METHODS

Trial design

This two-armed, parallel, investigator-blinded randomized controlled trial was the first of a two-staged peri-implantitis treatment approach consisting of (1) a single non-surgical treatment and (2) a surgical follow-up treatment if signs of peri-implantitis persisted at the 3-month evaluation after the non-surgical treatment. Patients with a successful treatment outcome at the 3-month evaluation (i.e., probing pocket depth (PPD) < 5mm, no bleeding / suppuration on probing (BoP) / (SoP) and no progressive marginal bone loss (MBL)) were enrolled in a peri-implant maintenance program and were additionally assessed at 6, 9 and 12 months post-treatment. The study was approved by the Medical Ethical Committee of the University Medical Center Groningen (METc, UMCG with study number 2016/355) and registered in the Dutch national trial register (www.trialregister.nl) under number NL8339. The CONSORT guidelines for reporting a randomized controlled trial were followed (Schulz, Altman, Moher, 2010).

Participants

Eligibility criteria

Between September 2016 and August 2018, 100 patients were screened by one and the same researcher (D.H.) for eligibility. The last follow-up visit took place in November 2019. Eligible participants had at least one dental implant with clinical and radiographical signs of peri-implantitis, which was defined as: probing pocket depth (PPD ≥ 5mm with concomitant bleeding and/or suppuration on probing (BoP/SoP) and progressive loss of marginal bone (MBL) ≥ 2mm, when compared to the baseline radiograph (after placement of the definitive restoration) ((de Waal et al., 2013). All the patients' eligible implants were included for clinical, radiographical and microbiological assessment. A patient was excluded when one of the following criteria was met: a history of local head and neck radiotherapy, pregnancy and/or lactation, uncontrolled diabetes mellitus (HbA1c > 7% or > 53 mmol/mol), chronic bronchitis and/or asthma, use of antibiotics within 2 months before the baseline assessment, known allergy to chlorhexidine, long-term use of anti-inflammatory drugs, incapability of performing basal oral hygiene measures, implants with bone loss exceeding 2/3 of the length of the implant, implant mobility, implants with no identifiable position for taking proper probing measurements. In addition, when the patient was subjected to a previous reconstructive or resective surgical treatment or previous non-surgical treatment of the peri-implantitis within the last 3 months, a patient was not included. Before participation, oral and written information about the study was provided. All the patients signed a written informed consent prior to enrolment.

Setting and location

All patients were recruited consecutively from the patient population of the Center of Dentistry and Oral Hygiene and the Department of Oral and Maxillofacial Surgery of the University Medical Center Groningen in the Netherlands. This single-center study was performed at the Department of Oral and Maxillofacial Surgery of the University Medical Center Groningen.

Intervention

One group of patients were treated once with an air-polisher using erythritol-based powder (grain size 14µm) containing 0.3% chlorhexidine (PLUS® powder, Electro Medical Systems (EMS), Nyon, Switzerland). The air-powder was applied subgingivally through a hand piece with a plastic nozzle (settings device: Perio, max liquid pressure 5.0 bar and 75% air-powder pressure, ≈7 bar, as recommended by the manufacturer). The nozzle contained a trilateral powder-outlet and an apical water-only spray. The other group patients were treated once with the piezoelectric ultrasonic scaler with a Polyether Ether Ketone (PEEK) coated plastic tip (PI instrument, EMS). Both interventions took place for 30 seconds per implant (5 seconds per site). Before subgingival decontamination, the implant surface was checked on hard deposits (i.e. calculus) and removed subsequently using hand instruments. The suprastructures remained fixed during the intervention and local anaesthesia was used as needed. Both groups' treatments were preceded by a 30-second mouth rinse with 0.12% chlorhexidine + 0.05% cetylpyridinium chloride without alcohol (Perio-aid®, Dentaid). Prior to peri-implant cleaning, but during the same session, a full mouth periodontal cleaning was applied using ultrasonic and/or hand instrumentation (EMS, Nyon, Switzerland / Hu-Friedy, Chicago, Illinois, US, scalers and curettes). Additionally, all patients received extensive oral hygiene instructions during the treatment appointment, including the use of an electric toothbrush and interdental brushes with the application of 0.12% chlorhexidine gel (PerioAid® gel, Dentaid Benelux, Houten, the Netherlands). All treatments were performed by three experienced dental hygienists. Reinforcement of oral hygiene instructions and supragingival cleaning of the included implant(s), using hand instrumentation, took place at 3, 6, 9 and 12 months (by the examiner, D.H.).

Outcomes

Primary outcome

The primary outcome was the mean percentage of peri-implant sites showing BoP at 3 month post-treatment.

Secondary outcomes

The secondary outcome parameters were mean peri-implant SoP (%), Plq (%), PPD (mm), MBL (mm), mean full mouth periodontal BoP (%), SoP (%), Plq (%), PPD (mm) and the presence and levels of 8 classical periodontal bacterial species at the 3 month evaluation. In addition, the midbuccal implant marginal soft tissue level between baseline and 3 month follow-up (i.e., recession (REC)), and the treatment pain/discomfort, were assessed.

Success criteria

The non-surgical therapy was considered successful at the 3 month evaluation when the implants demonstrated:

- Implant survival
- No pockets with a PPD ≥ 5mm, with or without concomitant BoP and no SOP
- Absence of radiographically assessed progressive marginal bone loss

Clinical assessment

The clinical parameters were assessed at 6 sites per tooth and implant (e.g., mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distoligual) using a Hu-Friedy PCPUNC156 periodontal probe and Shephaerds Hook Explorer EXS23. All assessments were carried out by one and the same examiner (D.H.) who was blinded regarding group allocation. The following clinical parameters were assessed binominally: BoP, visible presence of plaque and/or plaque on probing (Plq), SoP (1 = present or 0 = not present). Probing pocket depths were scored in absolute values to the nearest millimetre. To assess recession, a partial Vinyl Polysiloxane (VPS) impression (EXABITE™ II NDS, GC America Inc., Alsip, Illinois, US) was made of the suprastructure at the implant site and buccally trimmed to half way down the suprastructure (as a fixed reference point). The distance from the mid-buccal marginal mucosa to the margin of the VPS mould was assessed using a periodontal probe. In the case of an overdenture attachment system, the top of the suprastructure was taken as a fixed reference point. Peri-implant assessment took place at baseline, 3, 6, 9 and 12 months after therapy. Additional full mouth periodontal charts were made at baseline, 3 and 12 months.

Radiographical assessment

As approved by the Medical Ethical Committee, radiographs were taken at baseline, 3 and 12 months. To standardize the peri-apical radiographs and to assure perpendicularity (i.e., positioning of the film parallel to the long axis of the implant) the radiographs were taken using an individualized X-ray holder and paralleling technique (Planmeca Intra X-ray unit; Planmeca, Helsiniki, Finland) (Meijndert, Meijer, Raghoebar, Vissink, 2004).

When it was not possible to position the X-ray holder peri-apically in fully edentulous patients (painful to the floor of the mouth, or no position in which reproducible images could be made), panoramic images were taken. Peri-implant bone loss was measured using the DICOM software (DicomWorks 1.5). Calibration of each radiograph took place on a 3-point reference scale using the known implant length and/or diameter. Bone level differences were calculated for the mesial and distal site of the implant. The outer points of the implant connection plateau were taken as reference to which the initial bone level was present (in bone level implants). Measurement corrections were made in the presence of a smooth transgingival segment of the implant (1-stage implant systems i.e., tissue level implants). In order to calculate the inter-observer and intra-observer agreement, radiographic images of ten randomly selected implants were examined twice by the same researcher (D.H.) and once by another researcher (H.M.), both of whom were blinded regarding group allocation. Subsequently, D.H. measured all the X-ray images.

Microbiological sampling

A biofilm sample from the peri-implant sulcus was obtained at baseline, 3 and 12 months using sterile paper points. Before sampling supragingival plaque was mechanically removed. Samples were taken from four sites around the implant (mesiobuccal, distobuccal, mesioligual, distolingual). If a patient had more than one implant, sampling of the deepest pocket per implant took place. The samples collected from each patient were pooled in an empty vial. In dentate patients, bacterial samples were also taken from the site with the deepest probing pocket depth in each quadrant. If no deepened pockets were present, samples were taken from the mesiobuccal pockets of the teeth numbers 16, 26, 36 and 46. Outcome variables were the presence and numbers of the following putative periodontal pathogens; *Aggregatibacter actinomycetemcomitans (*Aa*), Porphyromonas gingivalis* (Pg*), Prevotella intermedia* (Pi)*, Tannerella forsythia* (Tf)*, Fusobacterium nucleatum* (Fn)*, Parvimonas micra* (Pm)*, Treponema denticola* (Td)*,* and *Filifactor alocis* (Fa)*.* Microbial samples were sent to Lab*Oral* Diagnostics (Houten, the Netherlands) and analysed using real time-PCR (quantitative polymerase chain reaction - qPCR).

Visual Analog Scale score

Immediately after the treatment all patients scored the level of pain and discomfort they had experienced during both the peri-implant therapy and periodontal cleaning using a Visual Analog Scale (VAS) ranging from 0 to 10.

Sample size calculation

The sample size calculation for the present study was based on the total number of patients required for a two-staged trial design, so that enough patients from the non-surgical part would be available for the surgical part. Literature on sample size and a power calculation of multilevel analyses shows that at least 50 patients should be included for there to be a relevant statistic difference, since a total amount of less than 50 will lead to biased estimates of the second-level standard errors (Maas & Hox, 2005). Scherbaum et al. (2009) pointed out the relationship of different levels in accordance to an adequate sample size and power. Translation of this relationship to our research protocol means a sample size (amount of patients) in combination with implants nested in patients. With a mean group size of 2 infected implants per patient and a minimum amount of 50 patients, it was estimated to detect a medium effect size with 80% power at a significance level of α=0.05. Since our study focused on clinical relevant effects, small effect sizes were less important and detection of medium effect sizes were supposed to be sufficient for our study.

According to the non-surgical peri-implantitis literature at the time of the study design, we estimated a 20% success rate for our non-surgical patient treatment phase (Muthukuru, Zainvi, Esplugues, Flemmig, 2012). Therefore, it was assumed that 80% of the patients would need surgical follow-up. To compensate for patient withdrawal and losses to follow up (10%), a sample size of 80 patients (40 in the air-polishing and 40 in ultrasonic group) was used. This was an intentional slight overestimation in order to assure enough available participants for the surgical phase of the study.

Randomization

Randomization was performed using sealed envelopes which contained a code ranging from AA to CZ alongside with a note saying either 'air-polishing' or 'ultrasonic therapy'. The dental hygienist performing the procedure opened the envelope, wrote down the code, read the note and performed the procedure. A decoding list saying which code belongs to which procedure was kept sealed until data analysis. This way, the investigator did not know which procedure was performed.

Statistical analysis

To analyse the difference in clinical and radiographical efficacy between both treatments, generalized linear mixed models (GLMMs) were used (IBM SPSS Statistical software, version 23.0. for Windows, Armonk, NY: IBM Corp). A three-level structure was chosen with patient implant and time as level 1, 2 and 3, respectively. The patient was considered unit of analysis, whereas the implant the unit of observation. First, the T3 clinical and radiographical outcomes were analysed while controlling for the

corresponding baseline parameters BoP, SoP, Plq, PPD and MBL (i.e., crude analysis). Then, the primary and secondary outcomes were analysed while controlling for the baseline values and confounding effects (i.e., adjusted analysis). The following a priori defined confounders were used in the adjusted mixed model: history of periodontitis (dichotome), smoking, prosthetic design (nominal) and mean periodontal plaque level at T3 (linear). For skewed data (SoP and Plq) a gamma distribution was used. The full mouth periodontal outcomes, VAS scores, midbuccal recession were analysed using an independent sampled t-test. A paired sampled t-test was applied to analyse differences in overall mean full mouth periodontal outcomes before and after therapy. The log-transformed mean peri-implant and periodontal microbiological outcomes were analysed at T3 using a Mann-Whitney U test was used (for between group differences). The data collected at 6, 9 and 12 months (for successfully treated patients at 3 month evaluation) is presented with descriptive statistics.

RESULTS

The flow of patients throughout the present study is depicted in Figure 1. The overall baseline patient and implant characteristics are shown in Table 1. Baseline characteristics of the successful subjects are described in Table 2. Patients, aged between 25-77 years (mean age 58 years, SD±12.3), were randomly allocated to receive air-polishing (n = 39) or ultrasonic scaling (n = 40). Four patients (6 implants) were lost to follow-up between baseline, intervention and 3 month evaluation (see Figure 1), yielding 76 patients with 133 implants, i.e., 38 patients/63 implants in the air-polishing group and 38 patients/70 implants in the ultrasonic group, available for analysis. Patients' baseline and 3 month follow-up clinical and radiographical outcomes are shown in Table 3a. An overview of the successful patient outcomes (at baseline, T3, T6, T9 and T12) is presented in Table 3b. Mixed model outcomes for the mean difference in BoP, SoP, Plq, PPD and MBL between both groups at T3 are shown in Table 5. The log-transformed mean (SD) of the selected putative periodontal pathogens of the pooled peri-implantitis samples and pooled periodontal samples (in partial edentulous patients) is presented in Table 6. The number of patients with positive samples (%) before and after therapy are presented in Figure 2.

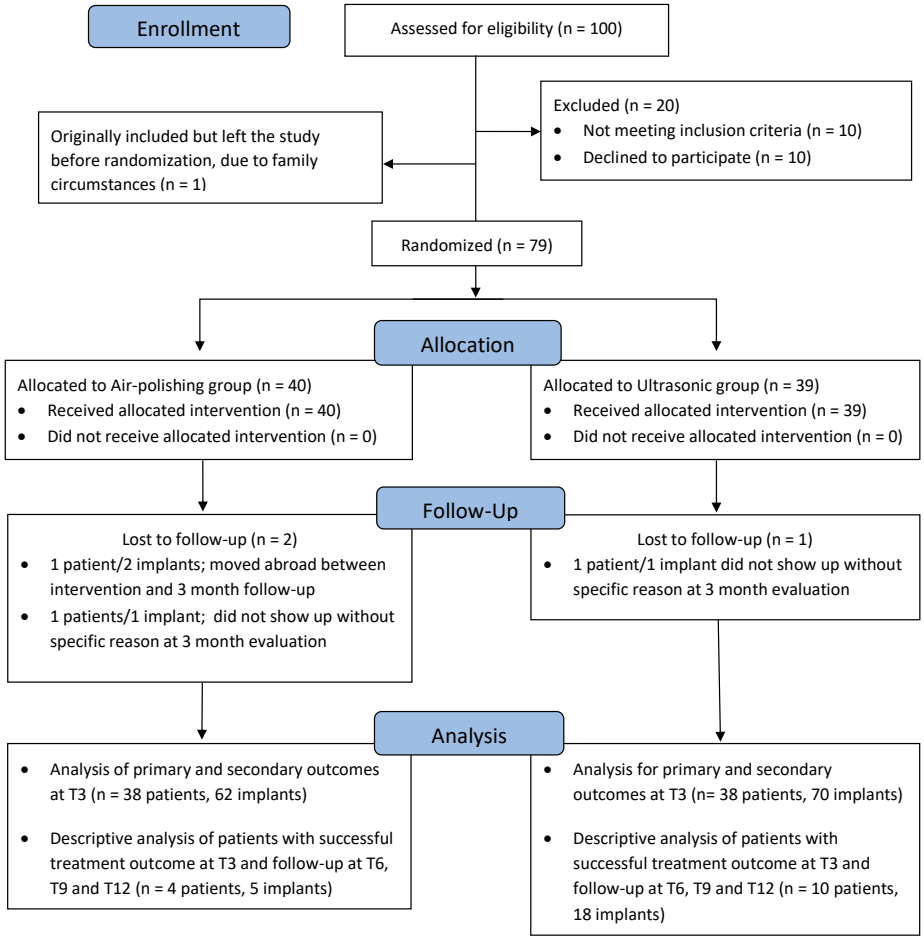

Figure 1. Flow diagram

Table 1. Baseline patient and implant characteristics

	Air-polishing	Ultrasonic
Patient characteristics		
Total number of patients	40	40
Age [years; mean (SD)]	62(8.9)	55(14.1)
Gender; F (female) / M (male)	15F/25M	20F/20M
Smoking; n subjects (%)		
Current	7 (17.5)	8 (20)
Never	26 (65)	23 (57.5)
Former	7 (17.5)	9 (22.5)
History of periodontitis; n subjects (%)		
Yes	17 (42.5)	10 (24)
No	23 (57.5)	30 (76)
Diabetes; n subjects (%)		
Yes (but controlled; HbA1c < 7% or < 53 mmol/mol)	2 (5)	0 (0)
No	38 (95)	40 (100)
Parafunction (bruxism/clenching); n subjects (%)		
Yes	6 (15)	8 (20)
No	34 (85)	32 (80)
Dental status, n patients (%)		
Fully edentulous	10 (25)	9 (22.5)
Partially edentulous	30 (75)	31 (77.5)
Implant characteristics		
Total number of implants included	66	73
Total number of implants presenting peri-implantitis (range)	(1-6)	(1-6)
Time in function [years; mean (SD)]	8.6 (6.1)	9.7 (4.8)
Implant type; n implants (%)		
Nobel Biocare	25 (37.9)	35 (47.9)
Straumann	26 (39.4)	21 (28.8)
Biomet 3i	4 (6.1)	7 (9.6)
MegaGen	4 (6.1)	1 (1.4)
Astra Tech	2 (3.0)	2 (2.7)
Camlog	2 (3.0)	2 (2.7)
Other (Simpler,IMZ, Dentsply Friadent, Pitt-easy, Smeden-Martina, Trinon Q)	3 (4.5)	5 (6.8)
Implant surface roughness (Sa)		
Minimally rough (turned, machined) ≥0.5, <1.0 µm	9 (13.6)	9 (12.3)
Moderately rough ≥1.0, <2.0 µm	56 (84.8)	59 (80.8)

Table 1. (Continued)

	Air-polishing	Ultrasonic
Rough ≥2.0 µm	1 (1.5)	5 (6.8)
Type of restoration; n implants (%)		
Single crown	20 (30.3)	38 (52.1)
Fixed partial denture	23 (34.8)	12 (16.4)
Overdenture	23 (34.8)	23 (31.5)
Screw- or cement-retained restoration; n implants (%)		
Screwed	47 (71.2)	52 (71.2)
Cemented	19 (28.8)	21 (28.8)
Implants placed in maxilla or mandible; n implants (%)		
Maxilla	36 (54.5)	46 (63.0)
Mandible	30 (45.5)	27 (37.0)
Implants placed anterior posterior; n implants (%)		
Anterior (central incisor to cuspid)	28 (42.4)	29 (39.7)
Posterior (premolar/molar)	38 (57.6)	44 (60.3)

At 3-month evaluation, 14 patients (18%) showed a successful treatment outcome: 4 patients (5 implants) in the air-polishing group and 10 patients (18 implants) in the ultrasonic group. Peri-implant assessment of these 14 patients took place at 6, 9 and 12 months follow-up. The distribution of sites with BoP in successful implants is shown in table 4. The remaining 62 patients with an unsuccessful treatment outcome at the 3-month evaluation discontinued the current study but were invited to continue in a surgical follow-up protocol.

Primary outcome

At 3 month evaluation, no statistical significant difference for mean BoP was found between air-polishing (49.8%±31.5) and ultrasonic therapy (48.1% ±29.0).

Secondary outcomes

No significant differences between both groups at 3 month evaluation were found for the secondary clinical peri-implant parameters; SoP, Plq and PPD, neither in the crude nor in the adjusted analysis, see Table 5. In addition, patients succeeded to lower mean levels of periodontal full mouth BoP and plaques scores (BoP reduced from 11.8% ±10.5 to 9.2% ±7.0 at T3, $p = 0.032$, plaque score reduced from 27.3% (±17.9) to 22.6 (±16.8), $p = 0.013$, at T3) (see Table 3a). No group differences were seen for mean marginal bone loss (at the mesial and/or distal site) or microbiological outcomes at 3 month evaluation (see Table 3a & 5). Patients that showed more than 0.5mm progressive bone loss at T3

all had probing pocket depths ≥ 5mm. At baseline, the most frequent isolated species from the peri-implant pocket were Fn, Pm and Tf (air-polishing group: 97.5%, 85%, 80% and ultrasonic group: 97.5%, 87.5%, 70%, respectively). Three months after treatment, in both groups almost unchanged levels for all periodontal bacterial species were found (see Table 6).

Table 2. Characteristics of successfully versus unsuccessfully treated patients

Patient characteristics	Successful	Unsuccessful
Number of patients (%) / implants (%)	14 (18.4) / 23 (17.3)	62 (81.6) / 110 (82.7)
Air-polishing; n subjects (%) / n implants (%)	4 (28.6) / 5 (21.7)	34 (54.8) / 58 (52.7)
Ultrasonic; n subjects (%) / n implants (%)	10 (71.4) / 18 (78.3)	28 (45.2) / 52 (47.3)
Age (years; mean (SD))	59.7 (12.0)	58.8 (12.0)
Gender; female (%) / male (%)	8 (57.1) / 6 (42.9)	26 (41.9) / 36 (58.1)
Smoking; n subjects (%)		
Current	0 (0)	13 (21.0)
Never	10 (71.4)	38 (61.3)
Former	4 (28.6)	11 (17.7)
History of periodontitis; n subjects (%) Yes / No	3 (21.4) / 11 (78.6)	22 (35.5) / 40 (64.5)
Diabetes; n subjects (%) Yes (but controlled) / No	0 (0) / 14 (100)	2 (3.2) / 60 (96.8)
Implant characteristics		
Time in function (years; mean (SD))	7.2 (4.0)	9.5 (5.6)
Jaw (upper/lower); n implants	12 (52.2) / 11 (47.8)	68 (61.8) / 42 (38.2)
Position (anterior/posterior); n implants	10 (43.5) / 13 (56.5)	45 (40.9) / 65 (59.1)
Edentulous (partial/fully); n patients	10 (71.4) / 4 (28.6)	48 (77.4) / 14 (22.6)
Screw/cement retained; n implants	20 (87.0) / 3 (13.0)	75 (68.2) / 35 (31.8)
Single crown/ fixed partial denture (FPD) / overdenture; n implants	9 (15.5) / 8 (22.9) / 6 (13.0)	45 (40.9) / 27 (24.5) / 38 (34.5)
Implant surface roughness (Sa); n implants		
Minimally rough (turned, machined) ≥0.5, <1.0 µm	2 (8.7)	15 (13.6)
Moderately rough ≥ 1.0, <2.0 µm	19 (82.6)	91 (82.7)
Rough ≥ 2.0 µm	2 (8.7)	4 (3.6)

Table 3a. Clinical and radiographical peri-implant outcomes and periodontal full mouth scores

Outcomes†			Air-polishing		Ultrasonic therapy		Overall	
Peri-implant	N = 80 patients / 139 implants		T0 (40 / 66)	T3 (38 / 63)	T0 (40 / 73)	T3 (38 / 70)	T0 (80 / 139)	T3 (76 / 133)
	mean BoP (%)	% of sites (SD)	58.1 (30.3)	49.8 (31.5)	56.2 (28.8)	48.1 (29.0)	57.1 (29.4)	48.9 (30.1)
		% of implants (n)	93.9 (62)	88.9 (56)	91.8 (67)	92.9 (65)	92.8 (129)	91.0 (121)
	mean SoP (%)	% of sites ((SD)	15.4 (20.7)	13.0 (19.5)	14.4 (21.6)	13.3 (22.2)	14.9 (21.1)	13.2 (20.9)
		% of implants (n)	54.5 (36)	44.4 (28)	42.5 (31)	35.7 (25)	48.2 (67)	39.8 (53)
	mean Plq (%)	% of sites ((SD)	23.2 (33.2)	15.9 (30.7)	16.0 (22.1)	12.3 (23.2)	19.4 (28.1)	14.0 (27.0)
		% of implants (n)	45.5 (30)	30.2 (19)	43.8 (32)	31.9 (22)	44.6 (62)	31.1 (41)
	PPD (mm)	mean (SD)	4.8 (1.2)	4.3 (1.3)	5.0 (1.5)	4.7 (1.8)	4.9 (1.4)	4.6 (1.6)
	Marginal bone loss (mm)‡	mean (SD)	4.0 (1.9)	4.0 (1.8)	3.9 (1.8)	4.0 (1.8)	4.0 (1.8)	4.0 (1.8)
Periodontal	Full mouth BoP (%)	mean (SD)	9.4 (7.0)	8.6 (6.4)	14.2 (12.9)	10.0 (7.7)	11.8 (10.5)	9.2 (7.0)*
	Full mouth SoP (%)	mean (SD)	2.7 (15.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.3 (10.7)	0.0 (0.0)
	Full mouth Plq (%)	mean (SD)	27.6 (18.3)	20.7 (16.5)	27.2 (17.9)	24.5 (17.1)	27.3 (17.9)	22.6 (16.8)*
	Full mouth PPD (mm)	mean (SD)	2.1 (0.27)	2.1 (0.31)	2.0 (0.26)	1.9 (0.48)	2.1 (0.27)	2.0 (0.40)

†measured on a 6 point scale
‡measured at the mesial and distal implant site
* significant difference for within overall group analysis (paired sampled t-test)

Table 3b. Descriptive statistics of successful patients' (air-polishing and ultrasonic group combined) clinical and radiographical outcomes

Outcomes†	N = 14 patients / 23 implants		T0 (14/23)	T3 (14/23)	T6 (12/19)	T9 (14/23)	T12 (14/23)
Peri-implant	mean BoP (%)	site level (SD)	49.3 (23.8)	31.9 (16.6)	28.1 (20.8)	18.1 (18.7)	23.9 (20.0)
		implant level (n)	95.7 (22)	95.7 (22)	73.7 (14)	60.9 (14)	73.9 (17)
	mean SoP (%)	site level (SD)	6.5 (16.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
		implant level (n)	17.4 (4)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	mean Plq (%)	site level (SD)	17.4 (19.8)	15.9 (20.4)	12.3 (15.6)	12.3 (12.5)	12.2 (15.3)
		implant level (n)	52.2 (12)	52.2 (12)	52.6 (10)	56.5 (13)	47.8 (11)
	PPD (mm)	mean (SD)	4.0 (0.9)	3.2 (0.6)	3.1 (0.5)	3.0 (0.7)	2.9 (0.6)
	Marginal bone loss (mm)‡	mean (SD)	3.0 (0.8)	2.9 (0.9)	*NA*	*NA*	3.0 (1.1)
Periodontal	N = 10 patients (partial edentulous)		T0	T3	T6	T9	T12
	Full mouth mean BoP (%)	patient level (SD)	14.6 (8.9)	9.9 (7.9)	*NA*	*NA*	9.4 (4.0)
	Full mouth mean SoP (%)	patient level (SD)	0.0 (0.0)	0.0 (0.0)	*NA*	*NA*	0.0 (0.0)
	Full mouth mean Plq (%)	patient level (SD)	33.5 (21.5)	31.3 (19.6)	*NA*	*NA*	21.9 (14.9)
	Full mouth mean PPD (mm)	patient level (SD)	2.0 (0.25)	2.0 (0.29)	*NA*	*NA*	2.0 (0.23)

†measured on a 6 point scale
‡measured at the mesial and distal site

Table 4. Distribution of sites with BoP in implants with pocket depths < 5mm at 3, 6, 9 and 12 months

N = number of successfully treated implants				
Sites with BoP	**T3 (N = 23)**	**T6 (N = 19)**	**T9 (N = 23)**	**T12 (N = 23)**
	N (%)	*N* (%)	*N* (%)	*N* (%)
0 out of 6	1 (4.3)	5 (26.3)	9 (39.1)	6 (26.1)
1 out of 6	7 (30.4)	2 (10.5)	6 (26.1)	6 (26.1)
2 out of 6	10 (43.5)	7 (36.8)	6 (26.1)	5 (21.7)
3 out of 6	3 (13.0)	4 (21.0)	1(4.3)	5 (21.7)
4 out of 6	2 (8.7)	1 (5.3)	1(4.3)	1 (4.3)
5 out of 6	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
6 out of 6	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Table 5. Generalized linear mixed model outcomes for mean difference in BoP, SoP, Plq, PPD and MBL between both groups at T3, using the ultrasonic therapy as reference arm.

	Crude analysis¶		**Adjusted analysis§**	
Outcome variable	β (95% CI)	*p*-value	β (95% CI)	*p*-value
Mean BoP†	-0.037 (-0.147; 0.073)	0.380	-0.023 (-0.165; 0.119)	0.746
Mean SoP‡	0.048 (-0.048; 0.143)	0.320	0.059 (-0.015; 0.134)	0.114
Mean Plq‡	0.034 (-0.103; 0.171)	0.623	-0.009 (-0.154; 0.136)	0.897
Mean PPD‡	0.054 (-0.253; 0.361)	0.728	0.140 (-0.249; 0.529)	0.478
MBL†	0.126 (-0.370; 0.623)	0.618	0.239 (-0.296; 0.775)	0.380

† Normal distributed data analysed with linear model distribution
‡ Non-normal distributed data analysed with gamma distribution
¶ Adjusted for baseline and time
§ Adjusted for baseline, time, smoking, history of periodontitis, mean periodontal full mouth plaque score at T3 and type of suprastructure.

No difference in mean pain/discomfort level (VAS scores) was found between both groups. However, patients reported low VAS scores for both therapies (air-polishing (2.1 (±1.9), ultrasonic (2.6 (±1.9), $p = 0.222$) as well as low periodontal pain/discomfort scores (VAS score ai-polishing (1.0 (±1.1) versus ultrasonic 1.4 (±1.5) respectively, $p = 0.425$). No significant difference in midbuccal recession was found between both groups, but both groups showed a slight increase in recession (air-polishing group 7.2 mm (±2.0) to 7.4mm (±2.0), ultrasonic group 6.6 (±1.8) to 6.7mm (±1.9), p = 0.552). Treatment of both therapies went uneventful; no emphysema could be detected after air-polishing treatment or any adverse reaction to ultrasonic treatment was reported.

Within the successful subgroup a continued reduction after 3 months of therapy was seen for peri-implant parameters BoP, Plq, PPD, and periodontal full mouth BoP and Plq. In addition, successful patients showed lower clinical scores at baseline (BoP, SoP, Plq, PPD, MBL), a shorter implant time in function compared to the overall group and all successful patients were non-smokers. The majority of successfully treated implants at T3 showed 2 out of 6 sites with BoP, with none of the implants showing 5 or 6 out of 6 sites with BoP.

Table 6. Log-transformed mean (SD) of selected putative periodontal pathogens. Pooled patient peri-implantitis samples and periodontal samples (of partially edentulous) per group.

Peri-implant outcome	**Air-polishing**		**Ultrasonic therapy**	
N = 40 (T0), N = 38 (T3)	T0	T3	T0	T3
Aa	6.7 (0.9)	6.5 (0.8)	4.2 (1.5)	5.6 (1.1)
Pg	5.9 (2.5)	5.3 (1.8)	4.8 (2.3)	6.3 (1.6)
Pi	4.6 (1.9)	5.3 (1.0)	4.8 (2.0)	5.3 (1.3)
Tf	5.1 (1.3)	5.0 (1.1)	4.8 (1.2)	4.9 (1.1)
Pm	4.1 (1.0)	4.2 (1.0)	3.9 (1.2)	4.1 (1.0)
Fn	4.9 (0.9)	4.7 (0.9)	4.4 1.3)	4.6 (1.0)
Td	4.7 (1.0)	3.9 (1.2)	4.7 (0.9)	4.9 (1.1)
Fa	5.2 (1.1)	5.0 (0.9)	4.4 (1.1)	4.7 (1.0)
Periodontal outcome				
N = 29 (T0), N = 29 (T3)	T0	T3	T0	T3
Aa	4.7 (1.0)	3.9 (1.1)	4.9	6.6
Pg	4.3 (1.4)	3.7 (1.6)	4.5 (2.2)	5.1 (1.3)
Pi	4.6 (1.2)	4.4 (2.0)	4.2 (1.6)	4.5 (1.4)
Tf	4.1 (1.2)	4.2 (1.7)	3.9 (1.3)	4.1 (1.3)
Pm	3.6 (0.9)	3.8 (0.8)	3.5 (0.9)	3.8 (1.0)
Fn	4.1 (0.9)	4.1 (1.0)	3.8 (1.3)	4.1 (1.0)
Td	3.8 (0.9)	3.8 (1.5)	3.7 (0.9)	3.8 (1.0)
Fa	4.1 (1.2)	3.9 (1.3)	4.1 (0.9)	3.6 (1.4)

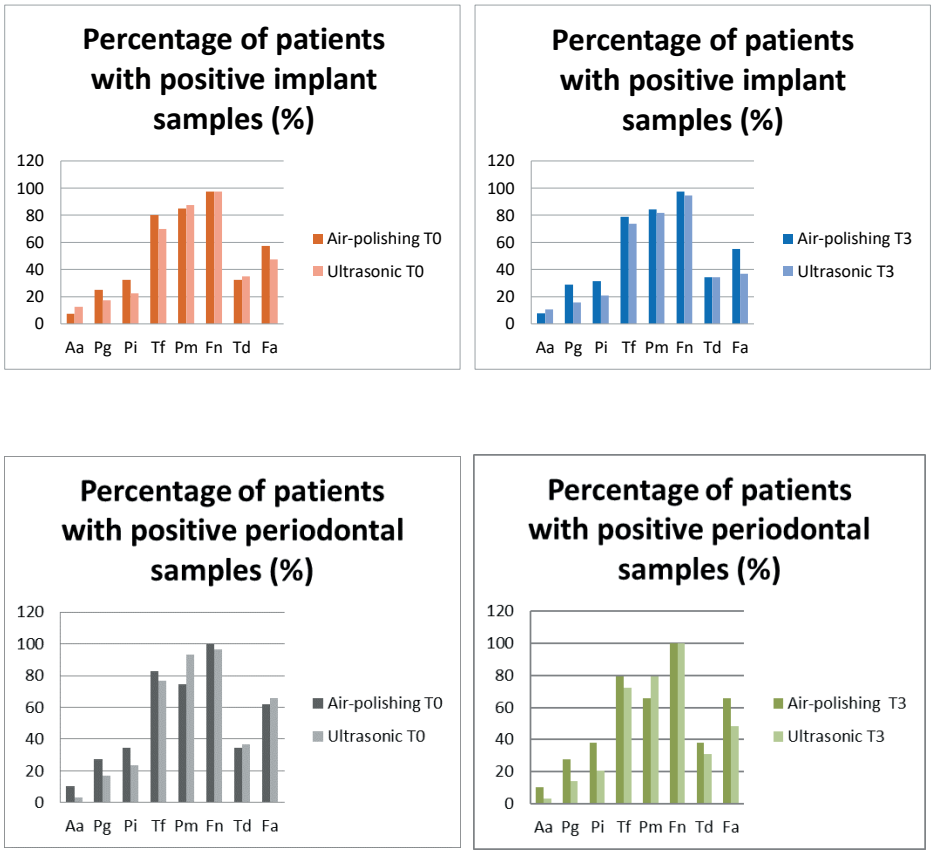

Figure 2. Number of patients in the air-polishing group and ultrasonic group with positive pooled peri-implant and periodontal samples, before and 3 months after therapy.

DISCUSSION

Key findings

This randomized controlled trial compared the clinical, radiographical and microbiological outcomes of erythritol air-polishing and piezoelectric ultrasonic scaling with a PEEK plastic tip in the non-surgical treatment of peri-implantitis. Three months after therapy, there was no significant difference between both therapies for the primary outcome mean BoP (%). Other clinical, radiographical or microbiological parameters neither showed any difference between both groups. Therefore, in terms of our null-hypothesis air-polishing seems to be as effective as ultrasonic scaling in the reduction of inflammatory signs (BoP, SoP, Plq and PPD). Both therapies however resulted in limited success with most of the patients showing persistent signs of inflammation at 3 month

follow-up. Interestingly, follow-up of successful patients showed gradual improvement of peri-implant parameters up to 12 months when supportive peri-implant therapy (supragingival instrumentation when plaque/calculus was visible) and oral self-care re-inforcement were applied at 6 and 9 months. In addition, both therapies were considered minimally painful without one of both being significantly less painful.

Comparison with relevant findings from other published studies

To date, no studies have evaluated erythritol air-polishing as monotherapy for the non-surgical treatment of peri-implantitis. Only two previous studies report on a single non-surgical intervention in peri-implantitis patients with glycine air-polishing therapy (John et al. 2015; Renvert et al. 2011). When glycine powder air-polishing was compared with mechanical debridement + local antiseptic therapy using chlorhexidine in a study by John and coworkers, a significant higher reduction in mean BoP scores at 3 months was found (BoP reduced from 99.0% ±4.1 to 57.8%±30.7 in the air-polishing group and from 94.7%±13.7 to 78.1%±30.0 in the mechanical debridement group). Compared to the present study, glycine air-polishing also seemed to result in a greater reduction of BoP. However, the study by John et al. included patients with as initial or moderate forms of peri-implantitis (probing pocket depths of ≥4mm compared to ≥5mm in our study and the loss of supporting bone as ≤ 30% compared to ≥ 2mm in our study), implying that implants with a less severe state of inflammation might have been studied. In addition, only non-smoking patients were included and a high risk of bias on several items was reported (e.g., allocation concealment, blinding of participants and selective reporting) in the recent systematic review (Suárez-López Del Amo et al. 2016). Therefore, interpreting these results should be done cautiously.

In comparison with Renvert et al., no statistical differences in clinical parameters (BoP, SoP, Plq and PPD) and bone level changes were found when glycine air-polishing (Perioflow®) was compared to laser therapy (Er:YAG). Also, the range of pocket depth reduction in the present study was comparable to the reductions in the study by Renvert et al. (between 0.1mm and 1mm at 6-months in the majority of patients). Moreover, comparable changes in average marginal bone loss were found for air-polishing (0.1mm (±0.8)) at 3 months. This despite the fact that suprastructures were removed, a sonic toothbrush was provided with a new brush head at the 3 month follow-up, and the treatment time was double as compared to our study (1 minute versus 30 seconds). Therefore, although it could be hypothesized that these measures might have led to a more effective removal of the peri-implant biofilm, it did not result in a better treatment outcome. Nevertheless, it might be reasonable to extend the subgingival treatment time and remove the suprastructure to secure a thoroughly cleaned peri-implant area, especially in more advanced lesions (Mensi et al. 2020).

None of the ultrasonic scaling studies in the current literature, evaluated the same piezoelectric ultrasonic scaler with plastic PEEK tip in the non-surgical treatment of peri-implantitis. Two studies with a comparative study design however were found evaluating subgingival instrumentation using an ultrasonic device (Vector® system) (Karring et al., 2005; Renvert et al., 2009). That ultrasonic device showed to be more effective in the reduction of BoP when compared to carbon fiber curettes and titanium curettes, respectively. However, no significant differences between the groups in clinical improvements (i.e., BOP, PPD, and bone level changes) were found. In accordance, our study showed a similar limited clinical effect of ultrasonic debridement. Therefore, from the data in the present study neither air-polishing nor ultrasonic cleaning could be considered a superior therapy in terms of our primary outcome (i.e., mean BoP at T3).

Regarding the microbiological results in this study, comparable outcomes were found in two studies by Persson et al. 2010 and Persson et al. 2011. Both studies showed no difference in bacterial counts when using an air-polishing, ultrasonic scaling or laser therapy (Er:YAG), including no significant changes in bacterial load or in bacterial composition. Reduced bacterial counts of *P. aeruginosa, S. aureus,* and *S. anaerobius* were seen 1 month after the air-polishing therapy, but the bacterial counts did not decline further at the 6 month evaluation after air-polishing and laser therapy. As compared to these studies, the limited clinical effect observed in the present study seems to be underlined by the unchanged levels of periodontal pathogens. Success at 3 months after therapy was defined without BoP (%) being a discriminating factor. Rightly so, because if previously used success criteria would have been applied (e.g., criteria by Heitz Mayfield and Mombelli 2014, Carcuac et al. 2016), implants with PPD < 5 with concomitant BoP would be considered unsuccessful. According to the current treatment protocol patients subsequently would have been invited for a surgical follow-up. Looking at the gradual decline in clinical parameters (i.e., mean BoP, PPD) within the successful group of implants, it seemed that stable bone levels and absence of progression of disease could be attained in implants showing PPD < 4mm with the presence of BoP up to 12 months. Therefore this study underlines that the sensitivity of BoP for the prediction of disease progression is quite low and that strict success criteria need to be cautiously interpreted and applied.

To decide which therapy could be considered preferable, next to the clinical, radiographical and microbiological parameters, treatment pain/discomfort of both therapies was assessed . In contrast to the periodontal literature, in which a low degree of discomfort for erythritol air-polishing was found compared to ultrasonic scaling, no difference in discomfort between both therapies in our study was found (Bühler et al. 2016). For both therapies an equal low level of pain was reported. Therefore

neither this parameter seems to be a discriminating factor to decide which therapy to apply. However, it should be kept in mind that, for air-polishing systems, the risk for emphysema may be increased in difficult to reach areas. Especially when it is needed to tilt the air-polishing nozzle. Moreover, air-polishers are limited to the removal of attached biofilms whereas hard deposits should be removed by hand. Interestingly, as reported by the experienced dental hygienists in this study, access of the peri-implant pocket appeared more challenging using a thick nozzle compared to the lean ultrasonic tip. Hence, these factors may indicate to recommend a different decontamination method in specific cases.

At last, when baseline characteristics of the successful group of patients were compared with these of the unsuccessful ones, interesting differences regarding PPD (4.0mm vs 4.9mm, respectively), MBL (3.0mm versus 4.0mm, respectively) and time in function before therapy took place (7.2 versus 9.5 year) were seen. Considering the success of these patients up to 12 months after therapy, these parameters might indicate the importance of early diagnosis and therefore early commencement of non-surgical therapy.

Limitations

The following limitations should be addressed when interpreting the results of this study. First, suprastructures were not removed during this study which might have led to inadequate peri-implant accessibility and inadequate clinical measurements. In addition, hampered access (e.g., due to overcontoured suprastructures) of the peri-implant pocket could have complicated the insertion of the ultrasonic or air-polishing tip, and therefore led to an inadequate therapy effect.

Second, this study might lack a true control therapy. However, to date, no non-surgical intervention seems to be the gold standard in the treatment of peri-implantitis. As a means of non-surgical treatment, mechanical debridement of the implant surface is primarily recommended (Renvert et al. 2019). Therefore a randomized study design in which two promising mechanical interventions were compared was chosen. This so, to analyze if the aforementioned treatment interventions could lead to appointing a superior standard therapy.

Third, the marginal bone level measurements were done on peri-apical radiographs as well as on panoramic pictures. In the latter case, a standardized angulation of the picture could not be secured. Therefore, the measurements on the overview x-ray pictures might not have been as accurate for comparison purposes. However, given

this study's outcomes, it seems unlikely that different bone levels would have been encountered when only peri-apical standardized pictures were used.

Lastly, the included patients showed large variations in implant characteristics (i.e., different implant brands, with different implant surfaces and suprastructures, placed in the anterior and posterior part of the mouth as well as in the lower and upper jaw), and peri-implantitis disease severity (varying from mild to severe peri-implantitis). Although such a heterogeneous group of patients and implants might represent a true cross-section of the society, it makes it very difficult to compare the effect of the therapies in specific subgroups of patients, e.g. cases with mild versus severe peri-implantitis or smokers versus non-smokers. Future studies are needed to evaluate the effect of therapy in these specific groups of cases.

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CHAPTER 4

ERYTHRITOL AIR-POLISHING IN THE SURGICAL TREATMENT OF PERI-IMPLANTITIS; A RANDOMIZED CONTROLLED TRIAL

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This chapter is an edited version of the accepted article in: *Clinical Oral Implants Research (November 2021)*

ABSTRACT

Objectives

To compare erythritol air-polishing with implant surface cleansing using saline during the surgical treatment of peri-implantitis.

Material and Methods

During a resective surgical intervention, implant surfaces were randomly treated with either air-polishing (test group n= 26 patients/53 implants) or saline-soaked cotton gauzes (control group n= 31 patients/ 40 implants). Primary outcome was change in mean bleeding on probing (BoP) from baseline to 12 months follow-up. Secondary outcomes were changes in mean suppuration on probing (SoP), plaque score (Plq), probing pocket depth (PPD), marginal bone loss (MBL), periodontal full mouth scores (PFMS) and levels of 8 classical periodontal pathogens. Clinical and radiographical parameters were analyzed using multilevel regression analyses. Microbiological outcomes were analyzed using the Mann-Whitney U test.

Results

No differences between the test and control group were found for BoP over 12 months of follow-up, nor for the secondary parameters Plq, PPD and MBL. Between both groups, a significant difference was found for the levels of SoP ($p = 0.035$). No significant effect on microbiological levels was found. A total number of 6 implants were lost in the test group and 10 in the control group. At 1-year follow-up, a successful treatment outcome (PPD<5mm, max 1 out of 6 sites BoP, no suppuration and no progressive bone loss >0.5mm) was achieved for a total of 18 implants (19.2%).

Conclusions

Erythritol air-polishing as implant surface cleansing method was not more effective than saline during resective surgical treatment of peri-implantitis in terms of clinical, radiographical and microbiological parameters. Both therapies resulted in low treatment success.

Trial registry: www.trialregister.nl; identifier: NL8621

INTRODUCTION

Implant surface decontamination and/or debridement is considered a critical component for the successful surgical treatment of peri-implantitis (Sanz, Chapple, Working Group 4 of the VIII European Workshop on Periodontology, 2012). Over the last decade, various interventions (i.e., chemical, mechanical or light-mediated) have been studied to eliminate the biofilm and resolve inflammation (Garaicoa-Pazmino, Sinjab, Wang, 2019; Ramanauskaite, Obreja, Schwarz, 2020). However, no clinical, radiographical and microbiological data favors any cleansing approach (Khoury, et al., 2019). To determine the superiority of a decontamination and/or debridement method, clinical studies are needed (Koo, et al. 2019).

In order to assess the influence of a debridement method, a randomized clinical trial (RCT) focusing on a single intervention, not using augmentive or adjunctive therapy, is recommended (Esposito, Grusovin, Worthington, 2012; Khan, et al. 2020). Thus far, only a limited number of studies evaluated their implant cleaning protocol in such a way. These studies mainly focused on the effects of chemical agents such as chlorhexidine and phosphoric acid (de Waal, et al., 2013; de Waal, et al., 2015; Hentenaar, et al., 2017) or laser therapy (Papadopoulos, et al., 2015), but not on mechanical debridement methods. Although these studies showed significant reductions in implant surface microbial load, no significant clinical benefits of one method over another were found.

Since its introduction around 1945, the use of air-polishing devices have recently gained popularity in the field of dentistry (Petersilka, 2011). The cleaning potential of an air-polisher is based on the kinetic energy of abrasive powder particles, mixed in a spray with water and compressed air. Positive results in terms of cleaning efficacy, surface change and biocompatibility were found in *in-vitro* studies, comparing glycine air-polishing to other debridement methods (e.g., hand instrumentation and laser therapy) (Louropoulou, Slot, Wismeijer, 2014; Moharrami, et al., 2019). In addition, evaluation of different implant surface cleansing methods in an *ex-vivo* study, showed that air-polishing was superior to chemical decontamination (Pranno, et al., 2020). However, limited clinical research on the use of air-polishing as a single decontaminating method in treatment of peri-implantitis has been performed thus far. Just recently, superior effects to plastic curettes (reduction in PPD) but equal to titanium brush or implantoplasty were described (Toma, Brecx, Lasserre, 2019, Lasserre, Brecx, Toma, 2020). These results however came from studies with small sample sizes, short follow-up and the use of a single air-polishing powder (i.e. glycine).

A promising new low abrasive air-polishing powder, i.e., erythritol, has recently been introduced on the market. *In-vitro* studies on erythritol have shown stronger antimicrobial and antibiofilm activity than glycine (Drago, et al., 2014) and inhibitory effects on *Streptococcus gordonii* and *Porphyromonas gingivalis* (Hashino, et al., 2013). In addition, erythritol suppresses biofilm regrowth and improves cell attachment, cell viability, and proliferation of osteoblasts (Drago et al. 2017, Matthes, et al., 2017, Mensi, et al., 2018). Moreover, promising effects in terms of titanium cleaning efficacy were seen (Tastepe, et al., 2018; Drago, et al., 2017). When erythritol air-polishing was compared to scaling and rootplaning in periodontal maintenance studies and in non-surgical periodontitis treatment studies, comparable clinical and microbiological results were found (Müller, et al., 2014; Hägi, et al., 2015; Park, et al. 2018; Jentsch, et al., 2020; Mensi, et al., 2021). More recently, a study by Cosgarea et al. (2021) showed that erythritol air-polishing during periodontal surgery may represent a valuable adjunct following calculus removal or as minimally invasive treatment for root surfaces without calculus. However, clinical studies on the effect of erythritol air-polishing during the surgical treatment of peri-implantitis are lacking.

The aim of the present randomized clinical trial was to evaluate the clinical, radiographical and microbiological effect of erythritol air-polishing as implant debridement method and compare this with saline-soaked cotton gauzes as control intervention. Hence, the null-hypothesis of erythritol air-polishing being not better than saline soaked gauzes in terms of clinical, radiographical and microbiological parameters was tested.

MATERIALS AND METHODS

Trial design

This two-armed, investigator-blind randomized controlled trial is the surgical part of a two-staged peri-implantitis therapy protocol. Prior to participation, all patients received a non-surgical treatment (Hentenaar, et al., 2021). If signs of inflammation persisted 3 months after the non-surgical intervention, a surgical treatment was rendered. The study was approved by the Medical Ethical Committee of the University Medical Center Groningen (METc, UMCG with study number 2016/356) and registered in the Dutch national trial register (https://www.trialregister.nl/) with number NL8621. The CONSORT (Consolidated Standards of Reporting Trials) guidelines for reporting a randomized controlled trial were followed (Schulz, Altman, Moher, 2010).

Participants

Eligibility criteria

Between December 2016 and January 2019, 62 patients were screened for eligibility by one and the same researcher (D.H.). The last follow-up visit took place in February 2020. Eligible participants had at least one dental implant with persisting signs of inflammation 3 months after the preceding non-surgical intervention (probing pocket depth (PPD ≥ 5mm with concomitant bleeding and/or suppuration on probing (BoP/SoP) and progressive loss of marginal bone (MBL) ≥ 2mm, when compared to the baseline radiograph (after placement of the definitive restoration) (de Waal, et al., 2013). All the patients' eligible implants were included for clinical, radiographical and microbiological assessment. A patient was excluded when there was a history of local head and neck radiotherapy, pregnancy and/or lactation, uncontrolled diabetes mellitus (HbA1c > 7% or > 53 mmol/mol), use of antibiotics within 2 months before the baseline assessment, known allergy to chlorhexidine, long-term use of anti-inflammatory drugs, incapability of performing basal oral hygiene measures, implants with bone loss exceeding 2/3 of the length of the implant, implant mobility, chronic bronchitis and/or asthma. Periodontal full mouth plaque and bleeding levels were required to be ≤ 20%. Before participation, oral and written information about the study was provided. All patients signed a written informed consent prior to enrolment.

Setting and location

All patients were consecutively recruited from the patient population of the Center of Dentistry and Oral Hygiene and the Department of Oral and Maxillofacial Surgery of the University Medical Center Groningen in the Netherlands. This single-center study was performed at the Department of Oral and Maxillofacial Surgery of the University Medical Center Groningen.

Surgical intervention

Prior to the surgical intervention, all patients underwent a non-surgical treatment in which they received extensive oral hygiene instructions, periodontal cleaning and a single mechanical peri-implant supra- and submucosal debridement with either air-polishing or piezoelectric ultrasonic scaling. Screw retained implant suprastructures were removed before surgery if reasonably possible. Surgery was performed by two experienced implant clinicians within the group of authors (G.R., Y.d.W.). The surgical resective procedure was performed under local anesthesia. After the incision, one or more millimetres under the level of the marginal gingiva in order to remove the inflamed soft tissue collar and create pocket reduction, a full-thickness flap was elevated at the buccal and lingual aspect of the affected implants. Subsequently, granulation tissue was removed using handinstruments (Hu Friedy®, Chicago, IL, USA). Calculus, if

present, was removed carefully with a scaler tip, and mechanical debridement of the peri-implant surface followed. According to the randomization, patients were assigned either to the test group or control group. In the test group the implant surface was treated with air-polishing (Airflow®, using the Airflow Master Piezon® device, EMS, Nyon, Switzerland) with erythritol-based powder containing 0.3% chlorhexidine (14µm, PLUS Powder, EMS). In the control group the implant surface was mechanically cleaned with saline-soaked cotton gauzes. In both groups, therapy was applied until the implant surface was assessed as visually clean by the surgeon followed by local application of abundant amounts of sterile saline. The angulation under which the air powder spray was applied and the working distance of the air polisher were factors that were not standardized in this study, as both factors varied according to the area being cleaned. The bone was recontoured on indication. After debridement the gingival flap was repositioned and closed with single interrupted sutures in a slightly apical position after which suprastructures were reconnected. Patients were instructed to use an antiseptic mouthwash (0.2% chlorhexidine, Orasol®, ICM Pharma Pte. Ltd., Singapore) for 2 weeks after surgery, two times daily. Two weeks after surgery, sutures were removed and patients were instructed to perform adequate self-performed peri-implant oral hygiene measures (i.e., at least twice daily use of electric toothbrush and use of interdental brushes).

Assessments

Clinical assessment

Peri-implant assessment took place at baseline and 3, 6, 9 and 12 months after intervention. Additional full mouth periodontal charts were made at baseline and 12 months. The clinical parameters were assessed by one and the same experienced examiner (D.H.) who was blinded for group allocation. At 6 sites per tooth and implant (mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distoligual) BoP, visible presence of plaque and/or plaque on probing (Plq) and SoP were binominally assessed (1 = present or 0 = not present) using a Hu-Friedy PCPUNC156 periodontal probe and Shephaerds Hook Explorer EXS23. Probing pocket depths were scored in absolute values to the nearest millimeter. A partial Vinyl Polysiloxane (VPS) impression (EXABITE™ II NDS, GC America Inc., Alsip, Illinois, US) was made of the suprastructure and buccally trimmed to be used as fixed reference point to assess the marginal peri-implant mucosa level. The distance from the mid-buccal marginal mucosa to the margin of the VPS mold was assessed using a periodontal probe at baseline and 12 months after surgery to calculate the recession. The top of the suprastructure was taken as a fixed reference point in case of an overdenture attachment system. In addition, a periodontal probe was used to assess the midbuccal width of keratinized mucosa. Midbuccal keratinized mucosa (KM) levels were assessed at baseline and 12 months. During surgery, the

peri-implant bone defect was measured at four sites around the implant (mesial, distal, buccal, palatinal) taking the implant-abutment platform as reference and classified according to the bone defect morphology classification by Schwarz et al. 2007.

Radiographical assessment

Radiographs were taken at baseline and 3, 6 and 12 months after treatment (Planmeca Intra X-ray unit; Planmeca, Helsinki, Finland). To standardize the peri-apical radiographs and to assure perpendicularity (*i.e.,* positioning of the film parallel to the long axis of the implant) an individualized X-ray holder and paralleling technique were used. Panoramic images were taken if peri-apical radiographs were painful for the patient (*e.g.,* painful to the floor of the mouth), or if no position was possible in which reproducible images could be made. Peri-implant bone loss was measured using the DICOM software (DicomWorks 1.5). Calibration of each radiograph took place on a 3-point reference scale using the known implant length and/or diameter. Bone level differences were calculated for the mesial and distal site of the implant. The outer points of the implant connection plateau were taken as reference to which the initial bone level was present (in bone level implants). In the presence of a smooth transgingival segment of the implant (1-stage implant systems *i.e.,* tissue level implants) measurement corrections were made. In order to calculate the inter-observer and intra-observer agreement, radiographic images of ten randomly selected implants were examined twice by the same researcher (D.H.) and once by another researcher (H.M.), both of whom were blinded regarding group allocation. High intraclass correlation (0.98) was found after which D.H. measured all the X-ray images.

Microbiological assessment

Microbiological samples from the peri-implant sulcus were obtained before and 12 months after surgical therapy using 4 sterile paper points. Supragingival plaque was mechanically removed before sampling. Samples were taken from four sites around the implant (mesiobuccal, distobuccal, mesioligual, distolingual). If a patient had more than one implant, sampling was divided over the implants, taking the deepest pocket per implant. The samples collected from each patient were pooled in an empty vial. In dentate patients, bacterial samples were also taken from the periodontal sites with the deepest probing pocket depth in each quadrant. If no deepened pockets were present, samples were taken from the mesiobuccal pockets of the first molars. Outcome variables were the presence and numbers of the following periodontal marker species; *Aggregatibacter actinomycetemcomitans (*Aa*), Porphyromonas gingivalis* (Pg*), Prevotella intermedia* (Pi)*, Tannerella forsythia* (Tf)*, Fusobacterium nucleatum* (Fn)*, Parvimonas micra* (Pm)*, Treponema denticola* (Td)*,* and *Filifactor alocis* (Fa)*.* Microbial samples were sent

to Lab*Oral* Diagnostics (Houten, the Netherlands) and analysed using real time-PCR (quantitative polymerase chain reaction - qPCR).

Outcomes

Primary outcome

The change in the mean of 6 peri-implant sites (%) showing BoP was defined as the primary outcome.

Secondary outcomes

Peri-implant parameters SoP (%), Plq (%) and PPD (mm) and full mouth periodontal parameters BoP (%), SoP (%), Plq (%), PPD (mm) were defined as secondary clinical outcomes. The change in mean of 6 sites per implant and tooth was calculated. Mean marginal bone loss (mm) and the presence and levels of 8 classical periodontal bacterial species were other secondary outcomes.

Success criteria

The surgical implant therapy was considered successful at the 12 month evaluation when implant sites demonstrated:

- PPD < 5mm
- Max 1 out 6 sites BoP
- No suppuration on probing
- No progressive radiographic bone loss ≥ 0.5mm, compared to baseline study radiographs

Sample size calculation

The sample size calculation for the present study was based on the total number of patients required for the two-staged treatment design (*i.e.,* surgical therapy following non-surgical therapy in case of persisting peri-implantitis). A calculation was performed in such a way that a sufficient amount of patients from the non-surgical phase would be available for the surgical phase, taking into account a three-level mixed model structure. Additionally, the total number of patients was estimated from a sample size and power calculation for a three-level mixed model structure, with implants (level 1) nested in patients (level 2), which are analyzed over time (level 3). Literature on sample size and a power calculation of multilevel analyses has shown that at least 50 patients should be included for there to be a relevant statistic difference (Maas and Hox, 2005). Scherbaum and Ferreter (2009) pointed out the relationship of different levels in accordance to an adequate sample size and power (Scherbaum and Ferreter, 2009). Translation of this relationship to our research protocol means a sample size (amount of patients)

in combination with implants nested in patients. With a mean group size of 2 infected implants per patient and a minimum amount of 50 patients, it was estimated to detect a medium effect size with 80% power at a significance level of α=0.05. Since our study focused on clinical relevant effects, small effect sizes were less important and detection of medium effect sizes were supposed to be sufficient for our study. According to the non-surgical peri-implantitis literature at the time of the study design, we estimated a 20% success rate for our non-surgical patient treatment phase (Muthukuru, et al., 2012). Therefore, it was assumed that 80% of the patients would need surgical follow-up. To compensate for patient withdrawal and losses to follow up (10%), a sample size of 80 patients was used at baseline. This was an intentional slight overestimation in order to assure enough available participants for the surgical phase of our design.

Randomization

Patients were randomly assigned to one of the two groups (test and control) following stratified randomization, taking into account the preceding non-surgically performed treatment (air-polishing/ultrasonic). Predefined generated notes with either 'air-polishing' or 'conventional' were equally divided over coded (AA, AB, etc...), identically sealed envelopes. On the day of the intervention, an operator assistant opened a coded envelop to decide which therapy to apply. Accordingly, all included implants per patients were treated with the randomized therapy. The code was written down and a decoding list saying which code belongs to which procedure was kept sealed until data analysis. This way the investigator performing the clinical assessments and data analysis (DH), which was not present at the surgical procedure, did not know which therapy was applied.

Statistical analysis

To analyse the difference in clinical and radiographical effects between both treatments, generalized linear mixed models (GLMMs) were used (IBM SPSS Statistical software, version 23.0. for Windows, Armonk, NY: IBM Corp). A three-level structure was chosen with patient, implant and time as level 1, 2 and 3, respectively. The patient was considered unit of analysis, whereas the implant unit of observation. First, the clinical and radiographical outcomes were analysed while controlling for the corresponding baseline parameters BoP, SoP, Plq, PPD and MBL (*i.e.,* crude analysis). Then, the primary and secondary outcomes were analysed while controlling for the baseline values and confounding effects (*i.e.,* adjusted analysis). The following a priori defined confounders were used in the adjusted mixed model: history of periodontitis (dichotome), smoking, implant surface modification (nominal), mean periodontal plaque level at T12 and mean marginal bone loss at baseline (linear). For skewed data (SoP and Plq) a gamma distribution was used. Within- group differences of the peri-implant clinical parameters

(BoP, SoP, Plq, PPD and MBL) were also analysed using GLMM, whilst taking the multilevel structure into account. Differences in full mouth periodontal outcomes and midbuccal recession between groups were analysed using an independent samples t-test. A paired samples t-test was applied to analyse differences in overall mean full mouth periodontal outcomes before and 12 months after therapy. The log-transformed mean peri-implant and periodontal microbiological outcomes were analysed at T12 using a Mann-Whitney U test for microbiological between-group differences. A Wilcoxon signed rank test was used for within-group differences. The data collected at baseline, 3, 6, 9 and 12 months are presented with descriptive statistics (see Table 2).

RESULTS

The flow of patients throughout the study is depicted in Figure 1. A total of 62 patients were screened for eligibility. Four patients declined to participate after which 58 patients (mean age 58.9 ± 11.7, male N = 25, female N = 33) were randomized over the test and control group. Between baseline and 12 month follow-up, 22% of the patients and 18% of the implants (5 patients (7 implants) in the test group and 8 patients (10 implants) in the control group) discontinued the study, all due to implant removal because of persisting peri-implantitis. In total, 27 patients (n = 54 implants) in the test group and 31 patients (n= 40 implants) in the control group were available for analysis.

The overall baseline patient and implant characteristics are shown in Table 1. The clinical and radiographical peri-implant outcomes and periodontal full mouth scores are shown in Table 2. In Table 3, the unstandardized β coefficient and significance levels for the mean difference in BoP, SoP, Plq, PPD and MBL between the control and test group during follow-up are presented. The distribution of sites with BoP in implants with PPD < 5mm, without suppuration and without progressive bone loss > 0.5mm is shown in table 4. The prevalence of patients positive for the selected marker species for peri-implant and periodontal samples (in partial edentulous patients) are presented in figure 2 and 3. Both treatments went uneventful; no cases of emphysema after air-polishing therapy were reported.

Primary outcome

No statistical significant difference was found between the test and control group over 12-month time for mean BoP, neither in the crude nor in the adjusted analysis (Table 3). Within both groups a significant reduction in mean BoP was seen between baseline and 12-months follow-up (test group: $p < 0.001$ and control group: $p = 0.042$) (Table 2).

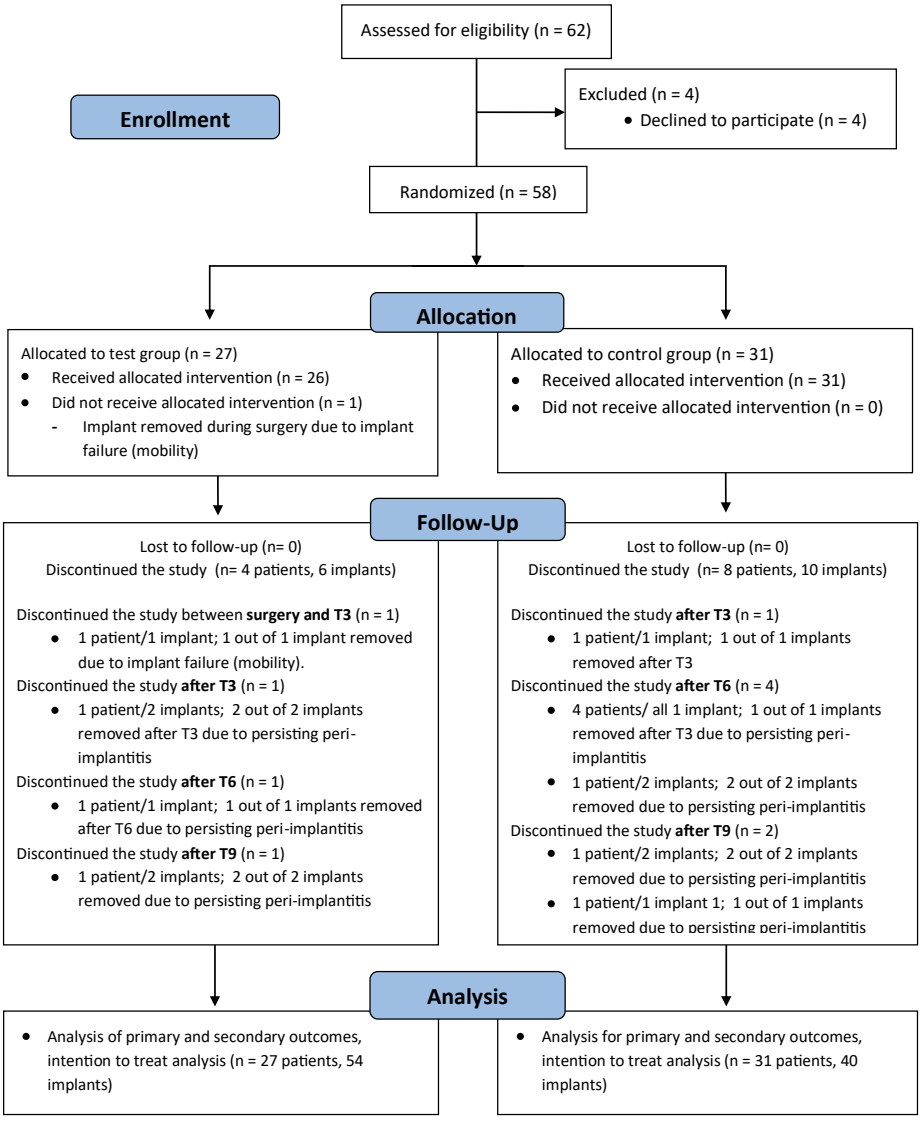

Figure 1. Flow diagram

Table 1. Baseline patient and implant characteristics

	Test	Control
Patient characteristics		
Total number of patients	27	31
Age [years; mean (SD)]	59.6 (13.6)	59.3 (10.0)
Gender; F (female) / M (male)	12 / 15	13 / 18
Smoking; n subjects (%)		
Current	5 (18.5)	8 (25.8)
Never	14 (51.9)	20 (64.5)
Former	8 (29.6)	3 (9.7)
History of periodontitis; n subjects (%)		
Yes	9 (33.3)	12 (38.7)
No	18 (66.7)	19 (61.3)
Diabetes; n subjects (%)		
Yes (but controlled; HbA1c < 7% or < 53 mmol/mol)	1 (3.7)	1 (3.2)
No	26 (96.3)	30 (96.8)
Dental status, n patients (%)		
Fully edentulous	7 (25.9)	6 (19.4)
Patially edentulous	20 (74.1)	25 (80.6)
Implant characteristics		
Total number of implants	80	83
Total number of implants presenting peri-implantitis (range)	54 (1-6)	40 (1-3)
Time in function [years; mean (SD)]	8.9 (5.8)	8.9 (6.1)
Implant type; n implants (%)		
Nobel Biocare	21	17
Straumann	22	14
Biomet 3i	7	0
Astra Tech	0	3
Other (Camlog, MegaGen, Simpler,IMZ, Dentsply Friadent, Smeden-Martina, Trinon Q)	4	6
Implant surface		
SLA + SLAactive	22	17
TiUnite	21	12
Other(Osseotite, Osseospeed, Xspeed, machined/turned, plasma sprayed HA)	11	11
Type of suprastructure; n implants (%)		
Single crown	14 (25.9)	28 (70.0)
Fixed partial denture (FPD)	18 (33.3)	2 (5.0)
Overdenture	22 (40.7)	10 (25.0)
Screw- or cement-retained restoration; n implants (%)		
Screwed	38 (70.4)	28 (70.0)
Cemented	16 (29.6)	12 (30.0)
Implants placed in maxilla or mandible; n implants (%)		

Table 1. (Continued)

		Test	Control
Maxilla		36 (66.7)	20 (50.0)
Mandible		18 (33.3)	20 (50.0)
Implants placed anterior posterior; n implants (%)			
Anterior (central incisor to cuspid)		22 (40.7)	16 (40.0)
Posterior (premolar/molar)		32 (59.3)	24 (60.0)
Bone defect configuration and grade (according Schwarz et al. 2007 and modified by Monje et. al 2019)			
Configuration			
1a	buccal dehiscence	2 (3.7%)	4 (10.0%)
1b	2/3 wall defect	9 (16.6%)	12 (30.0%)
1c	Circumferential	3 (5.5%)	2 (5.0%)
2	horizontal/supracrestal	8 (14.8%)	6 (15.0%)
3a	horizontal/supracrestal + buccal dehiscence	5 (9.3%)	4 (10.0%)
3b	horizontal/supracrestal + 2/3 wall defect	24 (44.4%)	10 (25.0%)
3c	horizontal/supracrestal + circumferential	3 (5.5%)	2 (5.0%)
Grade			
A	slight 3-4mm/<25% of the implant length	11 (20.4%)	15 (37.5%)
B	moderate 4-5mm/25-50% of the implant length	21 (38.9%)	19 (47.5%)
C	advanced >6mm/>50% of the implant length	22 (40.7%)	6 (15.0%)

Secondary outcomes

Clinical and radiographical outcome

No significant difference in PPD or MBL, neither in the crude nor in the adjusted analysis, between both groups was found over 12 months' time, see Table 3. Between both groups, a significant difference was found for the secondary clinical parameter SoP ((test 7.1%±15.4 versus control 11.1% ±19.8, β coefficient 0.211(0.017 to 0.406), $p = 0.035$) when taking into account the a priori defined confounders (adjusted analysis) (Table 3). In addition, a significant difference was found for mean levels of Plq ($p = 0.027$) while controlling for the baseline value and time (crude analysis). However, when all the predefined confounders were applied in the adjusted model the difference disappeared (p = 0.979). Full mouth periodontal plaque scores significantly reduced in the test group between baseline and 12 months follow-up (p = 0.023) (see Table 2). Midbuccal recession assessment showed a mean of 1.24mm and 0.76mm at 3 months and 0.97mm and 0.65mm and 12 months, in the test group and control group, respectively. Buccal keratinized mucosa levels at baseline were 3.37mm (±2.1) and 2.64mm (±2.1), in the group and control group respectively, and 1.96 (±2.0) and 1.88 (±1.6) in the test and control group respectively at 12 months.

Table 2. Descriptive statistics of clinical and radiographical outcomes test and control group

		Test					
N = 58 patients / 94 implants		Non-surgical phase		Surgical follow-up			
	Outcomes	Tpre (27/54)	Tpost* (27/54)	T3 (25/52)	T6 (23/44)	T9 (23/49)	T12 (22/47)
mean BoP (%)†	site level (SD)	59.6 (31.7)	52.2 (30.4)	40.0 (28.0)	33.4 (25.1)	31.5 (24.3)	34.0 (25.8)
	implant level (n)	92.6 (54)	90.7 (54)	83.3 (52)	79.5 (44)	77.5 (49)	80.1 (47)
mean SoP (%)†	site level (SD)	15.7 (20.3)	17.3 (22.2)	6.7 (15.2)	5.7 (16.1)	7.8 (17.4)	7.1 (15.4)
	implant level (n)	51.9 (54)	51.9 (54)	19.2 (52)	13.6 (44)	18.4 (49)	21.2 (47)
mean Plaq (%)†	site level (SD)	21.9 (34.7)	16.1 (34.6)	18.0 (21.8)	8.1 (12.2)	19.1 (25.2)	22.3 (37.3)
	implant level (n)	37.0 (54)	22.2 (54)	57.7 (54)	38.6 (44)	57.1 (49)	38.3 (47)
PPD (mm)†	mean (SD)	5.1 (1.4)	4.9 (1.6)	3.4 (1.1)	3.5 (1.2)	3.4 (0.9)	3.3 (0.8)
MBL (mm)‡	mean (SD)	4.4 (1.9)	4.3 (1.7)	4.5 (1.7)	4.3 (1.6)	NA	4.5 (1.7)
Full mouth mean BoP (%)	patient level (SD)	10.6 (9.3)	10.0 (7.3)	NA	NA	NA	14.8 (8.2)
Full mouth mean SoP (%)	patient level (SD)	0.0 (0.0)	0.0 (0.0)	NA	NA	NA	0.7 (2.8)
Full mouth mean Plaq (%)	patient level (SD)	24.9 (20.9)	19.9 (16.9)	NA	NA	NA	15.1 (14.4)
Full mouth mean PPD (mm)	patient level (SD)	2.1 (0.3)	2.0 (0.6)	NA	NA	NA	2.1 (0.23)

*outcome at 3 months after non-surgical treatment is baseline outcome (T0) for surgical treatment
†measured on a 6 point scale
‡measured at the mesial and distale site

Control					
Non-surgical phase		Surgical follow-up			
Tpre (31/40)	Tpost* (31/40)	T3 (31/40)	T6 (30/39)	T9 (25/32)	T12 (23/30)
59.0 (26.7)	58.3 (30.4)	42.4 (26.0)	41.0 (27.2)	39.6 (27.2)	44.4 (26.7)
97.5 (40)	95.0 (40)	90.0 (40)	87.2 (39)	90.6 (32)	86.6 (30)
16.7 (20.3)	15.0 (21.6)	8.0 (18.5)	15.0 (27.0)	10.9 (19.7)	11.1 (19.8)
65 (40)	50.0 (40)	27.5 (40)	30.8 (39)	28.1 (32)	30.0 (30)
18.3 (24.4)	8.8 (16.0)	20.5 (28.3)	10.3 (14.6)	11.1 (15.7)	11.7 (14.6)
47.5 (40)	30.0 (40)	55.0 (40)	43.6 (39)	46.9 (32)	46.7 (30)
4.7 (1.0)	4.6 (1.0)	3.5 (1.2)	3.7 (1.4)	3.5 (1.2)	3.5 (1.4)
3.5 (1.6)	3.7 (1.7)	3.9 (1.8)	3.7 (1.7)	NA	3.8 (2.0)
13.0 (12.8)	8.9 (6.9)	NA	NA	NA	11.3 (9.2)
3.5 (0.2)	0.0 (0.0)	NA	NA	NA	0.0 (0.0)
27.7 (13.0)	22.1(14.5)	NA	NA	NA	20.0 (10.8)
2.0 (0.2)	2.1 (0.3)	NA	NA	NA	2.2 (0.2)

Table 3. Generalized linear mixed model outcomes for mean difference in BoP, SoP, Plq, PPD and MBL between test and control group at T12

	Crude analysis¶		Adjusted analysis§	
Outcome	β (95% CI)	*p*-value	β (95% CI)	*p*-value
Mean BoP†	0.034 (-0.009 to 0.077)	0.120	0.037 (-0.016 to 0.089)	0.170
Mean SoP‡	0.157 (-0.000 to 0.314)	0.051	0.211 (0.017 to 0.406)	*0.035*
Mean Plq‡	-0.169 (-0.319 to -0.019)	*0.027*	-0.002 (-0.163 to 0.159)	0.979
Mean PPD†	0.083 (-0.018 to 0.184)	0.108	0.052 (-0.075 to 0.178)	0.423
MBL†	-0.019 (-0.063 to 0.025)	0.405	-0.030 (-0.098 to 0.037)	0.377

† Normal distributed data analysed with linear model distribution
‡ Non-normal distributed data analysed with gamma distribution
¶ Adjusted for baseline value and time
§ Adjusted for baseline value, time, smoking, history of periodontitis, mean periodontal full mouth plaque score at T12, mean marginal bone loss at T0 and implant surface

Table 4. Distribution of sites with BoP in implants with PPD < 5mm, without suppuration and progressive bone loss > 0.5mm

Sites with BoP	N (% of total implants)	Air-polishing	Saline-soaked gauzes
0 out of 6	9 (9.6%)	6 (25.0%)	3 (18.8%)
1 out of 6	9 (9.6%)	7 (29.2%)	2 (12.5%)
2 out of 6	9 (9.6%)	6 (25.0%)	3 (18.8%)
3 out of 6	6 (6.4%)	2 (8.3%)	4 (25.0%)
4 out of 6	6 (6.4%)	2 (8.3%)	4 (25.0%)
5 out of 6	1 (1.1%)	1 (4.2%)	0 (0.0)
6 out of 6	0 (0.0%)	0 (0.0%)	0 (0.0)
	40/94	24/40	16/40

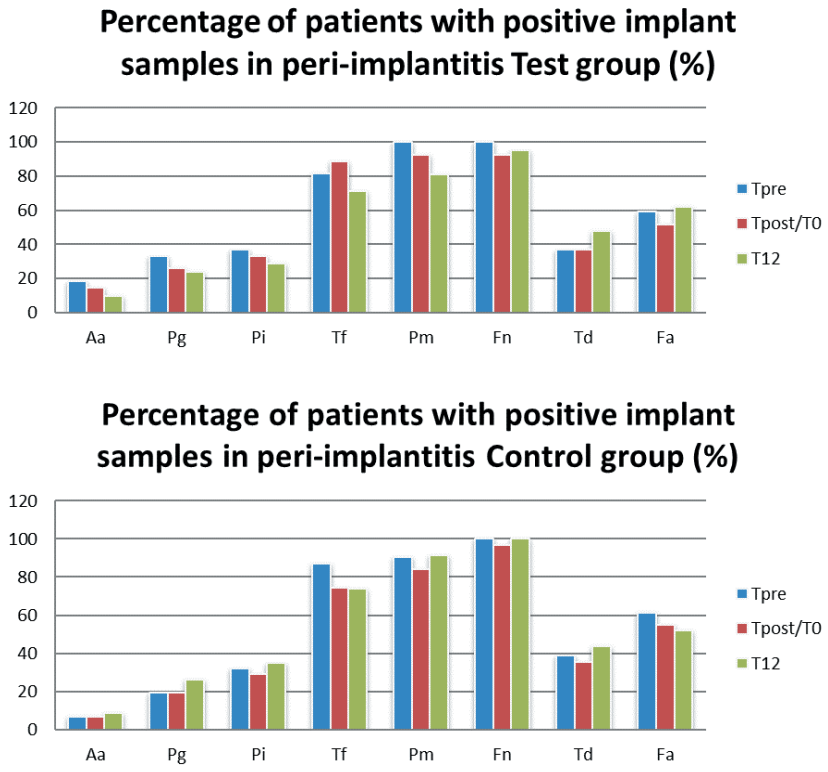

Figure 2. Percentage of patients (%) with positive peri-implant samples in test and control group for the presence of Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Tannerella forsythia (Tf), Fusobacterium nucleatum (Fn), Parvimonas micra (Pm), Treponema denticola (Td), and Filifactor alocis (Fa) before non-surgical intervention (Tpre), 3 months after the non-surgical intervention/before the surgical intervention (Tpost/ T0) and 12 months after the surgical intervention (T12)

Microbiological outcome

Samples of forty-four patients were available for analysis at 12 months after treatment (21 test group, 23 control group). No significant differences between both groups for mean peri-implant log-transformed bacterial counts were found for any of the bacterial marker species at 12 month evaluation (Mann-Whitney U test $p > 0.05$) (see figure 2). Within group analysis revealed no significant changes after therapy (Wilcoxon test $p > 0.05$). The majority of samples from the natural dentition showed no difference in mean counts 12 months after therapy in both groups (see figure 3). However, a significant difference in levels of *Pi*, *Td* and *Fa* was seen for the control group.

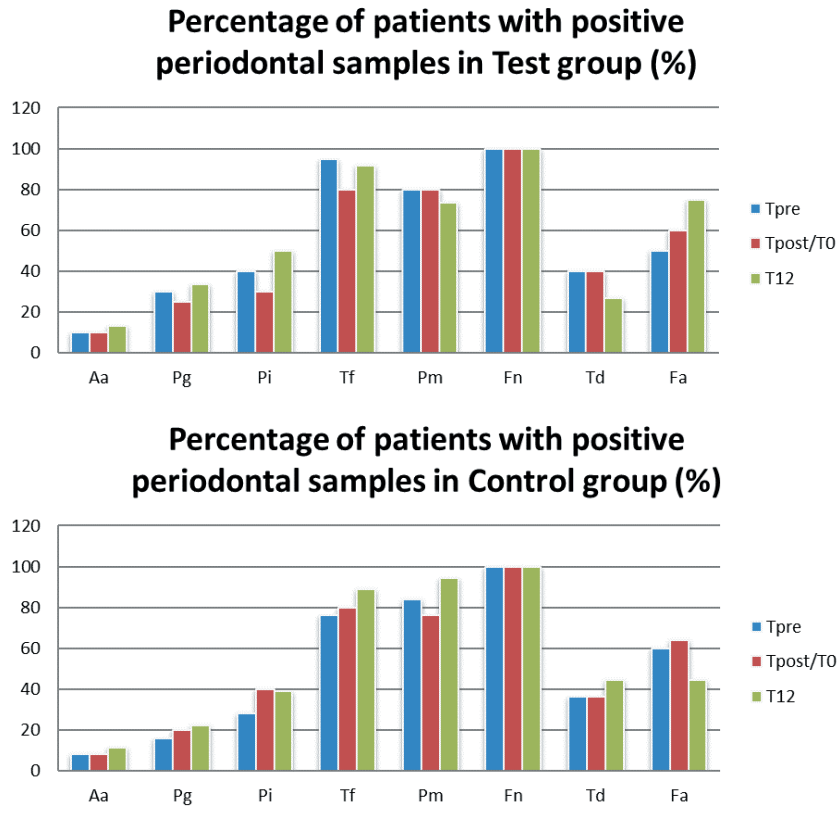

Figure 3. Percentage of patients (%) with positive periodontal samples in test and control group for the presence of Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Tannerella forsythia (Tf), Fusobacterium nucleatum (Fn), Parvimonas micra (Pm), Treponema denticola (Td), and Filifactor alocis (Fa) before non-surgical intervention (Tpre), 3 months after the non-surgical intervention/before the surgical intervention (Tpost/ T0) and 12 months after the surgical intervention (T12)

Treatment success

According to the success criteria applied, a total of 18 implants (19.1%) showed a successful treatment outcome at 12 months after surgery. Success was achieved for 13 implants (32.5%) in the test group and 5 implants (12.5%) in the control group. The overall survival rate (i.e. presence of patients/implants at T12, no explantation) was 81.9% and 74.0% at implant level and at patient level, respectively.

DISCUSSION

To the best of our knowledge, this is the first study that compared the use of erythritol powder air-polishing with saline soaked gauzes as implant surface debridement methods during a resective surgical treatment of peri-implantitis. The results showed no significant clinical differences between both groups in terms of our primary outcome BoP and secondary outcomes PPD, Plq and marginal bone loss, up to 1-year after therapy. Neither microbiological differences nor differences in full mouth clinical parameters were found between the groups. Only levels of SoP differed after 12 months follow-up. Hence, our null-hypothesis of erythritol air-polishing being not better than saline sauked gauze as cleansing method in terms of clinical, radiographical and microbiological effects could be adopted.

Comparable clinical studies using erythritol air-polishing as implant surface decontamination method were not found in the literature. However studies that evaluated the use of air-polishing as single decontaminating method in a resective peri-implantitis treatment approach, as such, recently appeared in the literature (Toma, et al. 2019; Lasserre, et al. 2020). Both previous studies evaluated the use of glycine powder and applied this through a handpiece with plastic (subgingival) nozzle insert. After 6 months follow-up, it was concluded that glycine air-polishing and the use of a titanium brush both were more effective than plastic curettes (Toma, et al. 2019) and glycine air-polishing was as effective as implantoplasty (Lasserre, et al. 2020). As compared to the present study, glycine air-polishing did not appear significantly more effective than control therapies in terms of BoP reduction. Neither for the secondary parameter 'presence of plaque' differences were found, which also seems to corroborate our findings. Regarding PPD reduction, the study by Lasserre et al. (2020) showed no difference in PPD reduction between both groups. A significant result was however found (mean ±2.2mm vs ± 1.7mm) in study by Toma et al. (2019) favoring the use of air-polishing. Whether these differences with the present study could be explained by the use of a different powder, different handpiece insert or shorter length of follow-up remains to be found. For levels of SoP, of which no data was found in the studies by Toma et al. (2019), no difference between both groups was found in the study by Lasserre, et al. (2020). Since the present study found a significant difference between both groups, the literature seems inconclusive thus far with regard to SoP. Why air-polishing more than saline sauced gauzes caused this reduction remains unclear. To better understand the role of suppuration in peri-implant health, future studies should include this parameter more often. Considering that stable marginal bone levels and comparable (low) success rates were found (at implant level; 29%, 26%, respectively) this might suggest that mechanical cleaning with air-polishing in a resective surgical

approach is able to stop progression of bone loss. In addition to the previous 6-month results, possibly up to 1-year after therapy as shown in our study. On the other hand, the sensitivity of BoP in the present study seemed quite low to predict further bone loss. It could therefore be questioned if the total absence of BoP as part of the success criteria used in previous studies, is not too strict. In order to truly evaluate the influence of BoP on therapy success on the long term, future studies should consider to present a more detailed overview of BoP levels (at implant level). Furthermore, the absence of relevant changes in radiographic marginal bone levels between the 3-month intervals suggest that future studies should extend this radiographic evaluation interval to justify a balanced risk (radiation exposure) to benefit ratio.

Only recently, a similar comparison of decontamination methods was evaluated in an in-vitro setting by the group of Amate-Fernandez, et al. (2021). It was shown that erythritol had the same antibiofilm and antibacterial capacity on a 14 day grown multi-species biofilm as mechanical removal with saline-soaked gauzes which might be an explanatory basis of the clinical findings in the present study. Translation of these preclinical findings to a clinical situation should however be done with utmost care, considering that *in-vitro* studies using specimens and biofilm contaminants may not simulate actual clinical situations. Patients characteristics, the presence of suprastructures and anatomical limitations of the oral cavity (e.g. the tongue) are confounders in a clinical setting which could overshadow possible beneficial in-vitro effects. Hence, this might also explain why the favorable *in-vitro* effects of eryhtritol/chlorhexidine powder, in terms of bacterial growth suppression (e.g., *P. gingivalis* and *S. gordonii*) (Soderling, et al., 2010; Hashino, et al. 2013) and prevention of bacterial regrowth (Drago, et al. 2017, Amate-Fernandez, et al. 2021) could not be clinically underlined by the present study. Namely, microbiologically, erythritol air-polishing did not lead to significantly lower bacterial counts 12 months after therapy. One could however advocate that earlier sampling should have been performed to find a related effect, however the present findings indicate that even though there might be a beneficial effect on bacterial suppression/ regrowth (on the short term) it does not lead to a clinically relevant effect. The exact mechanism underlying the antibiofilm activity of eryhtritol remains poorly understood.

Up to date, it remains unknown which powder is favorable in terms of cleaning efficacy, surface change and the ability to restore the biocompatibility. A myriad of *in-vitro* studies evaluating different powders (e.g., sodium bicarbonate, glycine, erythritol, calcium carbonate, calcium phosphate, hydroxyl apatite, tricalcium phosphate etc.) having different sizes, forms and hardness, used with different devices (in different settings) in custom made defect models with different morphologies have emerged in the recent literature (Moharrami, et al., 2019). Both larger particles (i.e. sodium

bicarbonate; 40-60 µm) and smaller particles (i.e. eryhtritol; 14µm and glycine; 25µm) have shown to exert beneficial effects in *in-vitro* studies. Where larger particles may seem to provide a greater cleaning capacity, they do cause more alterations of the implant surface (crater-like defects on smooth surfaces, rounding or removal of sharp edges on rough surface). Although smaller particles on the other hand only cause almost no observable change of the implant topography at SEM analysis, they might have a reduced capacity to remove implant contaminants (Matsubara, et al., 2020). However, these smaller particles are more likely to reach areas in the rough implant surface inaccessible by larger particles. Hence, to which extent these different effects impact on peri-implant health recovery remain to be found. In addition to powder difference, implant thread geometry and apically facing thread parts were found to impact the air-polishing decontaminating efficacy (Sanz-Martín et al. 2021). The most effective biofilm removal could be achieved in implants having low thread pitch and low thread depth values and on the non-apical facing parts. Also implant defect morphology might be an important factor contributing to a successful outcome (Tuchscheerer et al. 2021). The group by Tuchscheerer, et al. showed that although glycine air-polishing was significantly more efficient in a surgical simulated setting than in a non-surgical setting, in none of the bone defects an entirely clean surface could be achieved. Significant difference appeared between bone defects of 30° (8.26 ± 1.02% color remnant) and 60° (5.02 ± 0.84% color remnant) which might suggest that less wide (intraosseous) bone defects might leave more biofilm remnants as trigger for peri-implant inflammation.

Taken together, a positive influence of erythritol air-polishing on the reduction of inflammatory parameters could be expected on the short term (up to 1 year). As single decontaminating approach it does however not seem to improve the clinical outcome more than saline soaked gauzes. Therefore saline rinsing still might be regarded the gold standard for implant surface decontamination. Hence, when not already present in a daily practice, it seems questionable if one should invest in an expensive mechanical treatment method/device. Nevertheless, the use of an air polisher could be regarded the most easy to handle device when trying to decontaminate the implant surface in a surgical approach and thus advocated when present. Moreover, RCTs evaluating the use of erythritol air-polishing in combination with chemical decontamination are needed.

The present study has some limitations. First, optimal accessibility of the peri-implant bone defect might not have been reached in all cases considering that cemented restorations were not removed prior to the surgical intervention. Hence, the implant surface might have been insufficiently cleaned.

Second, irrespective of the bone defect morphology, a resective approach was chosen with the aim to evaluate the single influence of mechanical implant surface debridement. In some cases (i.e., 3/4 wall or circumferentially bone defect) a regenerative approach could have been a more successful therapy. However, at the start of this study, research data comparing the outcomes of resective and regenerative approaches in a randomized clinical trial was scarce and did not (and still does not) per se favor a regenerative approach (Tomasi et al 2019).

Third, recent microbiological research using metagenomic techniques have revealed a microbiological profile of peri-implantitis which appears more diverse than previously thought (Charalampakis and Belibasakis 2015). Therefore, other microorganisms which we did not target with the qPCR technique in our study might be important in the etiology and disease progression of peri-implantitis.

At last, considering the low number of cases showing therapy success a subanalysis on confounding factors (e.g. implant surface, implant position, buccal keratined gingiva, type of suprastructure, history of periodontitis and smoking) appeared not feasible.

To conclude, within the limitations of the present study, cleansing of the implant surface using erythritol air-polishing seems as effective as the use of saline-soaked cotton gauzes in terms of clinical, radiographical and microbiological effect during the surgical resective treatment of peri-implantitis. The overall treatment success of air-polishing as single debridement method in a resective surgical approach however remains low. To improve the treatment success and prevent disease recurrence on the short term studies evaluating new potential (combination of) strategies are needed.

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5

CHAPTER 5

IMPLANT DECONTAMINATION WITH PHOSPHORIC ACID DURING SURGICAL PERI-IMPLANTITIS TREATMENT; A RANDOMIZED CONTROLLED TRIAL

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This chapter is an edited version of the accepted article: *Internationl Journal of Implant Dentistry* 2017, 3, 33 doi: 10.1186/s40729-017-0091-5.

ABSTRACT

Background

Peri-implantitis is known as an infectious disease that affects the peri-implant soft- and hard tissue. Today, scientific literature provides very little evidence for an effective intervention protocol for treatment of peri-implantitis. The aim of the present randomized controlled trial is to evaluate the microbiological and clinical effectiveness of phosphoric acid as a decontaminating agent of the implant surface during surgical peri-implantitis treatment.

Methods

Peri-implantitis lesions were treated with resective surgical treatment aimed at peri-implant granulation tissue removal, bone recontouring and pocket elimination. Fifty-three implant surfaces in 28 patients were mechanically cleaned and treated with either 35% phosphoric etching gel (test group) or sterile saline (control group). Microbiological samples were obtained during surgery; clinical parameters were recorded at baseline and at 3 months after treatment. Data were analysed using multi-variable linear regression analysis and multilevel statistics.

Results

Significant immediate reductions in total anaerobic bacterial counts on the implant surface were found in both groups. Immediate reduction was greater when phosphoric acid was used. The difference in log-transformed mean anaerobic counts between both procedures was not statistical significant (p = 0.108), but there were significantly less culture-positive implants after the decontamination procedure in the phosphoric acid group (p = 0.042). At 3 months post-surgery 75% of the implants in the control group and 63% of the implants in the test group showed disease resolution. However, no significant differences in clinical and microbiological outcomes between both groups were found.

Conclusion

The application of 35% phosphoric acid after mechanical debridement is superior to mechanical debridement combined with sterile saline rinsing for decontamination of the implant surface during surgical peri-implantitis treatment. However, phosphoric acid as implant surface decontaminant does not seem to enhance clinical outcomes on a 3-month follow-up.

Trial registry: www.trialregister.nl; identifier: NTR5185

INTRODUCTION

Triggered host defense responses initiate inflammation of the peri-implant soft tissue (peri-implant mucositis), which can lead to loss of peri-implant supporting bone (peri-implantitis), and eventually, result in implant failure (Lang & Berglundh 2011). An increasing prevalence of peri-implantitis has been described in recent literature (Derks & Tomassi 2015), with current incidence ranging from 1 to 47%. A non-linear, accelerating pattern of progress is suggested for the majority of cases, with an occurring onset within 3 years of function (Derks et al. 2016). As for periodontal disease, the presence of micro-organisms is an important factor for the development of an inflammatory response in peri-implant tissue (Lindhe & Meyle 2008). In order to effectively treat the peri-implant inflammation, disruption of microbial adhesion and reduction of biofilm accumulation on the implant surface is probably of eminent importance.

A number of mechanical interventions (*e.g.* abrasive air powder, teflon curettes, ultrasonic devices) and chemical agents (*e.g.* chlorhexidine, hydrogen peroxide) solely or in combination, have been described as methods for implant surface decontamination in both in-vivo and in-vitro studies, in both a surgical and non-surgical setting (Leonhardt et al. 2003, Máximo et al. 2009, Serino & Turri 2011, Heitz-Mayfield et al. 2012, De Waal et al. 2013, Bassetti et al. 2014, De Waal et al. 2016, Riben-Grundstrom et al. 2015). According to different reviews on in-vivo and in-vitro mechanical debridement (Esposito et al. 2012, Subramani 2012, Louropoulou et al. 2014, Schwarz et al. 2015, Ramanauskaite et al. 2016) a gold standard mechanical debridement regimen still does not exists. Possibly, the implant surface roughness and screw-shaped design of dental implants may compromise an effective mechanical intervention. Therefore, the additional use of chemical agents for implant decontamination may be advocated.

Antimicrobial solutions have been studied in different clinical studies (Gosau et al. 2010, Heitz-Mayfield et al. 2012, De Waal et al 2013, De Waal et al. 2016). No superior clinical effectiveness has been shown in a single study for a specific chemical decontamination protocol (for reviews see: Ntrouka et al. 2011, Subramani 2012, Meyle 2012). However, studies using acids at low pH (< 2) have shown potentially beneficial antiseptic effects (Zablotsky et al. 1992, Dennison et al. 1994, Strooker et al. 1998, Mouhyi et al. 2000, Wohlfahrt et al. 2012, Wiltfang et al. 2012, Chen et al. 2016, Htet et al. 2016). Especially results on decontamination with phosphoric acid might be promising. Wiltfang et al. (2012) showed that surface decontamination with phosphoric acid (pH 1) in a surgical treatment protocol, resulted in complete elimination of the bacterial microflora. Also, results of a short-term clinical trial by Strooker et al. (1998) showed an instant greater reduction of colony forming units on the implant surface when using phosphoric

etching gel (pH 1). According to An et al. (2012), acid-etching of the implant surface might positively influence the epithelial seal around dental implants, as shown in their in-vitro study. In addition, animal studies (Kolonidis et al. 2003, Alhag et al. 2008) showed re-osseointegration and direct bone-to-implant contact when acids were used. Therefore, phosphoric acids might be considered a potentially feasible decontaminating agent.

Thus far, the use of phosphoric acid etching gel as decontaminating agent has not been evaluated in a randomized controlled trial. The aim of the present randomized controlled trial is to evaluate the short-term microbiological and clinical effectiveness of 35% phosphoric etching gel as a decontaminating agent of the implant surface during resective surgical treatment of peri-implantitis.

METHODS

Trial design

The present study is a double-blind randomized controlled trial evaluating the effect of 35% phosphoric etching gel (test group) compared to the effect of saline (control group) for implant surface decontamination combined with mechanical debridement during surgical peri-implantitis treatment. Patients were randomly assigned to the test or control group using a one-to-one allocation ratio. The study has been conducted in full accordance with the World Medical Association Declaration of Helsinki (version 2008) and was approved by the Institutional Review Board of the University Medical Center Groningen, the Netherlands (METc2013.005). Written informed consent was obtained from all participants before entering the trial. Clinical trial registration was done at the Netherlands National Trial Register (www.trialregister.nl, trial number NTR5185). The CONSORT guidelines for reporting a clinical trial were followed.

Participants

Patients participating in this study were consecutively selected from the patient populations of the Center of Dentistry and Oral Hygiene and the Department of Oral and Maxillofacial Surgery of the University Medical Center Groningen, Groningen, The Netherlands from October 2012 to April 2014. Adult patients with at least one endosseous implant with clinical and radiographical signs of peri-implantitis were included. Peri-implantitis was defined as a loss of marginal bone ≥ 2 mm in combination with bleeding and/or suppuration on probing and a peri-implant probing depth ≥ 5 mm (De Waal et al. 2015). Implants had to be in function for at least two years.

Exclusion criteria were:

- Contraindications for the surgical procedures;
- A history of local radiotherapy to the head and neck region;
- Pregnancy and lactation;
- Uncontrolled diabetes;
- Systemic use of antibiotics within 3 months before inclusion;
- Long-term use of anti-inflammatory drugs;
- Incapability of performing basal oral hygiene measures as a result of physical or mental disorders;
- Uncontrolled periodontitis (PPD > 5mm);
- Implants with bone loss exceeding 2/3 of the length of the implant or implants with bone loss beyond the transverse openings in hollow implants;
- Implant mobility;
- Implants at which no position could be identified where proper probing measurements could be performed;
- Previous surgical treatment of the peri-implantitis lesions.

Interventions

The study protocol was based on the study protocols of two previous studies evaluating the decontaminating effect of chlorhexidine during surgical peri-implantitis treatment (De Waal et al. 2013; De Waal et al. 2015), and is briefly described below.

Within one month before surgical treatment all patients received extensive oral hygiene instructions and mechanical non-surgical debridement of implants and remaining dentition using hand instrumentation and/or an ultrasonic device. Immediately before surgical treatment screw-retained suprastructures were removed. In order to obtain an optimal overview of the peri-implant area during surgery, prior to the procedure only screw-retained suprastructures were removed. Cemented single crowns or bridges on mesostructures were left in place to prevent any damage to these structures. Directly after surgery, the screw-retained suprastructures were placed back. Cemented single crowns or bridges on mesostructures were left in place to prevent any damage to these structures. Vertical releasing incisions extending into the alveolar mucosa were placed using a surgical blade (no. 15) and full thickness mucoperiosteal flaps were raised buccally and lingually. Flaps were designed to allow optimal access to the peri-implant bone defect. Granulation tissue was removed using titanium curettes (Gracey; Hu-Friedy®, Chicago, IL, USA). The implant surfaces were mechanically cleaned using titanium curettes and gauzes and cotton pellets soaked in saline. Next, the patients were randomly allocated to either the test or control group. Subsequently, implants

were cleaned with either local application of 35% phosphoric acid gel (pH 1) for 1 minute (Temrex gel, Temrex, Freeport, NY, USA) (test group) or by rinsing with an abundant amount of sterile saline for 1 minute (control group). Care was taken to apply the phosphoric etching gel precisely on the implant surface using a syringe with a small tip. During one minute the etching gel was continuously rubbed on to the implant surface with a small brush. In both groups, the intervention continued with rinsing of the implant surface with an abundant amount of sterile saline for 1 minute. Angular bony defects were eliminated and bone was recontoured using a rotating round bur under saline irrigation. Mucosal flaps were apically positioned and firmly sutured (Vicryl Plus 3-0; Ethicon Inc., Somerville, NJ, USA) and suprastructures were re-positioned. For both control and test group, surgery was followed by 2 weeks of mouth rinsing with 0.12% CHX + 0.05% CPC without alcohol two times daily for 30 s. Sutures were removed after 2 weeks. Follow-up visits were scheduled after 3 (T3) months. Patients were all surgically treated by one experienced oral- and maxillofacial surgeon (GR).

Outcomes

Primary outcome variable

The primary outcome variable was the difference in anaerobic bacterial load of the implant surface before and after mechanical and chemical debridement and decontamination. After flap deflection and granulation tissue removal a sample was obtained from the implant surface by rubbing a sterilized brush (Microbrush® International, Grafton, WI, USA) across the implant surface (Tpre). A second sample was obtained after mechanical debridement, decontamination of the implant surface with the test or control substance and subsequent rinsing with sterile saline (Tpost). After sampling the top part of the brush was cut off and collected in a vial containing reduced transport fluid (Syed & Loesche, 1972). From every implant presenting peri-implantitis separate samples were obtained. All microbiological samples were processed within 24 h (Van Winkelhoff et al. 1985). The total anaerobic bacterial load and the presence and numbers of the periodontal pathogens (Zambon 1996) *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Fusobacterium nucleatum, Parvimonas micra* and *Campylobacter rectus* were determined by laboratory technicians who were blind to treatment allocation.

Secondary outcome variables

Secondary outcome variables were percentage of sites with bleeding on probing (% sites BoP), percentage of sites with suppuration on probing (% sites SoP), mean probing pocket depth (mean PPD) and microbial composition of the peri-implant sulcus. Measurements were performed before (pre) treatment (baseline, T_0) and at 3 months (T_3) after surgery by one and the same examiner (DH) who was blind to

treatment allocation. Peri-implant pocket depth was measured at four sites per implant (mesial, buccal, distal and lingual) using a pressure sensitive probe (KerrHawe Click Probe, Bioggo, Switzerland) (probe force of 0.25 N). Bleeding and suppuration were scored up to 30s after pocket probing. Microbiological peri-implant sulcus samples were collected from each implant with peri-implantitis using 4 sterile paperpoints per implant. Paperpoints were collected in a vial containing RTF and were analyzed in the same manner as the intra-operative samples. Outcome variables were total anaerobic bacterial load and the presence and numbers of the periodontal pathogens *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Fusobacterium nucleatum, Parvimonas micra* and *Campylobacter rectus*.

Randomization

Fourteen notes with the word 'phosphoric acid' and 14 notes with the word 'saline' were put into 28 identical, sequentially numbered, non-transparent envelopes according to a randomization list generated by a computer program. The envelopes were irreversibly sealed. During the surgical procedure, after flap deflection and mechanical cleansing, the surgeon temporarily left the operating room. The surgical assistant opened an envelope and prepared the materials as needed according to the information on the note. A third person (YDW) performed the decontamination procedure according to group allocation. The materials were removed and the surgeon continued the surgical procedure. The researcher (performing the clinical measurements, DH) was blind to treatment allocation and did not have access to the randomization code until the end of the research period.

Statistical methods

Sample size

Sample size was based on the microbiological data from a previous study evaluating the effect of implant surface decontamination with a chlorhexidine-solution versus a placebo-solution (De Waal et al. 2013). The decontaminating effect of phosphoric acid was expected to be similar to the decontaminating effect of chlorhexidine (reduction in log-transformed mean anaerobic bacterial load = 4.21 (chlorhexidine group) versus 2.77 (placebo group), SD = 2.12). Assuming a two-sided two sample *t*-test with a significance level (α) of 0.05 and a power (β) of 80% required a sample size of 34 implants. A 20% compensation for dropouts was taken into account (34/0.8 = 42.5 implants). Based on a previous study (De Waal et al. 2013) it was expected that not all baseline microbiological samples would yield a detectable number of cultivable bacteria (De Waal et al. 2013, 19 out of 79 = 24% of samples showed no bacterial growth). Because 'negative' samples cannot be used to determine a decontaminating effect, the sample size was

compensated for these potential unusable samples (24%), yielding a sample size of 56 implants (42.5/0.76). According to the assumption that each patient has on average more than two implants with peri-implantitis (De Waal et al. 2013), a sample size of 28 patients was chosen (56/2, 14 patients per group).

Statistical analysis

For the analysis of the primary outcome variable and the secondary *microbiological* outcome variable linear regression analysis was performed. The implant was taken as the statistical unit. Total anaerobic bacterial loads at baseline (T_{pre} and $T_{0)}$ were distributed normally after logarithmic transformation. Baseline values were included in the regression model. For the comparison of the number of culture-positive implants after the decontamination period the chi-square test was used. The secondary *clinical* outcome variables were analyzed using a two-level hierarchical random intercepts model. The two-levels of analysis were implant-level and patient-level. With the crude analysis, the effect of the intervention was determined, while controlling for baseline value. Because a previous study (De Waal et al. 2016) has shown that mean bone loss at baseline and smoking are prognostic indicators for the outcome of resective peri-implantitis treatment, these factors were additionally included in the model (adjusted analysis). Descriptive data and data regarding the microbiological outcome variables were analyzed using IBM SPSS Statistics 22 Version 22.0 (IBM Corp. Armonk, NY: IBM Corp.). Multilevel models were analyzed using MLwiN version 2.12 (Centre for Multilevel Modeling, University of Bristol, Bristol, UK).

RESULTS

The progress of patients throughout the different phases of the study is illustrated in Figure 1. Table 1 depicts the baseline demographic patient and implant characteristics. The included patients had a total of 128 implants of which 53 implants showed signs of peri-implantitis. Different implant brands and types with different implant surfaces were present, including Straumann (Straumann AG, Basel, Switserland; SLA® and SLActive® surface), Nobel Biocare (Nobel Biocare AB, Göteborg, Sweden; TiUnite® surface), Biomet 3i (Biomet Inc., Warsaw, Indiana, USA; OSSEOTITE® surface), Frialit-2, (Dentsply Friadent, Mannheim, Germany; FRIADENT® plus surface) and Pitt-Easy (Sybron Implant Solutions GmbH, Bremen, Germany; Puretex® surface). Three patients with each one implant with peri-implantitis were lost to follow-up (2 patients from control group, 1 from test group).

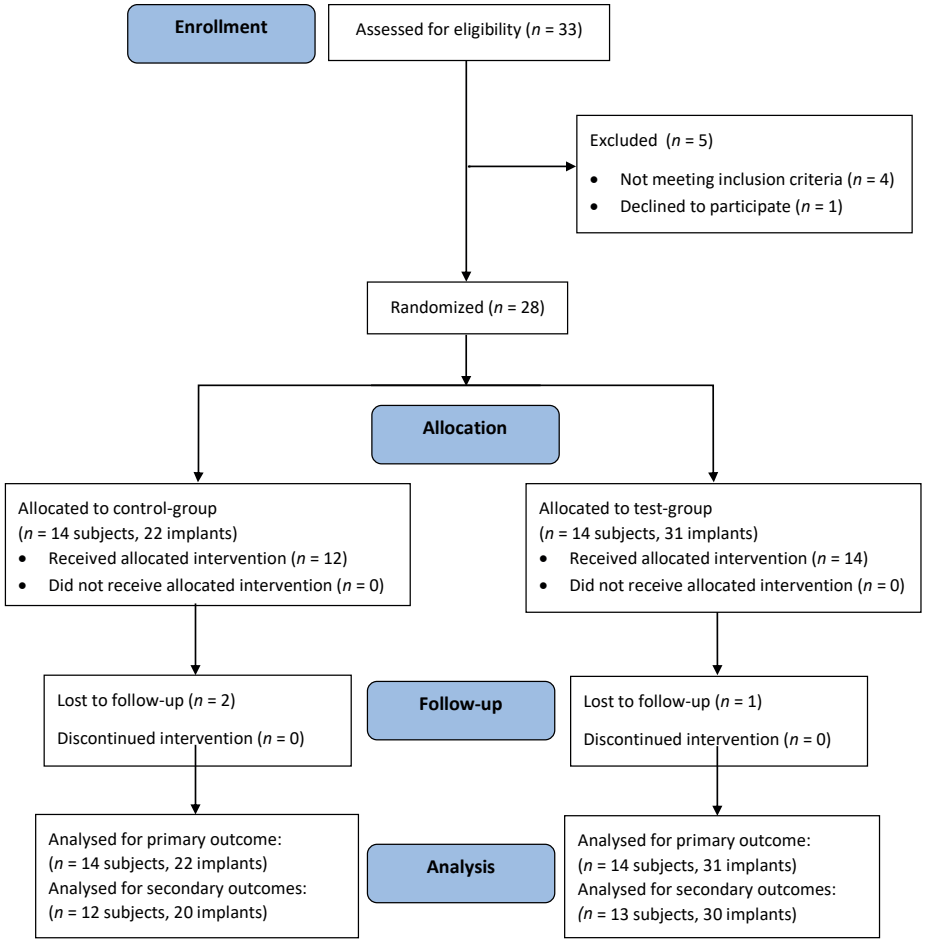

Figure 1. Flow diagram

Microbiological outcomes

[10]Log-transformed mean bacterial anaerobic counts of the culture-positive implants for the control and test group before and after debridement and decontamination of the implant surface during the surgical procedure are depicted in Table 2. In both groups, the debridement and decontamination procedure resulted in a significant immediate reduction in counts of anaerobic bacteria on the implant surface. Although the reduction in total anaerobic load was greater in the test group, the difference did not reach the level of statistical significance (p = 0.108). However, in the test group the total anaerobic load was significantly more often reduced below detection level than in the control group (20 out of 23 in the test group, 10 out of 17 in the control group,

p = 0.042). No significant differences were observed in the ^{10}Log-transformed mean bacterial anaerobic counts of the peri-implant sulcus, neither between control and test group, nor between baseline and three months after surgery (Table 3).

Table 1. Characteristics of included patients/implants.

Characteristics	Control	Test
Number of patients	14	14
Age (years; mean [SD])	57.0 (13.7)	60.9 (7.2)
Gender; M (male), F (female)	M5, F9	M7, F7
Smoking; n subjects (%)	1 (7%)	3 (21%)
History of periodontitis; n subjects (%)	4 (29%)	5 (36%)
Dental status; n subjects (%)		
• Partially edentulous	13 (93%)	12 (86%)
• Fully edentulous	1 (7%)	2 (14%)
Total number of implants (range)	68 (1-9)	60 (1-10)
Number of implants with peri-implantitis (range)	22 (1-4)	31 (1-5)
Mean bone loss at baseline in mm (SD)	2.73 (1.49)	3.58 (1.57)

Table 2. Log-transformed mean bacterial anaerobic counts (SD) of culture-positive implants for the control and test group before (Tpre) and after (Tpost) debridement and decontamination of the implant surface (intra-operative microbrush samples) crobrusD, standard deviation; from control group, 2 from test group).mple size of 28 patients. g the implant surface with a

N = 40*	Total anaerobic bacterial load Log-transformed mean (SD)				
	Tpre	Tpost	Difference	b (95% CI)**	p-value
Control	5.57 (0.93)	2.25 (2.98)†	2.68 (3.25)		
	[17]	[7]‡		-1.39 (-3.09 - 0.32)	0.108
Test	5.35 (0.98)	0.81 (2.25) †	4.19 (3.31)		
	[23]	[3]‡			

*Implants with baseline values of 0 excluded from analysis; SD, standard deviation; [n], number of culture-positive implants;
**Linear regression analysis, adjusted for baseline values.
†Significant difference from baseline
‡Significant difference in number of culture-positive implants after decontamination between test and control group (p = 0.042

Clinical outcomes

Descriptive statistics of the clinical outcomes at baseline and follow-up are depicted in Table 4. At 3-month follow-up 75% of the implants (66.7% of the patients) in the control

group and 63.3% of the implants (53.8% of the patients) in the test group showed no clinical signs of inflammation (PPD ≤ 4mm without bleeding and/or suppuration on probing) (Table 4). The results from the multilevel analyses regarding the effects of the intervention on BoP, SoP and PPD are shown in Table 5. No significant differences in BoP, SoP and mean PPD were detected between control and test group at 3 months after surgery, neither in the "crude" nor in the "adjusted" analysis.

Table 3. Log-transformed mean bacterial anaerobic counts (SD) for the control and test group before (T0) and 3 months after (T3) the surgical treatment (paperpoint samples)

N = 47*	Total anaerobic bacterial load Log-transformed mean (SD)				
	T0	T3	Difference	b (95% CI)**	*p*-value
Control	6.69 (1.32)	6.31 (1.30)	0.38 (1.36)	-0.26 (-0.84 - 0.33)	0.377
Test	6.53 (1.06)	5.98 (0.94)	0.55 (0.99)		

*3 samples without bacterial growth and 3 samples without follow-up excluded from analysis; SD, standard deviation;
**Linear regression analysis, adjusted for baseline values.

Table 4. Descriptive statistics of clinical parameters

		Control		Test	
N =		T0 (n = 22)	T3 (n = 20)	T0 (n = 31)	T3 (n = 30)
Plaque	% of sites (SD)	4.5 (12.5)	10.0 (18.8)	4.0 (9.3)	2.5 (7.6)
	% of implants (n)	13.6 (3)	25.0 (5)	16.1 (5)	9.7 (3)
BoP	% of sites (SD)	86.4 (18.5)	28.8 (35.6)	66.1 (29.3)	39.2 (31.3)
	% of implants (n)	100 (22)	50 (10)	96.8 (30)	76.7 (23)
SoP	% of sites (SD)	22.7 (24.3)	5.0 (15.4)	30.7 (20.1)	8.3 (20.1)
	% of implants (n)	54.5 (12)	10.0 (2)	80.6 (25)	20.0 (6)
Mean PPD	Mean (SD)	5.3 (1.1)	3.5 (1.5)	5.2 (1.1)	4.1 (1.6)
PPD ≥ 5 mm	% of sites (SD)	67.1 (26.0)	18.8 (30.2)	61.3 (22.2)	28.3 (33.9)
	% of implants (n)	100 (22)	35.0 (7)	100 (31)	46.7 (14)
PPD ≥ 6 mm	% of sites (SD)	50.0 (27.8)	12.5 (26.3)	46.8 (26.4)	24.2 (33.1)
	% of implants (n)	100 (22)	25.0 (5)	90.3 (28)	40.0 (12)
PPD ≥ 5 mm +	% of sites (SD)	65.9 (26.2)	12.5 (25.0)	54.8 (22.7)	20.0 (29.7)
BoP/SoP (same site)	% of implants (n)	100 (22)	25.0 (5)	100 (31)	36.7 (11)
	% of patients (n)	100 (14/14)	33.3 (4/12)	100 (14/14)	46.2 (6/13)
PPD ≥ 6 mm +	% of sites (SD)	50.0 (27.8)	8.8 (20.3)	41.1 (24.6)	17.5 (28.7)
BoP/SoP (same site)	% of implants (n)	100 (22)	20.0 (4)	90.3 (28)	33.3 (10)
	% of patients (n)	100 (14/14)	33.3 (4/12)	100 (14/14)	46.2 (6/13)

Table 5. Average differences in BoP, SoP, and PPD between control and test group at 3-month follow-up

	Crude model*		Adjusted model**	
Outcome variable	**β (95% CI)**	***p*-value**	**β (95% CI)**	***p*-value**
% Sites BoP	16.2 (-7.9 to 40.3)	0.743	7.9 (-16.4 to 32.3)	0.821
% Sites SoP	0.0 (-10.9 to 10.9)	1.000	0.7 (-10.1 to 11.4)	0.882
Mean PPD	0.6 (-0.6 to 1.8)	0.205	0.2 (-1.0 to 1.3)	0.470

The reference category for intervention effect is the control group. The regression coefficients (β) indicate the average differences in clinical outcomes between control and test group at three month follow-up. BoP, bleeding on probing; SoP, suppuration on probing; PPD, probing pocket depth; 95% CI, 95% confidence interval.
*Adjusted for baseline values.
**Adjusted for baseline values, smoking and mean bone loss at baseline

DISCUSSION

This randomized controlled trial aimed to determine the effect of 35% phosphoric etching gel on decontamination of the implant surface during resective surgical treatment of peri-implantitis. Both decontamination procedures (mechanical debridement with curettes and gauzes combined with phosphoric acid 35% and mechanical debridement combined with sterile saline) resulted in a significant immediate reduction in counts of anaerobic bacteria on the implant surface. This immediate reduction was greater when phosphoric acid was used. Although the difference in log-transformed mean anaerobic counts between both decontaminating procedures did not reach the level of statistical significance ($p = 0.108$), there were significantly less culture-positive implants after the decontamination procedure in the phosphoric acid group ($p = 0.042$). As our study focused on the decontaminating effect of phosphoric acid on implant surfaces we used the microbiological parameter as primary outcome variable. To evaluate the effect of the intervention on this microbiological parameter an in-vivo situation was chosen to benefit the influence of a clinical situation. In addition we evaluated secondary outcome parameters indicating the clinical effect of the treatment procedure, i.e. disease resolution 3 months after active treatment. At 3 months post-surgery disease resolution was more frequently observed in the control group (75% of implants) than in the test group (63.3% of implants)). However no significant differences in clinical and microbiological outcomes between control and test group were found. Although the study was 'a priori' not powered to detect clinical differences, no trend was observed for superior results of one decontamination procedure over the other.

To our knowledge, this is the first randomized controlled clinical trial reporting on the effect of phosphoric acid in relation to peri-implantitis treatment. The reason for

choosing phosphoric acid as decontaminating agent was that acids with low pH exert a strong bactericidal effect (Héritier 1984, Chen et al. 2016) and phosphoric acid does not seem to chemically damage titanium implant surface (Tastepe et al. 2013). A gel as application mode has the great advantage of being precisely applicable with minimal touching of the surrounding bone or connective tissue. A disadvantage of a gel might be the limited flow in deeper areas of the rough implant surface. To overcome this problem it was decided to continuously rub the etching gel onto the implant surface with a small brush during the decontamination period. Phosphoric acid gel as agent for implant surface decontamination has only been investigated in two other clinical studies (Strooker et al. 1998, Wiltfang et al. 2012). Strooker et al. (1998) used phosphoric acid 35% for peri-implant supportive therapy and found greater reductions in bacterial load, but no significant clinical differences compared to conventional mechanical supportive therapy. They concluded that local application of 35% phosphoric acid gel can be as effective as conventional mechanical therapy in the professional supportive care of oral implants. In the study of Wiltfang et al. (2012), 20% etching gel was used for implant surface decontamination in a combined surgical protocol for treatment of peri-implantitis. Thirty-six implants with peri-implantitis in 22 patients were followed for 1 year. The implants were decontaminated with etching gel and the defects were filled with autologous bone mixed with an osteoinductive material for regenerative treatment of bone defects. In their study previous microbiological tests (not published) of implants *in situ* had revealed complete elimination of the bacterial microflora after decontamination with etching gel, which is close to our results of 'complete' elimination (reduction below detection level) in 20 out of 23 implants. They concluded that their surgical protocol in combination with phosphoric etching gel provides a reliable method to treat peri-implant bone defects.

Phosphoric acid used in an *in vitro* setting has only been described in a study by Tastepe et al. (2013). The use of an air abrasive device with four different powders was compared to phosphoric acid. In contrast to our study and the previous described clinical studies the use of phosphoric acid was not efficient in removing biofilm. The residual biofilm area was significantly greater after treatment with phosphoric acid compared to air abrasive treatment with powder or even control treatment without powder. Apparently only water and air might be effective in reducing the biofilm. Nonetheless, when the titanium surface was viewed under a Scanning Electron Microscopy (SEM) no visible titanium surface change was seen after phosphoric acid application while some minor changes (dependent on the character and size of the particles) were observed after air powder abrasive treatment.

Recent studies that zoom in on titanium surface physico-chemistry reveal interesting results (Kotsakis et al. 2016, Wheelis et al. 2016). Kotsakis et al. (2016) hypothesized that chemical residues alter the titanium surface physicochemistry and subsequently compromise cellular response to these decontaminated surfaces. However, they report on effective restoring of biocompatibility when sterile saline, citric acid and EDTA/sodium hypochlorite (NaOCl-EDTA) were used, in contrast to chlorhexidine. Therefore they propose the use of sterile saline, citric acid and NaOCl-EDTA in the treatment of peri-implantitis not only for their antimicrobial properties but also for the preservation of the titanium material properties. In contrast, a study by Wheelis et al. 2016 found noticeable morphological changes and corrosion on the titanium surface when the synergistic effect of acidic environments (*i.e.* citric acid, 15% hydrogen peroxide, tetracycline, peroxyacetic acid) and mechanical forces (rubbing with cotton swabs) was investigated. Dissolution of the oxide layer (which can result in corrosion) was observed when using peroxyacetic and citric acid. It is therefore hypothesized that surface damage of dental alloys may potentially be induced after detoxification and maintenance treatments with acidic solutions and subsequently might hinder re-osseointegration. No visibly evident damage of the surfaces was shown by Wheelis et al. 2016 when neutral or basic treatments such as sodium fluoride 0.12%, 0.20%, and 1.10% were used, which might be explained by the neutral electrochemical environment (Suito et al. 2013).

Interpreting the results of these *in vitro* studies has to be done cautiously since the results among the studies are not homogenous and the effects of the chemical environment coupled with mechanical force in the oral environment has to be further evaluated. In our study however, phosphoric acid neither seemed to have a positive nor a negative effect on clinical outcomes. The current study is based on a follow up time of 3 months and therefore the long-term results on the use of phosphoric acid remain unclear.

CONCLUSION

Implant surface decontamination is considered a highly susceptible step in the treatment of peri-implantitis. The application of 35% phosphoric acid after mechanical debridement is superior to mechanical debridement combined with sterile saline rinsing for decontamination of the implant surface during surgical peri-implantitis treatment. However, phosphoric acid as implant surface decontaminant does not seem to enhance clinical outcomes on a 3-month follow-up. Larger studies with a longer follow-up period are needed to validate these findings.

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6

CHAPTER 6

INFLUENCE OF CERVICAL CROWN CONTOUR ON MARGINAL BONE LOSS AROUND PLATFORM-SWITCHED BONE-LEVEL IMPLANTS: A 5-YEAR CROSS-SECTIONAL STUDY

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This chapter is an edited version of the accepted article: *International Journal of Prosthodontics* 2020, 33,373-379. doi: 10.11607/ijp.6365.

ABSTRACT

Aim

The aim of the present cohort study was to evaluate the influence of the cervical crown contour on marginal bone loss and soft tissue health around platform-switched, posteriorly placed, two-piece implants.

Methods

A dataset from two previously conducted studies was used. Patients with single two-piece, platform-switched implants in between two natural teeth or adjacent to one natural tooth were included. Clinical parameters and standardized peri-apical radiographs taken at 1 month and 5 years after final crown placement were assessed. A new measurement method was developed to analyse geometric values of the cervical crown contour. The inter and intra-examiner reliability was assessed. Emergence angles were measured at 1, 2 and 3 mm above implant shoulder. The linear correlation between variables was determined by calculating the Pearson correlation coefficient.

Results

A total of 64 patients with 67 posteriorly implants met the inclusion criteria. At 1, 2 and 3 mm above the implant shoulder, mean emergence angles at the mesial implant sites were 0.5±2.8, 12.8±12.8 and 18.0±11.3 degrees, respectively. At the distal sites corresponding values were 2.8±8.3, 16.2±16.6 and 18.7±13.8 degrees. Mean marginal bone loss between 1 month and 5 years evaluation was 0.14±0.34mm at the mesial and 0.26±0.47 at the distal aspect of the implants. No correlation with peri-implant bone loss and soft-tissue health could be found. No implants showed signs of peri-implantitis.

Conclusion

The cervical crown contour at platform-switched, posteriorly placed, two-piece implants showed no correlation with peri-implant marginal bone loss and soft-tissue health up to 5 year after implant placement.

INTRODUCTION

Preservation of peri-implant marginal bone and maintaining healthy soft tissues are important for long-term implant success. However, this success can be influenced by a variety of prosthetic aspects. For example implant-abutment connection type, platform-switched or matched connections, screw-retained or cement-retained restorations, occlusal prosthesis design, implant-crown micro-gap level or abutment material, height and surface texture can be local predisposing factors that contribute to peri-implant disease (i.e. peri-implant mucositis and peri-implantitis) (1-9). In general, it is recommended that implant suprastructures should be designed in such a way that oral hygiene measures can be performed effectively, plaque accumulation is prevented and implants are accessible for probing (10). Early studies on subgingival crown contour and overhangs of restorations on natural teeth showed that when the crown contour is overly thick because of excessive bulk of tooth structure or restorative material, the free marginal gingivae are crowded, circumferential fibers are torn, and the gingival tissues are pushed beyond their physiologic limits of accommodation (11). Leading to increased plaque accumulation, gingival swelling and a detrimental effect on gingival health and marginal bone loss (12-17). However it is unclear whether this also applies to dental implant suprastructures. To date, no studies have been conducted on how to design the crown contour in terms of emergence angle and emergence profile with respect to preserving marginal bone level and peri-implant soft tissue, despite publication of prosthetically focused studies which mainly concern aesthetic outcome (15,18,19). Only one study thus far has focused on the implant crown contour (20). However, a heterogenic dataset consisting of several implant brands, with anterior and posteriorly placed, non-platformed switched, one and two-piece implants and peri-implantitis as primary outcome, was evaluated. Moreover, the most mesial/distal point of the crown contour was taken as intersection to measure the restoration emergence angle. Hence, the influence of the *cervical* crown contour on marginal bone loss remains unknown. Therefore the aim of this study was to evaluate the cervical crown contour on dental implants in relation to the peri-implant marginal bone level and peri-implant soft-tissue health. The null-hypothesis formulated was that the crown contour (in terms of emergence angle) in the first 3 mm, measured from the implant platform of platform-switched, posteriorly placed, two-piece implants, has no correlation with marginal bone level and peri-implant health.

MATERIAL AND METHODS

Subjects

Data used in the present study consisted of patient data from two studies previously performed at the department of Oral and Maxillofacial Surgery, University Medical Center Groningen (21,22). Both studies included non-smoking patients who needed one or more dental implants in the posterior region. Between 2005 and 2010, 122 implants were placed in a total of 96 patients. All patients received individually designed suprastructures. Up to 5 years after placement of the implant restoration patients were seen for clinical and radiographic follow-up examination. Inclusion criteria and results from clinical and radiographical analyses of both study populations were reported previously (21,22). Eligibility criteria used for the present study were as follows: patients with non-splinted, two-piece implants and platform-switched abutment connections, placed in posterior healed sites (i.e. 3 months healing of extraction sites after tooth removal) of maxilla and mandible, in between two natural teeth or adjacent to one natural tooth. Exclusion criteria were as follows; patients with two or more adjacent implants, implants in the anterior region (inter premolar region), implant-abutment matched connections (non-platform-switched), soft-tissue level implants or implants with a poor restoration connection (i.e. abutment-restoration gap), patients that were smokers. Implants that were not fully depicted or showed a buccal-lingual over-angulation on peri-apical radiographs were also excluded.

Radiographic analysis

Peri-apical radiographs (Planmeca Intra X-ray unit; Planmeca, Helsiniki, Finland) taken at one month (T1, baseline) and 5 years (T60) after placement of the final implant crown were used for radiographic assessment of the crown contour and marginal bone level. To standardize radiographs and assure perpendicularity (i.e. positioning of the film parallel to the long axis of the implant) an individualized X-ray holder and paralleling technique were used (23).

Bone level change measurement

Peri-implant bone level change was determined on radiographs taken on T1 and T60. The distance from the implant reference point (most outside point of implant neck) to the level of bone-to-implant contact was measured at both the mesial and distal aspect of the implant. Radiographs were calibrated using the known dimensions of the implant as reference values. Illustrator software (Illustrator CS; Adobe Systems Inc, San Jose, CA, USA) was used for calibration of the implants. The difference in bone level between one month and five years after crown placement was calculated and used for correlational analysis.

Crown contour assessment

A new measurement protocol using image processing software (Rasband, W.S., Image J, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997-2018) was developed. The protocol was as follows: first, calibration of the peri-apical radiograph in Image J was performed using the known implants lengths. After this, the apical-coronal direction of the implant position on the radiograph was assessed. For this purpose a vertical line was drawn through the middle of the implant axis, after which the angulation was computed. Parallel to the implant axis, on the first, second and third mm of the crown length, angles were assessed by calculating α (see Figure 1). A tangent function [tan(α) ='opposite side'/'adjacent side'] was used with the known lengths of the 'adjacent side' (1 mm, 2 mm, 3 mm respectively) and the measured lengths, using image J, of the 'opposite side'. Negative 'opposite side' values (crown contour on the inside of the 'adjacent side') were considered as '0' degree angle, since we assumed no additional influence from under-contoured crown areas compared to 0 degree angles. An online calculating tool (https://www.rapidtables.com/calc/math/Arctan_Calculator.html) was used for inverse tangent ($\tan^{-1}$) calculation. Accordingly, angle α could be calculated.

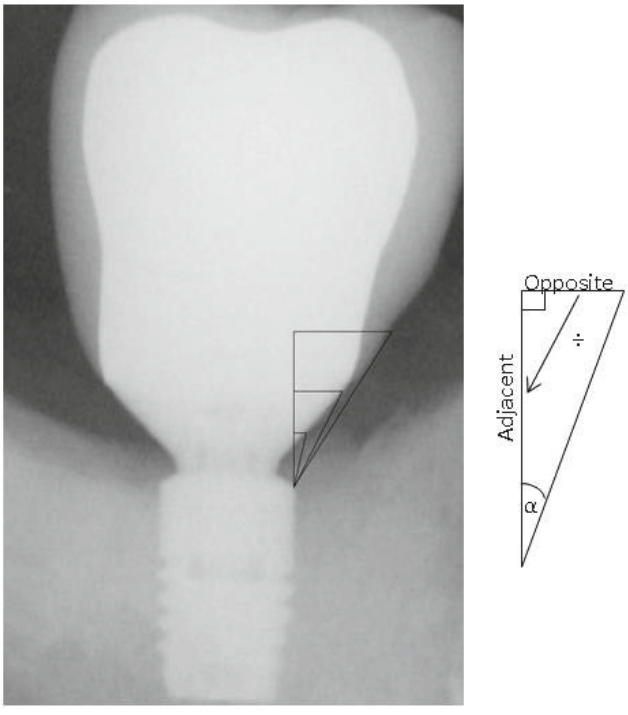

Figure 1. Example of cervical crown emergence angle measurement on a peri-apical radiograph using tangent geometry.

Clinical assessment

Clinical evaluation of the peri-implant soft tissue was performed 1 month (baseline, T1) and 5 years (T60) after placement of the final implant crown. The sulcus bleeding index (24), the gingival/mucosal index (25,26) and the probing depth using a manual periodontal probe (Williams Color Coded Probe; Hu-Friedy, Chicago, IL, USA), were clinically recorded. At four points around the implant (mesiobuccal, distobuccal, mesiolingual, distoligual), probing depths were assessed to the nearest millimeter.

Statistical analysis

Sample size calculation

Since this is the first study evaluating the influence of cervical crown contour on marginal bone loss we could not use an effect size from the literature to perform a proper sample size calculation. Therefore we used a pilot study design in which all eligible implants (122 implants in 96 patients) from two previous studies were used (21,22). Taken into account the eligibility criteria a total of 67 implants and 64 patients were included. A significance level in statistical analysis of $p < 0.05$ was chosen.

Inter-rater and intra-rater reliability

To assess the accuracy and reliability of the new protocol for crown contour measurement, an intra-class correlation coefficient for the crown angles was calculated. Twelve radiographs (from the current dataset) were randomly selected and assessed twice by two independent examiners (DFMH&HJAM) and twice by one and the same examiner (DFMH) for the inter-rater and intra-rater reliability assessment.

Pearson correlation

Pearson correlation coefficients were calculated using Statistical Package for Social Sciences (version 25; SPSS Inc, Chicago, IL, USA) to determine whether the observed changes in peri-implant bone level at the mesial and distal aspects of the implant (change between one month and five years) were correlated with the measured crown angles on respectively the first 1 mm, 2 mm and 3 mm of crown height.

RESULTS

Patients

A combined total of 96 patients (37 patients from study Telleman et al. 2014 (22) and 59 patients from study Guljé et al. 2013 (21)) with 122 bone-level implants were available for evaluation. Fifty-five implants were excluded, of which 40 implants were placed adjacent to another implant and 15 implants were not fully depicted on a peri-apical radiograph. A total of 64 unique patients with 67 bone-level implants were included

in the present study. Two implant brands (Biomet 3i & Astra Tech) and three implant lengths (6 mm, 8.5 mm & 11 mm) were evaluated. All implants were placed at equicrestal level and restored with porcelain crowns cemented on customized titanium abutments. See Table 1 for patient and implant characteristics.

Table 1. Patient and implant characteristics

		N	Total N (%)
Total implants/patients		67/64	100%/100%
Gender, n(%)	Female	38	56.7%
	Male	29	43.3%
Implant region, n(%)	Premolar	16	23.9%
	Molar	51	76.1%
Jaw, n(%)	Mandible	25	37.3%
	Maxilla	42	62.7%
Implant positioning, n(%)	Interdental	53	79.1%
	Non-interdental	14	20.9%
Implant type/surface, n(%)	Astra 'Osseospeed TX 4.0 S'	37	55.2%
	Biomet 3i 'Full Osseotite XP Certain'	30	44.7%
Implant/abutment connection, n (%)	Connical seal design connection	30	44.7%
	Quick seat connection	37	55.2%
Implant length, n(%)	11 mm	10	14.9%
	8.5 mm	33	49.3%
Diameter of implant, n(%)	6 mm	24	35.8%
	4.0 mm	55	82.1%
Abutment fabrication, n (%)	5.0 mm	12	17.9%
	Customized	67	100%
	Stock	0	0%

Inter-rater and intra-rater reliability

The correlation coefficients for the inter-observer and intra-observer agreement (Cronbach's a) on emergence angle calculation were a = 0.975 and a = 0.981, respectively. Both outcomes can be interpreted as an almost perfect agreement (27).

Emergence crown angle

Mean crown angles ranged from 0.5 degrees to 18 degrees at the mesial aspect of the implant crown and from 2.8 degrees to 18.7 degrees at the distal aspect, with the largest angles at 2 mm and 3 mm height. Crown angles increased with increasing

height, but the largest increase in crown angles was measured between 1 mm and 2 mm height (see Table 3).

Table 2. Clinical characteristics at baseline (T1) and 5 years (T60)

		T1		T60	
		N	*%*	*N*	*%*
Bleeding score	0	45	67%	45	67%
	1	19	28%	17	25%
	2	3	5%	5	7%
Gingiva index	Healthy gingiva	59	88%	65	97%
	Light inflammation	8	12%	2	3%
	Mild inflammation	0	0%	0	0%
	Severe inflammation	0	0%	0	0%
Deepest pocket	1	1	2%	0	0%
	2	19	28%	15	22%
	3	34	51%	32	48%
	4	6	9%	8	12%
	5	3	5%	5	8%
	≥6	4	5%	7	10%

Table 3. Crown angle on heights 1 mm, 2 mm and 3 mm at mesial and distal implant site

	Height	N	Mean (°)	Min(°)	Max(°)	St. Dev.
Mesial	1 mm	67	0.5 °	0.0 °	19.8 °	2.8
	2 mm	67	12.8 °	0.0 °	56.5 °	12.8
	3 mm	67	18.0 °	0.0 °	40.5 °	11.3
Distal	1 mm	67	2.8 °	0.0 °	39.4 °	8.3
	2 mm	67	16.2 °	0.0 °	53.4 °	16.0
	3 mm	67	18.7 °	0.0 °	46.1 °	13.8

Marginal bone loss

The average marginal bone loss between T1 and T60 was 0.14 mm ± 0.34 and 0.26 mm ± 0.47 at the mesial and distal implant sites, respectively (see Table 4). No correlation was found between the crown angles on different crown heights and marginal bone loss (Table 5).

Table 4. Marginal bone loss difference between T1 and T60

	Total N	Min (mm)	Max (mm)	Mean (mm)	Median (mm)	St. Deviation
Mesial implant site	67	0.00	1.84	0.14	0.00	0.34
Distal implant site	67	0.00	1.92	0.26	0,00	0.47

Table 5. Pearson correlation of mesial and distal crown contour (angle) and marginal implant bone loss

	Difference in bone loss between T1 and T60			
	Height	Pearson Correlation	Sig. (2-tailed)	N
Mesial site	1 mm	0.072	0.562	67
	2 mm	-0.043	0.731	67
	3 mm	-0.01	0.937	67
Distal site	1 mm	-0.031	0.801	67
	2 mm	0.057	0.646	67
	3 mm	0.029	0.816	67

Clinical parameters

The clinical parameters at baseline and 5-year evaluation generally indicated healthy peri-implant soft tissues. At both evaluation moments, 67% of the implants showed no bleeding on probing as measured by the sulcus-bleeding index (24). The gingival index (25) indicated healthy soft tissue in 88% and 97% of the implants respectively. At baseline 5% of the implants showed probing pockets depth deeper than 5 mm whereas at the 5-year evaluation in 10% of the implants probing depths of 6mm and more were found (see clinical characteristics, Table 2). The amount of patients showings signs of peri-implant mucositis (bleeding score 1,2 and marginal bone loss ≤ 2mm) was 32% at 5 year evaluation. No implants showed signs of peri-implantitis (marginal bone loss > 2mm combined with bleeding and/or suppuration on probing). A significant correlation (p = 0.003) between probing depth and emergence angles on the mesial 1 mm height was found. No other correlations were found regarding the different clinical parameters and crown contour (Table 6).

Table 6. Pearson correlation of mesial and distal crown contour (angle) and clinical parameters

		Deepest pocket		Gingiva index		Sulcus bleeding	
Height		mesial	distal	mesial	distal	mesial	distal
	Pearson Correlation	0.36	-0.065	-0.03	-0.059	0.094	0.041
1 mm	Sig. (2-tailed)	*0.003*	0.601	0.811	0.635	0.448	0.741
	N	67	67	67	67	67	67
	Pearson Correlation	-0.171	-0.126	0.029	-0.178	0	-0.024
2 mm	Sig. (2-tailed)	0.166	0.309	0.814	0.149	0.998	0.845
	N	67	67	67	67	67	67
	Pearson Correlation	-0.189	-0.166	0.059	-0.214	0.033	-0.05
3 mm	Sig. (2-tailed)	0.126	0.178	0.637	0.082	0.791	0.686
	N	67	67	67	67	67	67

DISCUSSION

To the best of our knowledge, this is the first cross-sectional study with a 5-year evaluation that focussed on the influence of cervical crown contour on peri-implant marginal bone loss and soft tissue health in patients with platform-switched, posteriorly placed, two-piece implants. No correlation was found between marginal bone loss and the implant crown emergence angles for any of the evaluated heights. Neither correlations between the different emergence angles and clinical parameters were found at the 5-year evaluation, apart from a weak correlated incidental finding between the crown contour at the mesial 1mm height and deepest probing depth. Clinical parameters showed highly desirable levels of peri-implant health both at baseline and 5 years thereafter. Considering previous studies on marginal bone loss around platform-switched implants, showing bone loss ranging from 0.20 to 0.65 mm, outcomes of this study corroborate on those outcomes (3,4,8).

A new measurement method was developed attempting to geometrically map the cervical crown contour. The measurement method described by Katafuchi et al. 2018 (20), based on a stone cast model measurement method of Yotnuengnit et al. 2008 (28) and designed for (natural) front teeth, used a tangent line to the most mesial/distal point of the implant crown for emergence angle calculation. Inherently, a variety in restoration heights were used for correlational analysis. Since the influence of the first 3 mm above implant level was of specific interest in this study, the former measurement method could therefore not be adopted. Using the new measurement method, on different crown heights (1,2,3 mm) angles were assessed with respect to the implant interface,

taken into account the implant inclination. In contrast to the study by Katafuchi et al. 2018 (20) in which average emergence angles of 30 degrees for bone as well as soft-tissue level implants were found, the average emergence angle in this study did not exceed 18.7 degrees on both implant sites. A restoration emergence angle >30 degrees was found by Katafuchi et al. 2018 (20) to be correlated to peri-implantitis in bone-level, non-platform switched implants. Consequently, they assumed that platform-switched implants might have a larger emergence angle and therefore an increased risk for peri-implantitis. Average angles found in this study did not exceed 30 degrees on any of the 3 heights and therefore an increase in emergence crown angles on platform-switched implants compared to platform-matched implants could not be confirmed. Besides, high desirable levels of peri-impant health were seen at 5 year evaluation in this study. No clinical signs of moderate/severe inflammation (marginal bone loss > 2mm combined with bleeding and/or suppuration on probing) indicating a state of peri-implantitis, were found at any of the implants. Hence, these results do not indicate an increased risk for developing peri-implantitis in bone-level platform-switched implants as suggested by Katafuchi et al. 2018 (20). Also the number of patients which showed signs of peri-implant mucositis (bleeding score 1,2 and marginal bone loss ≤ 2mm) at the 5 year evaluation seemed comparable to prevalences found in recent systematic reviews and meta-analyses (31,32). The pilot study design and small sample size of this study, however, should be taken into account. Besides, the different intersection used (3 mm intersection versus most mesial/distal point of the crown), as well as the steep slope crown design in this study could have resulted in different angles and favourable peri-implant health outcome (20). Namely, steep slope crown designs may favour peri-implant accessibility for biofilm removal and presumably give more space to the soft tissue dimension (epithelial and connective tissue) to be present, preventing implants from inflammation (29,30). A design which in this study might already have been taken into account at the time of individually designing the implant crowns. Additionally, all patients in the studies were included under strict inclusion criteria and appeared a priori to be exceptionally healthy: none had a history of periodontal disease and all were reported non-smokers. Factors which both might positively influence peri-implant health and stability on the long term. Lastly, platform-switched implant connections might have influenced the peri-implant clinical outcomes. Previous studies evaluating platform-switched implant-abutment connections versus matched connections showed favourable marginal bone levels for platform-switched designs (1,3,4,8).

Drawbacks of the present study could be related to the fact that only the mesial and distal aspects of the crown contour were assessed, since the emergence angles on the lingual or buccal contour could also influence the outcome parameters. Although three-dimensional records (cone beam computed tomography, CBCT) might be of

additional use to geographically map the crown contour in a 360 degree aspect, thus including the lingual or buccal contour, monitoring implants using CBCT cannot be used as standard follow-up examination, considering the ALARA (as low as reasonably achievable)-principle.

Further, changes of the interproximal bone levels could be superimposed across radiographs. In a study by Malloy et al. 2017 inaccurate results were reported when measuring bone-levels on radiographs of cadaver specimens as a result of x-ray angulation (33). Moreover perceived bone level changes on radiographs less than 1mm are more likely due to human error than to actual change because of inaccuracy of examiners (34). Considering this, the amount of implants then showing actual change in this study (>1mm) seems very low, accentuating the healthy status of the majority of implants assessed. At last, given the two different implant brands, with two different diameters placed on premolar and molar position included in this study, a relatively heterogeneous data set was evaluated. However, it seems unlikely that the aforementioned differences would have influenced the marginal bone level, taken into account the small amount of implants showing a small amount of marginal bone loss. To confirm our data and more profoundly understand the mechanisms by which the crown contour could influence peri-implant health, future research on the influence of the crown contour using the measurement method described in this study, should consider to evaluate various confounding factors (e.g. implant depth, width of occlusal table, implant diameter of implant design, implant abutment connections of abutment design) in a larger, homogeneous group of implants.

Conclusively, within the limitation of this study, no correlation appears to exist between the cervical crown contour i.e. first 3mm above the implant platform and peri-implant marginal bone loss around two-piece platform-switched implants after 5 years of function. Platform switched implants seem to perform well in terms of peri-implant hard and soft-tissue health up to 5 years, taken into account a priori periodontal healthy, non-smoking patients, and treatment by experienced implant and restorative specialists.

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CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

At the start of this PhD thesis 6 years ago, clinical research on potential interventions for treating peri-implantitis was highly needed considering the absence of a gold standard protocol. Back then, a wide variation of in-vitro and preclinical studies had been published, evaluating different implant surface decontaminating agents/methods or combination of methods in different situations/conditions, making it difficult to discriminate between effective and ineffective interventions (Ntrouka et al. 2011, Meyle 2012, Mellado-Valero et al. 2013, Louropoulou 2014). Clinical studies to put these non-clinical data into perspective remained scarce (Esposito et al. 2012, Heitz-Mayfield & Mombelli 2014). Therefore, in line with previous research performed by the current research group on the treatment of peri-implantitis (de Waal et al. 2013, de Waal et al. 2015), the aim of the research presented in this thesis was to perform randomized clinical trials to clinically, radiographically, microbiologically and immunologically evaluate the influence of a single implant surface decontaminating/peri-implant debridement intervention. In addition, the aim was to add knowledge on the peri-implant diagnosis and on the influence of the implant supported crown contour with regard to the peri-implant condition.

Biomarkers in peri-implant diagnostics

Dental implants are foreign bodies and can initiate inflammation in the surrounding tissues due to infection or a foreign body reaction. Inflammation is characterized by the production of an array of pro- and anti- inflammatory proteins. To assess whether implants with peri-implantitis and implants in healthy state are accompanied by different inflammatory biomarker levels in peri-implant crevicular fluid (PICF), a comparative study was conducted (**chapter 2**) in which several potentially diagnostic biomarker candidates were evaluated (Duarte et al. 2016). The results of this study revealed that PICF of implants with peri-implantitis contains significantly higher levels of IL-1β and MMP-8 compared to PICF obtained from healthy peri-implant sites. Other inflammatory markers including IL-6, TNF-α, MIP-1α/CCL3, MCP-1, OPG and G-CSF showed no difference between both conditions. Levels of sRANKL and INF-γ appeared to be under limit of detection.

Up to date, IL-1β belongs to the most investigated pro-inflammatory cytokines in PICF (Duarte et al. 2016). Evidence suggests that IL-1β acts synergistically with TNF-α to initiate and propagate inflammation (Dinarello 2000). Also, when IL-1β is inhibited, reduced tissue breakdown and progression of tissue inflammation has been reported (Delima et al. 2001). The enhanced levels of IL-1β found in our study corroborate the majority of previous studies on IL-1β, strengthening the evidence that IL-1β is currently one of the most promising proteins to be used as marker in PICF to distinguish peri-implantitis from peri-implant health (Ghassib et al. 2019). Whether this marker also

exerts further potential to predict/determine disease progression or to distinguish peri-implant mucositis from peri-implantitis remains to be established in longitudinal clinical studies.

Our study is one of the first to compare levels of MMP-8 in peri-implantitis implants with healthy implants. Previously, MMP-8 was detected in peri-implant sites with ongoing bone loss (Arakawa et al. 2012). Classic pro-inflammatory cytokines IL-1β and TNF-α have shown to induce the synthesis and secretion of MMP-8 which in part might explain the elevated levels of MMP-8 found in our study (Siwik et al. 2000). Comparable to what is found in patients with periodontal disease, there seems moderate evidence in the literature showing upregulated levels of MMP-8 in PICF of implants with peri-implant disease (Salvi et al. 2012, Ghassib et al. 2019, Alassy et al. 2019). It therefore might be hypothesized that, in addition to IL-1β, MMP-8 could also serve a promising role to differentiate between peri-implant health and disease (Arakawa et al. 2012, Thierbach et al. 2016, Al-Majid et al. 2018, Alassy et al. 2019). Polymorphism in the promoter region of MMP-8 might be another reason for elevated levels of MMP-8 in PICF around implants with peri-implantitis. Polymorphisms might explain varied responses between different individuals with the same disease category (Ghassib et al. 2019). For individuals who are at high risk of peri-implantitis, this finding could be of great benefit considering that genetic polymorphisms are constant and can be measured before disease onset or implantation. Futures studies should however more often include this marker to further elucidate its contribution to the complex immune response around inflammated implants.

No significant differences in quantitative outcomes for the other markers investigated were found in the PICF between both groups, suggesting no discriminating potential. A finding which seems in line with the current literature (Dursun et al. 2016, Duarte et al. 2016, Theodoridis et al. 2021). However, considering that the study in **chapter 2** included a small sample size with a variety of implant brands, studies with greater sample size, evaluating patients with similar implant brands and designs are needed to confirm our outcomes. Moreover, considering the fact that peri-implant diseases present a cyclic evolution, the immunoinflammatory events responsible for tissue breakdown may not always be active in a cross-sectional studies design with a single moment of fluid collection. Therefore, future research should explore cytokine levels at multiple time points to evaluate the role of the different cytokines and their signaling pathways in different peri-implant conditions. In addition, when researching 'new' potential biomarkers it is recommended to perform recoverability experiments, before using a Luminex assay, to assure the optimal sampling method with matching elution protocol, per separate biomarker.

Furthermore, it is interesting to note that significant higher levels of PICF volume were found at peri-implantitis sites compared to healthy implant sites. This finding corroborates the results of the studies by Bevilacqua et al. (2016) and Tözüm et al. (2007) who also showed significant elevated levels of fluid in a diseased state compared to health, as well as around natural teeth compared to implants. Moreover, in the study by Bevilacqua et al. (2016), in a periodontal and peri-implant healthy state the probing pocket depth seemed directly related to the amount of fluid produced (up to 3 mm pocket depth). Although this relation was however not present in the case of active peri-implant disease, this might suggest that a volumetric threshold level might exist that could be of importance in assisting in the (early) diagnosis of peri-implant disease.

In **chapter 2**, the influence of the non-surgical peri-implantitis therapy on immunological biomarker levels present in PICF at 3 month follow-up was additionally evaluated. The study showed that none of the biomarker levels had significantly improved after non-surgical therapy and levels of IL-1β and MMP-8 remained high. This finding seems in accordance with a previous non-surgical study by Renvert et al. (2011) who neither found any differences in the majority of the studied cytokines (6 out of 9) when using either an air-abrasive device or Er:YAG laser. A surgical intervention study performed by Thierbach et al. (2016), also did not show a difference in MMP-8 levels 6 months after the treatment when compared to baseline, in smokers and non-smokers. Hence, after treatment of peri-implantitis, MMP-8 levels still seems to reflect an intensified host response around implants and immunologically indicate the challenge of controlling peri-implantitis. It therefore might be speculated that the limited clinical effect of a non-surgical therapy seems to be immunologically underlined. However, with our study, it seems not possible to truly support or deny the potential use of a change in biomarker as a monitor to assess the effectiveness of a peri-implantitis treatment with PICF analysis.

Collectively, the outcomes of the study presented in **chapter 2** seem to demonstrate capacity for biomarkers to improve clinical diagnosis of peri-implant conditions and indicate a role of tissue fluid volume in the diagnosis of peri-implant disease. Interpretation of the study results should be done cautiously since the results are based on a limited sample size, on which no sub-analyses could be performed for several possible confounding factors (e.g., smoking, age, gender). Clinical evaluation of peri-implant tissues and radiographic assessment still form the foundation for detection of peri-implant disease. However, although the evidence is still limited, a shift from clinically based towards a more biologically supported definition of peri-implant conditions seems to be imminent.

Treatment of peri-implantitis

In pursuit of the main objective of this thesis we performed three peri-implantitis treatment studies on different implant surface decontamination methods. Two studies were performed with the aim to evaluate mechanical implant surface decontamination (efficacy of air-polishing), in a non-surgical and surgical setting, and one with the aim to evaluate chemical decontamination (phosphoric acid).

Mechanical implant surface debridement: air-polishing

Given the scarcity of clinical trials on the single use of air-polishing in the treatment of peri-implantitis (Schwarz et al. 2016), including the identified sources of bias in previously performed studies on peri-implantitis treatment using air-polishing, we decided to conduct two large sample size randomized controlled trials comparing erythritol air-polishing with conventional debridement methods in both a non-surgical and surgical setting.

From the non-surgical study (**chapter 3**) results it was concluded that air-polishing (EMS, Airflow Master Piezon) is as effective as ultrasonic scaling (piezo-electric ultrasonic scaling with PEEK plastic tip) in the improvement of clinical parameters. However, in both groups only a limited number of patients could be considered successfully treated. No effect on marginal bone levels or microbiology was noticed and low pain scores were reported for both interventions (mean VAS scores < 3), without one being less painful than the other. At 3 month follow-up, patients who were considered unsuccessfully treated were invited to participate in a surgical follow-up study (see **chapter 4)**. The aim of that randomized controlled trial was to compare the effect of erythritol air-polishing as mechanical implant surface cleansing method to saline during a resective surgical protocol. It was concluded that erythritol air-polishing was as effective as saline in terms of clinical, radiographical and microbiological parameters. At 1-year follow-up, a successful treatment outcome (PPD<5mm, max 1 out of 6 sites BoP, no suppuration and no progressive bone loss >0.5mm) was only rarely attained (19%). During 1 year follow-up a total number of 16 implants (17%) were lost due to persistent signs of severe inflammation. Air-polishing did not appear to be significantly more successful.

It is hard to explain the variation in treatment outcome between patients. The great variety of implant and patient characteristics as well as device specific parameters may possibly account for these observations. For example, implant thread geometry seems to influence the access of decontamination devices and in turn its efficacy (Steiger-Ronay et al. 2017). The most favorable implant characteristic for optimal mechanical biofilm removal was recently shown to be low thread pitch and low thread depth (Sanz-Martin et al. 2021). Also, the design of the air-polishing tip/nozzle, the insertion depth

and movement of the air-polishing nozzle, as well as device settings such as drive air-pressure, water ejection and powder emission are factors which seems to be related to the clinical effect of an air polisher (Tastepe et al. 2017). Moreover, according to the manufacturer's manual, particles should impact at an angle of 30–60° at an ideal working distance of 3–5 mm for the kinetic energy of the powder to remove the biofilm. Considering that suprastructures were not removed during non-surgical therapy and during the surgical intervention cemented restorations were kept in place, the insertion of the cleaning devices into the peri-implant pocket might have been hampered and proper working angel and distance might not have been achieved in every situation. We found no indications that the pre-treatment composition of the bacterial biofilm could be indicative for the treatment outcome.

Furthermore, the effectiveness of air-polishing seems to depend on the abrasiveness of the powder particles used (hardness, size, and shape) (Cobb et al. 2017) and the shape of the bone defect (Sahrmann et al. 2015, Ronay et al. 2017, Keim et al. 2019, Tuchscheerer et al. 2021). Larger, coarser powder types (atrium bicarbonate ±70µ) seem to provide a higher cleaning efficacy than finer ones, but at the same time do cause more alterations of the implant surface (crater-like defects, rounding or removal of sharp edges). Smaller particles (e.g. erythritol ±14µ and glycine ±25µ) on the other hand, are less damaging and exert only a minor effect on the implant surface topography. However, although these smaller particles were more likely to reach areas in the rough implant surface inaccessible by larger particles, reduced capacity to remove implant contaminants was seen when using these smaller particles (Cha et al. 2019, Matsubara et al. 2020). Hence, this reduced capacity might have played a role in the limited clinical effects found in our studies. Regardless of the type of bone defect (30, 60 or 90 degrees), the effect of an air polisher in a laboratory open/surgical approach seems to be significant over a closed/non-surgical approach (Tuchscheerer et al. 2021). Wider implant bone defects (60,90 degrees) seem to be more effectively cleaned than narrow defects (30 degrees) but no differences between intraosseous (30, 60 degrees) and supraosseos defects (90 degrees) in a surgical approaches were found (Sahrmann et al. 2015, Keim et al. 2019, Tuchscheerer et al. 2021).

One might argue that the implants were not cleaned long enough. It appeared that when the treatment time was increased from 5 to 45 seconds, considerable more efficient implant cleaning was achieved with an air-polishing device in a pre-clinical setting (Mensi et al. 2020). During the non-surgical intervention, implants were cleaned up to 30 seconds in total (5 seconds per site) and during surgery the therapy was applied until the implant surface was assessed as visually clean. Compared to the literature, a non-surgical treatment study by Renvert et al. 2011, evaluating the use of glycine

air-polishing after a treatment time up to 1 minute, showed a range of pocket depth reductions (between 0.1 mm and 1mm) and changes in average marginal bone loss (0.1mm (±0.8)) comparable to the results of our non-surgical study. In general, it should however be kept in mind that patients characteristics, the presence of suprastructures and anatomical limitations of the oral cavity (e.g. the tongue) are confounders in a clinical setting which could overshadow possible beneficial in-vitro effects. Hence, the true effect of the different parameters mentioned above on the clinical outcome remain to be found.

Microbiologically, no significant difference between therapies, neither after the non-surgical nor after the surgical intervention was found. Also no difference in levels of periodontal pathogens were seen when the successfully treated patients in the non-surgical setting were compared to the unsuccessful ones. These relatively unchanged counts 3 and 12 months after respectively a non-surgical and surgical intervention are difficult to understand. Whether for example fast bacterial regrowth or the method of bacterial sampling are underlying causes remain to be found. Moreover, in our studies we used targeted quantitative PCR analysis to investigated the presence of a number of putative bacterial species, both in natural teeth and implants. Although the investigated periodontal species may be considered marker species for periodontitis (Griffen et al., 2012), open-ended microbiome studies have shown that the microbiomes associated with periodontitis and peri-implantitis show major differences (Kumar et al., 2012; Dabdoub et al., 2013; Lafaurie et al., 2017; Sahrmann et al., 2020). The microbiome in peri-implantitis seems associated with predominantly non-cultivable Gram-negative species and is not associated with a uniform microbial profile. Considering this, with our studies an incomplete picture of the potential changes in composition of the peri-implant and periodontal microbiome could have been found.

Although for the majority of patients a non-surgical approach seemed to have a limited effect, a small number of patients did seem to benefit from the non-surgical phase in such a way that the burden of surgical follow-up could be prevented. When baseline characteristics of the patients who were considered successfully treated at 3 month follow-up were compared with the characteristics of the unsuccessful ones, lower probing pocket depths, less marginal bone loss and shorter time of implant function before therapy were seen. Moreover, follow-up of the *successful* patients showed gradual improvement of peri-implant parameters up to 12 months when supportive peri-implant therapy (supragingival instrumentation when plaque/calculus was visible) and oral self-care reinforcement were applied at 6 and 9 months. Hence, the findings of our study underline the importance of early diagnosis and early commencement of non-surgical therapy. Moreover, as shown in the study presented in **chapter 3**, and

underlined by recent study of De Waal et al. 2021, when a full-mouth non-surgical treatment is meticulously performed, combined with a high level of daily oral hygiene and healthy periodontal tissues, the starting position of the subsequent (surgical) peri-implantitis treatment phase can significantly be improved.

Overall, a large number of patients having implants with considerable amounts of marginal bone loss, up to two-thirds of the implant length, were included. Hence, the state of disease might have exceeded the capacity of a mechanical decontamination method in a non-surgical as well as a surgical resective approach to be successful. Moreover, one might question whether the extensiveness of disease did not exceed the recoverability capacity of the human body. Considering the low success of treatment approaches described in the literature and a tendency of disease recurrence after more years of observation following surgical treatment of peri-implantitis defects, irrespective of the chosen approach (i.e., reconstructive vs. resective) (Carcuac et al. 2020; La Monaca et al. 2018) a guideline which could help the clinician to decide whether it is still feasible to treat the disease or one should decide to remove the implant, seems urgently needed. Therefore, in contrast to the general goal by dental clinicians of trying to save natural teeth 'as long as reasonably possible', one could advocate to explant an implant 'as soon as reasonably possible' when diagnosed with multiple unfavorable factors. Factors which might play a role and therefore should be taken into account when making such a decision are for example: amount of marginal bone loss, implant mobility, implant malposition, soft tissue dehiscence and patient preference.

Chemical implant surface decontamination: phosphoric acid

Considering that implant surface characteristics may compromise an effective mechanical intervention, adjunctive use of chemical agents for implant decontamination has been advocated (Claffey et al. 2008). Previous in-vitro and in-vivo studies have failed to identify one chemotherapeutic agent as the gold standard for implant surface decontamination (Ntrouka et al. 2011) and therefore, we continued the search for other potentially beneficial chemical agents. Hence, in **chapter 5**, the effect of phosphoric acid 35% on the composition of the submucosal microbiome and the effect on clinical parameters in a resective surgical peri-implantitis approach was evaluated at 3 months post-treatment. It was concluded that the application of 35% phosphoric acid after mechanical debridement is superior to mechanical debridement combined with sterile saline rinsing for decontamination of the implant surface during surgical peri-implantitis treatment. However, no significant clinical or microbiological effect was found at 3 month follow-up.

Previously, studies evaluating the influence of acids on titanium implant surfaces were mainly performed in an in-vitro or animal setting mainly focused on the use of citric acid (Zablotsky et al. 1992, Dennison et al. 1994, Mouhyi et al. 2000, Htet et al. 2016, Dosti et al. 2017). Clinically, phosphoric acid was, as far as we know, only used in a peri-implant maintenance study (Strooker et al. 1998) and a case series by Wiltfang et al. (2012) on the surgical regenerative treatment of peri-implantitis. Hence, our study is the first to show that the use of phosphoric acid as implant surface decontaminant did not seem to enhance clinical outcomes on a 3-month follow-up more than sterile saline. However, our findings are in line with previous studies on acid decontamination showing an antibacterial potential of phosphoric acid. A recent in-vitro study by Dosti et al. (2017), which for the first time evaluated SLA implant disks with multilayer and multi-species 3-week-old biofilm, found that when the implant surface was rinsed twice with sterile saline (i.e., pre-rinse, followed by 2 minute rinsing) this 'double rinse group' was the only group to have significantly more bacteria removed from the SLA disks than a single rinse control group. The use of phosphoric acid in that study did not appear to result in a superior effect compared to the 'double rinse group'. In addition, a study by Wheelis et al. (2016) found noticeable morphological changes when the synergistic effect of acidic environments (*i.e.* citric acid, 15% hydrogen peroxide, tetracycline, peroxyacetic acid) and mechanical forces (rubbing with cotton swabs) were applied. To which extent implant surface changes affect the biological response in terms of peri-implant hard and soft tissue cell (i.e., fibroblast, epithelial cells and/or osteoblasts) re-attachment, remains to be found. However, considering the possible detrimental effect of mechanical and chemical combined influences on the implant surface, it seems advisable that care should be taken applying such combinations on the implant surface. Randomized clinical trials are needed to evaluate the influence of different combinations in different approaches. Hence, until no superior decontaminating approach has been appointed, one might advocate the use of only sterile saline as implant surface decontaminant.

Treatment considerations

Titanium particle release

In recent years, studies increasingly focused on evaluating implant surface physical and chemical properties (Kotsakis et al. 2016, Wheelis et al. 2016). It is expected that, as a result of synergism between different wear factors (e.g., cyclic implant loading, implant maintenance/cleaning procedures, oral biofilm and friction at the implant-abutment interface) there is an increased risk that the implant surface titanium dioxide layer might get damaged to such an extent that it diminishes the corrosion resistance. Subsequently, titanium particle release and ion leakage can occur, a phenomenon which is called bio-tribocorrosion (Kotsakis et al. 2021). Titanium particle release and ion leakage in turn have been suggested as triggers for marginal bone loss and peri-

implant infection (Costa et al. 2019, Han et al. 1998, Johansson et al. 1998). Hence these triggers might be another important factor that might partially explain the limited clinical effect found in the studies presented in chapter 3, 4 & 5. Recent reviews on titanium particle release, underline that titanium particles in peri-implant tissues are a common finding and that peri-implantitis sites revealed a higher number of particles compared to healthy conditions (Delgado-Ruiz R. & Romanos G. 2018, Noronha Oliveira et al. 2018, Suárez-López Del Amo et al. 2019, Romanos et al. 2021). In addition, titanium dissolution products have been shown to act as a modifier in the peri-implant microbiome structure and diversity (Daubert et al., 2018). However, even though there is an association between the presence of titanium particles and biological complications, evidence for a direct causal relationship is still missing (Mombelli et al. 2018). More research is needed to find out what factors cause destruction of the protective titanium dioxide layer and how particle release and corrosion of dental implants influences peri-implant tissues.

Regenerative approach and bone defect morphology

With the aim to evaluate the single influence of mechanical or chemical implant surface debridement, irrespective of the bone defect morphology, a resective approach was chosen in both surgical studies presented (see chapter 4, 5). One might advocate that a regenerative approach would have been more successful in circumferentially or infrabony defect configurations and preferable regarding maintenance of soft tissue height (Schwarz et al. 2010). However, although the literature is emerging, studies evaluating regenerative treatments thereby taking into account different bone defect morphologies remain scarce (Tomasi et al. 2019). Only recently, Renvert et al. (2021) compared the use of deproteinized bovine bone mineral and native bilayer collagen membrane in ≥ 3 wall defects to a non-regenerative surgical approach in a randomized clinical trial. The results showed no significant difference in terms of BoP, SOP and PPD reduction between both groups. Although low success rates were found, the regenerative approach appeared to achieve a successful outcome more often (32% versus 21%). Another recent study by Roccuzzo et al. (2021) evaluating a surgical regenerative approach recording different bone morphology defects, was able to recreate and maintain peri-implant healthy conditions around most of the treated implants for a period of 5 years' time, regardless of the initial defect configuration (according to Schwarz et al. 2007). Hence, one might expect considerable success from a regenerative approach although it should be kept in mind that the research on this topic remains limited. Whether a specific type of bone defect is more favorable to regenerate with stability of successful outcomes (on the long term) remains to be found.

Adjunctive use of antibiotics

From the literature on the treatment of periodontitis, full-mouth ecological change (suppression of periodontal pathogens and recolonization of the biofilm by host-compatible species) was found to be necessary to re-establish periodontal health (Feres et al. 2015). Hence, one might advocate the use of local and/or systemic antibiotics in the treatment of peri-implantitis. Although our studies were conducted to evaluate solely the effect of the decontaminating agent, different local and systemic antibiotic applications have previously been investigated (van Winkelhoff 2012). Recent randomized clinical trials evaluating the use of systemic amoxicillin plus metronidazole showed no improvement in clinical and microbiological outcomes after non-surgical peri-implantitis treatment (Shibli et al. 2019, de Waal et al. 2021). It was suggested that, as compared to planktonic bacteria, bacteria in (undisturbed) biofilms display an increased tolerance to antimicrobial agents (Stewart et al. 2015), and hence may cause adjunctive systemic antibiotics to be less effective. It therefore seems unlikely that the lack of antibiotics could have impacted the results found in the study presented in **chapter 3**. On the other hand, positive results of local minocycline use in the surgical treatment of peri-implantitis have been described (Cha et al. 2019). The repeated local delivery of minocycline showed significant benefits in terms of clinical parameters and radiographic bone fill, with a higher treatment success rate in the short healing period (6 months). In addition, the use of systemic antibiotics in the surgical treatment of peri-implantitis showed beneficial effects, especially in implants with non-modified surfaces (Carcuac et al. 2016). Although the implant surface characteristics seem to have a significant impact on 3-year outcomes, benefits of systemic antibiotics were limited to the first year of follow-up (Carcuac et al. 2017). Therefore, up to date, long term clinical efficacy of antibiotics in the treatment of peri-implantitis are lacking. Moreover, it remains unclear which patient characteristics are indicative for a beneficial systemic or local antimicrobial treatment.

Modifying iatrogenic factors

In order for a peri-implantitis treatment to be successful, local predisposing factors with a potential negative impact on the treatment outcome should be identified, and modified if possible before initiating an intervention (Monje et al. 2018). Such factors include for example: cement remnants, implant malpositioning and loose or improper fit of the prosthetic reconstruction. Additionally, the design of the implant supported suprastructure is considered an important factor which should allow patients to perform an optimal level of self-care. This factor was highlighted in a study by Serino and Ström (2009), in which a high proportion of implants with peri-implantitis were associated found to be associated with no accessibility/capability for appropriate oral hygiene measures. However, to date, little is known on how to design the optimal

crown contour. Previously, prosthetically focused studies mainly concern aesthetic outcome without paying attention to the emergence crown's angle and emergence profile. Hence, in **chapter 6** a study is presented in which the geometric influences of the cervical crown contour on marginal bone loss and soft tissue health was evaluated. From the results it was concluded that the cervical crown contour at platform-switched, posteriorly placed, two-piece implants were not correlated with peri-implant marginal bone loss and soft-tissue health up to 5 year after implant placement.

Several factors might explain the results found in the study in **chapter 6**. For example, all patients were included under strict inclusion criteria and appeared a priori to be exceptionally healthy: all patients were non-smokers and none had a history of periodontal disease. Moreover, all implants were placed by only two experienced implant-specialists and hence a correct three-dimensional implant position, which can be considered a prerequisite to achieve an optimal crown design, was therefore reasonably assured (Buser et al. 2004). In addition, all implants were restored with platform-switched restorations having customized titanium abutments. These customized abutments, which showed a gradual increase in width with a steep emergence profile or even concave/convergent design in the first 2 millimeters (i.e. a great amount of angles appeared zero or near zero at the first mm), might be part of a favorable trans mucosal design. Namely, ideally the design should respect the anatomical characteristics of the soft tissues and allow a smooth transition from the implant platform to the cervical margin (González-Martín et al. 2020). In our study, this profile might already have been taken into account at the time of individually designing the titanium abutment. Hence, this in turn could have created space for a protective biological soft tissue seal (epithelial and connective tissue) which might have positively influenced the marginal bone levels. A recent meta-analysis by the group of Valente et al. (2020) who found that concave/convergent implant transmucosal profiles are associated with less marginal bone loss seems to underline this finding. Further, all implants in the study presented in chapter 6 were restored with cemented restorations. It remains unclear whether the position of the cementation line between the titanium base and the suprastructure affects bone stability and tissue health. However, giving the fact the bone levels appeared stable over 5-years time, it did not seem to have influenced the outcomes.

Although no correlation was found, the study proved and presented a reliably measuring method to evaluate a possibly critical implant crown region. To confirm our data and more profoundly understand the mechanisms by which the crown contour in the transmucosal region could influence peri-implant health, future research group could adopt this method to evaluate larger, heterogeneous groups of implants. Thereby

taking into account various confounding factors such as implant depth, width of occlusal table, implant diameter or implant design, implant abutment connections or abutment design, in implants with marginal bone loss of 3 mm and more.

Concluding remarks and future perspectives

The current thesis underlines the notion that peri-implantitis is a difficult disorder to treat. To date, clinical approaches using single mechanical, chemical or combined methods in the (non-regenerative) treatment of peri-implantitis, remain (very) limited successful. With the evolution of titanium implant surfaces becoming increasingly complex, focused on better and faster osseointegration, returning a contaminated implant surface into a clean 'rejuvenated' pre-implantation status has turned out a challenging task (Sanz, Chapple 2012, Lee et al. 2018, Lollobrigida et al. 2020, Tong et al. 2021). New studies remain urgently required to find the optimum combination of different cleansing methods that compensate for each method's respective downsides, with study protocols combining non-surgical and (resective or regenerative) surgical procedures (Sanz et al. 2019, Alarcón et al. 2021). But, more importantly, peri-implant disease should be diagnosed early and ideally be prevented to save both patient and clinician a significant amount of time, money, effort and frustration. In addition, patients systematic implant aftercare programs are of critical importance (Ramanauskaite & Tervonen 2016). Patients should should be strongly recommended and frequently remotivated to comply with an aftercare program (Mitschke et al. 2020).

Methods which recently appeared in the literature with promising effects on reduction of the bacterial load on titanium surfaces, but rigorously needs to be confirmed in clinical trials, are for example the use of photodynamic therapy (Lopez et al. 2020), leukocyte- and platelet-rich fibrin (Schuldt et al. 2021), cold plasma (Hui et al. 2021, Jungbauer et al. 2021) or an electrolyte device (Schlee et al. 2019). Especially on this latter (electrolyte) device, which efficacy is based on the generation of hydrogen bubbles that lifts the biofilm off the implant surface, significant effects were reported in an in-vitro setting (Ratka et al. 2019). It was shown that the electrolyte approach inactivated the bacterial biofilm without leaving reproducible bacteria behind. Clinically, this device has thus far only been described one time in a regenerative approach, lacking a true control group (the control group consisted of the same device in a combined method with air-polishing instead of an approach without the device). Hence, greater sample size studies with longer follow-up needed to confirm the pre-clinical findings on this device. Another (resective) method which also gains more attention in the recent literature is removal of the implant threads by means of rotatory instruments and/or polishing stones, i.e. implantoplasty. Although some studies show promising outcomes (Monje et al. 2021), there is much concern regarding this procedure in terms

of potential cytotoxicity of nano-sized metal particles on soft tissue cells (Suárez-López Del Amo et al. 2019), biomechanical issues in terms of bending strength especially in narrow/regular diameter implants (Chan et al. 2013), influence on the implant-abutment connection designs (Gehrke et al. 2016) and implant/bone overheating (Sharon et al. 2013). Moreover, it is technically demanding and time consuming (Costa-Berenguer et al. 2018).

While early detection and diagnosis of peri-implant disease is critical to prevent peri-implantitis, current diagnostic procedures (e.g. bleeding on probing) can only assess past tissue destruction and do not provide any information about disease activity or the risk on future disease progression. As such, point-of-care technologies, which recently emerge as new tools to diagnose periodontitis and peri-implantitis at chair-side, are needed to pinpoint the crucial disease transition from peri-implant health to peri-implant mucositis to peri-implantitis (Golub et al. 2020). Currently, promising results regarding the use of an active form of MMP8 point-of-care test for periodontitis have been reported (Räisänen et al. 2019, Räisänen et al. 2020). However, to evaluate the potential use of diagnostic chair-side tests in identifying the onset and progression of peri-implant diseases, much research is needed. Another promising point-of-care technology which recently appeared in the literature on dental implants is the use fluorescence spectroscopy (Andrade et al. 2021, Hwang et al. 2021). With this technology, the amount of pathogenic bacteria or their metabolic activity on the implant surface could be visualized in-situ. Hence, although much more future research is needed to confirm the potential of this technology, it might play an important role in determining whether the implant surface/area has been successfully decontaminated after treatment .

At last, the studies presented in this thesis evaluated the inflammatory reaction solely around titanium dental implants. Whether the therapy outcomes also hold true for zirconia dental implants remains to be found. Currently, zirconia is increasingly being discussed in the literature as alternative for titanium implants (Cionca et al. 2017, Afrashtehfar & Del Fabbro 2020, Comisso et al. 2021). In-vitro studies have shown low affinity to bacterial plaque and some clinical studies show better soft tissue response with zirconia implants and less material corrosion, suggesting that this material could provide a protective effect against inflammation (Afrashtehfar & Del Fabbro 2020). However whether these properties lower the risk for peri-implant disease remains unknown. Thus far, only short term promising results in terms of clinical efficacy have been reported (Webber et al. 2021). The literature on peri-implantitis in patients with ceramic implants is still scarce. Hence, to gain insight in the prevalence,

etiopathogenesis, risk factors and treatment of inflammatory reactions around ceramic implants studies are needed.

Based on the various studies described in this thesis, the following specific conclusions can be drawn:

- Levels of IL-1β and MMP-8 in PICF show the potential to discriminate between peri-implant health and disease. Non-surgical therapy does not seem to influence the inflammatory immune response (**chapter 2**);
- Limited treatment success should be expected from non-surgical peri-implantitis treatment using either erythritol air-polishing or piezoelectric ultrasonic scaling. Hence, the majority of patients seem to require further surgical treatment after a non-surgical treatment (**chapter 3**);
- Erythritol air-polishing as implant surface cleansing method result the same treatment effect as saline soaked gauzes in the surgical resective treatment of peri-implantitis. However, both therapies seem to result in low treatment success up to 1-year after treatment. (**chapter 4**);
- The application of 35% phosphoric acid after mechanical debridement is superior to mechanical debridement combined with sterile saline rinsing for decontamination of the implant surface during surgical peri-implantitis treatment. However, phosphoric acid as implant surface decontaminant does not seem to enhance clinical outcomes on a 3-month follow-up. (**chapter 5**);
- The cervical crown contour at platform-switched, posteriorly placed, two-piece implants does not seem to show a correlation with peri-implant marginal bone loss and soft-tissue health up to 5 year after implant placement (**chapter 6**).

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ADDENDUM

SUMMARY

The gold standard for treatment of peri-implantitis remains to be found. The aim of the research presented in this thesis was to perform randomized clinical trials to clinically, radiographically, microbiologically and immunologically evaluate the influence of a single implant surface decontaminating/peri-implant debridement intervention. In addition, the aim was to add knowledge on the peri-implant diagnosis and on the influence of the implant supported crown contour with regard to the peri-implant condition.

The aim of the study presented in **chapter 2** was two-fold: first, to compare biomarker levels in peri-implant crevicular fluid (PICF) of implants with a peri-implant healthy status with levels in PICF of implants with peri-implantitis and second to compare biomarker levels before and after non-surgical treatment. In total, periopaper samples were taken from 20 healthy implants in 17 patients and from 20 implants with peri-implantitis in 19 patients, before and 3 months after non-surgical treatment using the Airflow Master Piezon® (EMS). For test group samples, patients from the study in presented in **chapter 3** were asked to additionally participate in the study described in **chapter 2**. A Luminex™ assay was used to evaluate pro-inflammatory and anti-inflammatory cytokines IL-1β, TNF-α, IL-6 & G-CSF, collagen degradation enzyme MMP-8, chemokines MCP-1 & MIP-1α/CCL3, bone markers OPG & sRANKL and interferon-γ. Clinical and radiographical characteristics were assessed at baseline and at 3 months. Results showed significantly elevated levels of IL-1β and MMP-8 levels in implants with peri-implantitis when compared to implants with a healthy status (p= .007; p=< .001, respectively). No difference in other biomaker levels (i.e., TNF-α, IL-6, MCP-1 and MIP-1α/CCL3, OPG & G-CSF) between healthy and diseased implants were found. Levels of sRANKL and INF-γ were under the level of detection. Evaluation of biomarker levels 3 months after non-surgical therapy did not significantly improve, levels of IL-1β and MMP-8 remained high. Hence, it was concluded that Implants diagnosed with peri-implantitis have higher levels of IL-1β and MMP-8 in PICF compared to healthy implants and non-surgical therapy did not seem to influence the inflammatory immune response.

In **chapter 3 and 4** two randomized controlled trials are presented which together describe a two-staged peri-implantitis treatment protocol evaluating the effect of mechanical debridement using an air-polisher in a non-surgical study (**chapter 3**) and a resective surgical approach (**chapter 4**). The aim in the study described in **chapter 3** was to evaluate the effect of a single non-surgical intervention using air-polishing with erythritol powder (test group) and compare the effect with piezo-electric ultrasonic cleaning (control group) on clinical, radiographical and microbiological parameters. Patients were assessed at baseline and 3 months follow-up. In patients which were

considered succesfull at 3 month follow-up, parameters were additionally assessed at 6, 9 and 12 months. Moreover, evaluation of patient percepted pain scores took place directly after intervention using a VAS-scale. A total of eighty patients having 139 implants with peri-implantitis were non-surgically treated. Patients were randomly assigned to the test group or control group. In both groups, a single session of full mouth periodontal cleaning was performed and the peri-implant area was treated with either eryhtritol air-polishing treatment or piezo-electric ultrasonic therapy with PEEK plastic tip. Three months post therapy no significant difference between both therapies for the primary outcome mean bleeding on probing (%) (BoP) was found. Moreover, other clinical parameters, including suppuration on probing (SoP), levels of plaque (Plq) and probinig pocket depth (PPD), marginal bone levels or microbiological parameters showed any difference between both groups. Both therapies resulted in limited success i.e. 18% of the patients was considered succesfull. Evaluation of patient percepted pain scores directly after intervention indicated that both therapies were considered minimally painful without one of both being significantly less painful. Therefore, it was concluded that air-polishing seemed to be as effective as ultrasonic scaling in the reduction of inflammatory signs without being perceived more or less painfull (BoP, SoP, Plq and PPD). Hence, neither erythritol air-polishing nor ultrasonic cleaning could be considered a superior therapy in terms of our primary outcome i.e., mean BoP at T3. When baseline characteristics of the successful group of patients were compared with the unsuccessful ones, lower PPD (4.0mm vs 4.9mm, respectively), less marginal bone loss (3.0mm versus 4.0mm, respectively) and shorter time in function before therapy took place (7.2 versus 9.5 year) were seen. Interestingly, follow-up of the *successful* patients showed gradual improvement of peri-implant parameters up to 12 months when supportive peri-implant therapy (supragingival instrumentation when plaque/calculus was visible) and oral self-care re-inforcement were applied at 6 and 9 months. Hence, considering the success of these patients up to 12 months after therapy, these parameters indicate the importance of early diagnosis and early commencement of non-surgical therapy. Moreover, it seemed that stable bone levels and absence of progression of disease could be attained in implants showing PPD < 4mm with the presence of BoP up to 12 months. Therefore the outome of **chapter 3** underlines that the sensitivity of BoP for the prediction of disease progression is quite low and that strict success criteria need to be cautiously interpreted and applied.

In **chapter 4** we aimed to evaluate the effect of mechanical implant surface decontamination using an air polisher with erythritol powder on clinical, radiographical and microbiological parameters. The parameters were assessed before treatment (baseline), 3,6,9 and 12 months follow-up. Patients which were considered unsuccessful at 3 month follow-up in the non-surgical peri-implantitis study of **chapter 3** were

invited to participate in the surgical follow-up study described in **chapter 4**. During a resective surgical intervention, consisting of bone recontouring, surface debridement and mechanical decontamination and apically repositioned flap the implant surfaces of 93 implants with peri-implantitis (n=57 patients) were randomly treated with air-polishing (test) or saline-soaked cotton gauzes (control). Clinical, radiographical and microbiological parameters were recorded. Before treatment (baseline) and at 3, 6, 9 and 12 months after therapy clinical parameters were assessed. Radiographic examination took place at 3, 6 and 12 month follow-up and microbiological samples were taken at 12 months after treatment. No differences between the test and control group were found for the primary outcome BoP over 12 months of follow-up, nor for the secondary parameters Plq, PPD and MBL. Between both groups, a significant difference was found for the levels of SoP was seen. No significant effect on 8 classical periodontal pathogen levels was found from one of both therapies. At 1-year follow-up, a successful treatment outcome (PPD<5mm, max 1 out of 6 sites BoP, no suppuration and no progressive bone loss >0.5mm) was achieved for a total of 18 implants (19.2%).

The randomized clinical trial presented in **chapter 5** aimed to evaluate the effect of implant surface decontamination with phosphoric acid during a surgical peri-implantitis treatment on clinical, radiographical and microbiological parameters. In total 28 patients with 53 implants with peri-implantitis were treated with a resective surgical approach consisting of bone recontouring, surface debridement and chemical decontamination and apically repositioned flap. Patients were randomly allocated to decontamination with phosphoric acid 35% (test group) and saline rinsing (control group). Microbiological parameters were recorded during surgery, whereas clinical parameters were recorded before treatment (baseline) and 3 months after treatment. Implant surface decontamination with phosphoric acid 35% led to a greater immediate suppression of bacterial colony forming units on the implant surface than saline rinsing (1 minute of abundent sterile saline rinsing). Comparing microbiological samples taken from the peri-implant sulcus 3 months after surgery to pre-surgical samples, there were significantly less culture-positive implants after the decontamination procedure in the phosphoric acid group ($p = 0.042$). However, between both groups no significant differences in clinical and microbiological outcomes were found. Hence, the use of phosphoric acid as implant surface decontaminant seemed to sort a similar clinical effect as saline rinsing.

The aim of the prospective cohort study presented in **chapter 6** was to evaluate the influence of the cervical crown contour on marginal bone loss and soft tissue health around platform-switched, posteriorly placed, two-piece (bone-level) implants. A dataset from two previously conducted studies was used to evaluate a total of 64

patients with 67 posteriorly implants with a 5 year follow-up. Patients with single two-piece, platform-switched implants in between two natural teeth or adjacent to one natural tooth were included. Clinical parameters and standardized peri-apical radiographs taken at 1 month and 5 years after final crown placement were assessed. Clinical evaluation of the peri-implant soft tissue was performed 1 month (baseline) and 5 years after placement of the final implant crown. The sulcus bleeding index, the gingival/mucosal index and the probing depth were clinically recorded. Peri-implant bone level change was determined by measuring the distance from the implant reference point (most outside point of implant neck) to the level of bone-to-implant contact, at both the mesial and distal aspect of the implant. Radiographs were calibrated using the known dimensions of the implant as reference values. The difference in bone level between one month and five years after crown placement was calculated. A new measurement method was developed to analyse geometric values of the cervical crown contour. The inter and intra-examiner reliability (Cronbach's a) was assessed showing an almost perfect agreement. Emergence angles were measured at 1, 2 and 3 mm above the implant shoulder. The linear correlation between variables was determined by calculating the Pearson correlation coefficient. The results showed no correlation between the mesial and distal cervical crown angles with peri-implant bone loss and soft-tissue health. It should however be noted that none of the implants showed signs of peri-implantitis. Therefore it was concluded that the cervical crown contour at platform-switched, posteriorly placed, two-piece implants showed no correlation with peri-implant marginal bone loss and soft-tissue health up to 5 year after implant placement.

SAMENVATTING

De goudenstandaard voor de behandeling van peri-implantitis is tot op heden nog niet gevonden. Het doel van het onderzoek dat in dit proefschrift wordt gepresenteerd, was om gerandomiseerde klinische onderzoeken uit te voeren naar de invloed van implantaatoppervlakte reinigingsmethode op klinische, röntgenologische, microbiologische en immunologische parameters. Daarnaast was het doel om kennis toe te voegen aan de diagnose van peri-implantitis en aan de invloed van de implantaat gedragen krooncontour op de peri-implantaire conditie.

Het doel van de studie gepresenteerd in **hoofdstuk 2** was tweeledig: ten eerste, het vergelijken van biomarkerniveaus in de peri-implantaire creviculaire vloeistof (PICF) van implantaten met een peri-implantaire gezonde status met niveaus in PICF van implantaten met peri-implantitis. Ten tweede, het vergelijken van biomarkerniveaus voor en na niet-chirurgische behandeling. In totaal werden van 20 gezonde implantaten in 17 patiënten peri-implantaire monsters genomen en van 20 implantaten met peri-implantitis in 19 patiënten. Tevens werd deze laatste groep 3 maanden na de niet-chirurgische behandeling met de Airflow Master Piezon® (EMS) nogmaals gesampled. Voor testgroepmonsters werden patiënten uit de studie in hoofdstuk 3 gevraagd om aanvullend deel te nemen aan de studie beschreven in hoofdstuk 2. Een Luminex™-assay werd gebruikt om de pro-inflammatoire en anti-inflammatoire cytokines IL-1β, TNF-α, IL-6 & G-CSF, collageenafbraak-enzym MMP-8, chemokinen MCP-1 & MIP-1α/CCL3, botmarkers OPG & sRANKL en interferon-γ te evalueren. Klinische en röntgenologisch kenmerken werden beoordeeld bij aanvang en na 3 maanden. De resultaten toonden significant verhoogde niveaus voor IL-1β en MMP-8 in implantaten met peri-implantitis in vergelijking met implantaten met een gezonde status (respectievelijk p=.007; p=<.001). Er werd geen verschil gevonden in andere biomakerniveaus (d.w.z. TNF-α, IL-6, MCP-1 en MIP-1α/CCL3, OPG & G-CSF) tussen gezonde en geïnfecteerde implantaten. Niveaus van sRANKL en INF-γ lagen onder het detectieniveau. Evaluatie van biomarkerniveaus 3 maanden na niet-chirurgische therapie verbeterde niet significant, niveaus van IL-1β en MMP-8 bleven hoog. Daarom werd geconcludeerd dat implantaten gediagnosticeerd met peri-implantitis hogere niveaus van IL-1β en MMP-8 in PICF hebben in vergelijking met gezonde implantaten en niet-chirurgische therapie leek de inflammatoire immuunrespons niet te beïnvloeden.

In **hoofdstuk 3 en 4** worden twee gerandomiseerde, gecontroleerde studies gepresenteerd die samen een tweetraps behandelprotocol voor peri-implantitis beschrijven, waarbij het effect van mechanisch reiniging met behulp van een air polisher wordt geëvalueerd in een niet-chirurgische (hoofdstuk 3) en resectieve chirurgische

benadering (hoofdstuk 4). Het doel van de studie beschreven in hoofdstuk 3 was om het effect van een enkele niet-chirurgische ingreep met behulp van een air polisher (EMS Airflow Master Piezon®) met eryhtritolpoeder (testgroep) te evalueren en het effect te vergelijken met piëzo-electrische ultrasone reiniging (controlegroep). In beide groepen werd tevens in een enkele sessie parodontale reiniging van de volledige mond uitgevoerd. In totaal werden tachtig patiënten met 139 implantaten met peri-implantitis niet-chirurgisch behandeld. Patiënten werden willekeurig toegewezen aan de testgroep of controlegroep. Het effect werd geëvalueerd op basis van klinische, röntgenologische en microbiologische uitkomsten. Patiënten werden beoordeeld bij aanvang en na 3 maanden follow-up. Bij patiënten die na 3 maanden follow-up als succesvol werden beschouwd, werden aanvullend beoordeeld na 6, 9 en 12 maanden. Bovendien vond evaluatie van de door de patiënt waargenomen pijnsensatie, direct na interventie, plaats met behulp van een score op een VAS-schaal. Drie maanden na de therapie werd geen significant verschil gevonden tussen beide therapieën voor de primaire uitkomstmaat bloeding na sonderen (%) (BoP). Bovendien vertoonden geen van de andere klinische parameters, d.w.z. pus na sonderen (SoP), plaqueniveaus (Plq) en sondeerpocketdiepte (PPD), marginale botniveaus of microbiologische parameters enig verschil tussen beide groepen. Beide therapieën resulteerden in beperkt succes (18% van de patiënten werd als succesvol beschouwd). Evaluatie van de door de patiënt waargenomen pijnscores direct na de interventie gaf aan dat beide therapieën als minimaal pijnlijk werden beschouwd zonder dat een van beide significant minder pijnlijk was. Daarom werd geconcludeerd dat air-polishing even effectief bleek te zijn als ultrasoon scaling in de niet-chirurgische behandeling van peri-implantitis. Bij patiënten die na 3 maanden follow-up als succesvol werden beschouwd, werden de parameters aanvullend beoordeeld na 6, 9 en 12 maanden. Wanneer baseline-kenmerken van de succesvolle groep patiënten werden vergeleken met die van de niet-succesvolle patiënten, werd een lagere PPD (respectievelijk 4.0 mm versus 4.9 mm), minder marginaal botverlies (respectievelijk 3.0 mm versus 4.0 mm) en kortere functieduur vóór therapie plaatsvond (7.2 versus 9.5 jaar) gezien. Interessant is dat de follow-up van de succesvolle patiënten een geleidelijke verbetering van de klinische parameters liet zien tot 12 maanden wanneer nazorg (supragingivale instrumentatie wanneer plaque/calculus zichtbaar was) werd toegepast en de zelfzorg werd aangemoedigd op 6 en 9 maanden. Gezien het succes van deze patiënten tot 12 maanden na de therapie, geven deze parameters het belang aan van een vroege diagnose en een vroeg begin van niet-chirurgische therapie. Bovendien leek het erop dat stabiele botniveaus en afwezigheid van ziekteprogressie konden worden bereikt bij implantaten die PPD < 4 mm vertonen mét de aanwezigheid van BoP tot 12 maanden. Daarom onderstreept de uitkomst van hoofdstuk 3 dat de gevoeligheid van BoP voor de voorspelling van ziekteprogressie vrij laag is en dat strikte succescriteria voorzichtig moeten worden geïnterpreteerd en toegepast.

In hoofdstuk 4 wilden we het effect evalueren van mechanische decontaminatie van het implantaatoppervlak met behulp van air-polishing met eryhtritolpoeder op klinische, radiografische en microbiologische parameters. De parameters werden beoordeeld vóór de behandeling (baseline) en op 3, 6, 9 en 12 maanden follow-up. Patiënten die na 3 maanden follow-up als niet succesvol werden beschouwd in de niet-chirurgische peri-implantitis studie van hoofdstuk 3 werden uitgenodigd om deel te nemen aan de chirurgische follow-up studie beschreven in hoofdstuk 4. Tijdens een resectieve chirurgische ingreep, bestaande uit botrecontouring, mechanisch oppervlaktereiniging en apicaal verplaatste flap de implantaatoppervlakken van 93 implantaten met peri-implantitis (n=57 patiënten) werden willekeurig behandeld met air-polishing (test) of in zoutoplossing gedrenkte gazen (controle). Voor de behandeling (baseline) en 3, 6, 9 en 12 maanden na de therapie werden klinische parameters bepaald. Röntgenologisch onderzoek vond plaats na 3, 6 en 12 maanden follow-up en microbiologische monsters werden op baseline en 12 maanden na de behandeling genomen. Er werden geen verschillen gevonden tussen de test- en controlegroep voor de primaire uitkomstmaat BoP over 12 maanden follow-up, noch voor de secundaire parameters Plq, PPD en MBL. Tussen beide groepen werd een significant verschil gevonden voor de niveaus van SoP. Er werd geen significant effect op 8 klassieke parodontale pathogeenniveaus gevonden, tevens zonder verschil in effect tussen beide therapieën. Na 1 jaar follow-up werd een succesvol behandelresultaat (PPD <5 mm, maximaal 1 van de 6 plaatsen BoP, geen pus en geen progressief botverlies >0,5 mm) bereikt voor in totaal 18 implantaten (19,2%).

De gerandomiseerde klinische studie gepresenteerd in **hoofdstuk 5** was gericht op het evalueren van het effect van chemische implantaatoppervlakte reiniging met fosforzuur tijdens een chirurgische peri-implantitisbehandeling op klinische, radiografische en microbiologische parameters. In totaal werden 28 patiënten met 53 implantaten met peri-implantitis behandeld met een resectieve chirurgische benadering bestaande uit botrecontouring, chemische oppervlaktereiniging en apicaal verplaatste flap. Patiënten werden willekeurig toegewezen aan reiniging met fosforzuur 35% (testgroep) en spoelen met zoutoplossing (controlegroep). Microbiologische parameters werden geregistreerd tijdens de operatie, terwijl klinische en radiografische parameters werden geregistreerd vóór de behandeling (baseline) en 3 maanden na de behandeling. Decontaminatie van het implantaatoppervlak met fosforzuur 35% leidde tot een grotere onmiddellijke onderdrukking van bacteriekolonievormende eenheden op het implantaatoppervlak dan spoelen met zoutoplossing (1 minuut overvloedig spoelen met steriele zoutoplossing). Bij het vergelijken van microbiologische monsters genomen van de peri-implantaire sulcus 3 maanden na de operatie met pre-operatieve monsters, waren er significant minder kweekpositieve implantaten na de decontaminatieprocedure in de fosforzuurgroep (p = 0,042). Tussen beide groepen werden echter geen significante

verschillen in klinische en microbiologische uitkomsten gevonden. Daarom leek het gebruik van fosforzuur als decontaminatiemiddel voor het implantaatoppervlak een vergelijkbaar klinisch effect te sorteren als spoelen met zoutoplossing.

Het doel van de prospectieve cohortstudie gepresenteerd in **hoofdstuk 6** was om de invloed van de cervicale krooncontour op marginaal botverlies en de gezondheid van zacht weefsel rond platform-geswitchte, posterieur geplaatste, bone-level implantaten te evalueren. Een dataset van twee eerder uitgevoerde onderzoeken werd gebruikt om in totaal 64 patiënten met 67 posterieure implantaten te evalueren met een follow-up van 5 jaar. Patiënten met enkele tweedelige, platform geswitchte implantaten tussen twee natuurlijke tanden of naast één natuurlijke tand werden geïncludeerd. Klinische parameters en gestandaardiseerde peri-apicale röntgenfoto's, genomen 1 maand en 5 jaar na de definitieve plaatsing van de kroon, werden beoordeeld. Klinische evaluatie van het peri-implantaire zachte weefsel werd 1 maand (baseline) en 5 jaar na plaatsing van de definitieve implantaatkroon uitgevoerd. De sulcus-bloedingsindex, de gingivale/mucosale index en de sondeerdiepte werden klinisch geregistreerd. De verandering in botniveau rond het implantaat werd bepaald door de afstand te meten van het implantaatreferentiepunt (meest buitenste punt van de implantaathals) tot het niveau van bot-implantaatcontact, zowel aan de mesiale als het distale zijde van het implantaat. Röntgenfoto's werden gekalibreerd met de bekende afmetingen van het implantaat als referentiewaarden. Het verschil in botniveau tussen één maand en vijf jaar na het plaatsen van de kroon werd berekend. Een nieuwe meetmethode ontwikkeld om geometrische waarden van de cervicale krooncontour te evalueren. De inter- en intra-beoordelaarsbetrouwbaarheid (Cronbach's a) werd beoordeeld met een bijna perfecte overeenstemming. Hoeken werden gemeten op 1, 2 en 3 mm boven de implantaatschouder. De lineaire correlatie tussen variabelen werd bepaald door de Pearson-correlatiecoëfficiënt te berekenen. De resultaten lieten geen correlatie zien tussen de mesiale en distale cervicale kroonhoeken met peri-implantaat botverlies en gezondheid van de weke delen. Er moet echter worden opgemerkt dat geen van de implantaten tekenen van peri-implantitis vertoonde. Daarom werd geconcludeerd dat de cervicale krooncontour bij platform geswitchte, posterieur geplaatste, bone-level implantaten geen correlatie vertoonde met peri-implantaat marginaal botverlies en gezondheid van de weke delen tot 5 jaar na plaatsing van het implantaat.

DANKWOORD

Het moment is daar om mensen te bedanken! Zonder steun, aanmoediging en inspiratie van mensen die bij mij zowel fysiek of in gedachte aanwezig waren in de afgelopen jaren was dit proefschrift niet tot stand gekomen. Allereerst gaat mijn dank uit naar de geoliede onderzoeksmachine waar ik afgelopen jaren deel van heb uit mogen maken. Hiertoe behoren zowel mijn begeleidingsteam, de afdeling MKA-chirurgie van het UMCG en de meer dan 150 onderzoekspatiënten die hebben deelgenomen aan de verschillende klinische studies. Het was door deze unieke gouden driehoekscombinatie mogelijk om op grote schaal klinisch onderzoek toe doen. Een waar voorrecht om met jullie te hebben samen gewerkt!

Zonder financiële ondersteuning was dit traject niet mogelijk geweest. Daarom gaat mijn dank ook uit naar de Graduate School of Medical Sciences (GSMS) van de Rijksuniversiteit Groningen die ons onderzoeksvoorstel honoreerde met een studiebeurs.

Prof. dr. G.M. Raghoebar, hooggeleerde eerste promotor, beste Gerry. Ik wil je bedanken voor de kans die je mij hebt geboden om onder jouw vleugels een bijdrage te leveren aan de wetenschappelijke kant van de implantologie. Maar natuurlijk ook dank voor jouw kenmerkende efficiënte manier van promovendibegeleiding. Het overleg was altijd to the point en je was ten allen tijde bereikbaar. Je hebt mij vrijgelaten waar het kon maar wist mij altijd op de juiste momenten te bevragen naar de voortgang van mijn studies of naderende deadlines. Mede door jouw gedrevenheid in het behandelen van patiënten hebben we snelheid in de promotie gehouden, heel veel dank! Tot slot, dank voor het vertrouwen dat je in mij had dat ondanks mijn keuze voor de 200kmwoon-werk afstand het traject tot een goed einde zou worden volbracht.

Prof. dr. A.J. van Winkelhoff, hooggeleerde tweede promotor, beste Arie Jan. Wat een geluk heb ik gehad dat je mij nog in de nadagen van je hoogleraarschap als een van je laatste promovendi wilde begeleiden. Jouw schat aan ervaring op het wetenschappelijke vlak heeft er zondermeer aan bijgedragen dat van iedere ruwe manuscriptschets die je van mij onder ogen kreeg toch weer een geaccepteerde versie kon worden gemaakt. Ondanks dat we elkaar niet vaak zagen was het contact per mail of telefoon altijd warm. Je hebt me met je positieve commentaar altijd het vertrouwen gegeven dat ik wetenschappelijk stappen maakte. Jouw idee om peri-implantitis van een hematologische kant te gaan onderzoeken achtte ik een stap te groot. Als tussenstap, en wellicht als opmaat naar de hematologie, hebben we een mooi immunologische stuk aan de wetenschap kunnen toevoegen.

Prof. dr. H.J.A. Meijer, hooggeleerde derde promotor, beste Henny. Binnen onze onderzoeksgroep hebben wij elkaar als eerste leren kennen. Als gemotiveerde tandheelkunde student kwam ik bij je om mijn master scriptie te schrijven. Het resulteerde in een plezierige samenwerking. Deze kennismaking met de wetenschap onder jouw begeleiding is voor mij zeer bepalend geweest om op de afdeling voor een promotietraject te solliciteren. Waarvoor dank! Jouw relaxte en ontspannen manier waarop je diverse problematiek weet te benaderen heeft bij tijden het doen van onderzoek mentaal een stuk lichter gemaakt. Samen hebben we gedurende mijn promotietraject succesvol een prothetische aspect van de implantologie nader onderzocht. Hier kijk ik met plezier op terug. Tot slot, bedankt voor alle woensdagmiddagen waar je hulp voorafgaand aan de chirurgie en terloops bij mijn patiëntevaluaties onmisbaar was.

Dr. Y.C.M. de Waal, zeergeleerde copromotor, beste Yvonne. Wat was het een voorrecht om met jou samen te werken! Vanaf dag 1 heb je mij aan de hand genomen en wegwijs gemaakt in de wetenschappelijke wereld en in het doen van klinisch onderzoek. Je hebt me onder andere geleerd METc aanvragen te schrijven, patiënten te beoordelen/evalueren, studieresultaten te interpreteren en manuscripten te schrijven en bovenal gepubliceerd te krijgen. Jouw kritische en objectieve blik, steeds weer opnieuw, is de reden dat dit boekje alleen maar een klein beetje in de buurt komt van de kwaliteit van jouw eigen proefschrift. Ik wil je danken, dat ondanks jouw drukke gezinsleven én het hebben van een eigen praktijk je altijd op een zeer prettige manier de tijd nam om mij de sturing te geven die ik nodig had. Ik ben heel dankbaar dat ik een vervolg aan de door jou ingezette onderzoekslijn heb mogen geven. Ik had mij als promovendus geen betere copromotor kunnen wensen!

Prof. dr. F. Abbas, prof. dr. H. de Bruyn, prof. dr. A. Visser, hooggeleerde leden van de boordelingscommissie, hartelijk dank voor de tijd die jullie hebben gestoken in de boordeling van het proefschrift en de bereidheid om zitting te nemen in de beoordelingscommissie.

Geachte leden van het dagelijks Bestuur van de afdeling MKA-chirurgie, geachte prof. dr. F.K.L. Spijkervet, dr. B. van Minnen, dr. H. Reintsema, mw. J.M. Baldi-Ekelhoff, dhr. R. van der Graaf. Hartelijk dank voor de mogelijkheid die ik heb gekregen om op de afdeling dit promotieonderzoek uit te voeren en te voltooien.

Prof. dr. F.M.G. Kroese en dr. S.C. Liefers, beste Frans, beste Sylvia. Dank voor jullie bereidheid om samen immunologisch onderzoek te doen! Frans, dank voor het sparren over de studie-opzet, het evalueren en interpreteren van de onderzoeksresultaten

en de mogelijkheid voor het gebruik van het lab. Je hebt me welkom laten voelen op jullie afdeling. Sylvia, we hebben samen uitgevonden op welke manier en in welke samenstelling de 'paper strips' het beste verwerkt konden worden. Je hebt vervolgens ook alle 'studie samples' verwerkt waarvoor heel veel dank! Tevens dank voor de door jou inzichtelijk gemaakte onderzoeksresultaten. Mede door jullie is er van een ruw onderzoeksidee een verfijnde studie tot stand gekomen met als kers op de taart een mooie publicatie (hoofdstuk 2)!

Dr. R.E. Stewart, beste Roy. Ik wil je bedanken voor jouw hulp met de 'multilevel analyses'. In jouw drukke agenda wist je toch nog tijd vrij te maken om vaak telefonisch met mij te overleggen, zelfs op zondagen! Jouw expertise was onmisbaar bij het analyseren van de klinische data! Bedankt voor jouw mede-auteurschap aan de hoofdstukken 3 en 4.

Drs. M.G.P. Hentenaar, drs. W.D.C. Derksen, beste Michiel en Wiebe, beste paranimfen. Wat een eer om jullie aan mijn zijde te hebben tijdens het slotstuk van mijn promotie! Jullie beiden zijn voor mij een inspiratie geweest om na mijn Bachelor geneeskunde te switchen naar de studie tandheelkunde. Vanaf het eerste moment bleek het een schot in de roos en sindsdien kan ik op belangrijke momenten bij jullie terecht om te sparren over ideeën of stappen die ik in mijn carrière wil zetten. Ik kan mij geen betere klankborden wensen, waarvoor heel veel dank. Michiel, sinds een aantal jaar zijn we bevoorrecht om op loopafstand van elkaar te werken in wat wij het mooiste gedeelte van Amsterdam vinden;). Ik hoop dat we nog lang met veel plezier elkaar kunnen blijven prikkelen om een betere tandarts te worden. Wiebe, ik ben je zeer dankbaar voor onze waardevolle vriendschap. Jouw hulp en advies zijn altijd van meerwaarde. Zonder jouw introductie van mij bij Kirsten, waarna het balletje is gaan rollen, was dit proefschrift er nooit geweest. Heel erg bedankt!

Dr. K. Slagter, beste Kirsten. Dank dat ik een bijdrage heb kunnen leveren als tandheelkundestudent aan jouw proefschrift. Als gevolg daarvan werd ik door jou geprikkeld om na te denken om mijn scriptie een wetenschappelijk vervolg te geven in de vorm van een promotie. Dat het nu zo ver is, is dus mede aan jou te danken!

Drs. J. Hakkers, beste Jarno. Met jou aan boord hebben we een waardig opvolger binnen de onderzoekslijn peri-implantaire infecties. Ik wens je alle succes met jouw onderzoek! En leve het Eurovisiesongfestival;).

Mw. Kempers, beste Lisa. Dank voor je ondersteuning bij mijn onderzoek. Je bent in je rol als researchcoördinator onmisbaar bij het doen van wetenschappelijk onderzoek.

Mw. H.H. Kooistra-Veenkamp, beste Ria. Dank voor het plannen van de ruim 700! onderzoeksafspraken. Jouw logistieke accuraatheid heeft er voor gezorgd dat mijn woensdagen altijd strak gepland waren en het onderzoek onverminderd snel door kon.

Mw. M.A. Bezema, beste Ans. In de afwezigheid van Ria kon ik altijd op jou rekenen voor het plannen van de onderzoeksafspraken, dank voor jouw hulp!

Beste Sanne, beste Barbara. Bedankt voor alle mondhygiëne-behandelingen die jullie in het kader van mijn onderzoek hebben uitgevoerd. De zorg en aandacht die jullie aan de patiënten besteden is top.

Beste dames van de röntgen, Charlotte, Mariëlle, Lilian & Yvonne. Dank voor jullie hulp op de woensdagen bij het maken van foto's van mijn onderzoekspatiënten. Ondanks dat ik het jullie moeilijk heb gemaakt om met die rare gekleide houdertjes te werken, bleven jullie altijd heel behulpzaam. Dank daarvoor!

Dr. A. J. Tuin, beste Jorien. Ruim 4 jaar lang had ik het genoegen om op woensdag en donderdag met jou kamer S3.220 te delen. Door jouw aanwezigheid vanzelfsprekend de leukste kamer van de afdeling! Dank voor alle gezellige pauzemomenten waarin jij altijd benieuwd was naar een 'sappige roddel'. Helaas heb je het vaak moeten doen met het aanhoren van mijn geklaag over verschillende dagelijkse futiliteiten, waaronder het maar niet meer te noemen hightech onderzoeks- apparaat uit hoofdstuk 2 ;).

Dr. J. Kraeima, dr. K. Boeve, dr. T. van der Meulen, dr. van Nimwegen, dr. M. Filius, beste Joep, Koos, Taco, Wouter en Marieke. Dank voor alle gezellige momenten, maar bovenal voor de weekenden weg. Het was bijzonder om samen getuige te zijn van Apollo-lanceringen en om dolfijnen te spotten;).

Beste medeonderzoekers en EAO congresgangers, beste Caroline, Pieter, Carina, Elise, Wim, Charlotte, Christiaan. Dank voor de gezellige en goed georganiseerde weekenden samen op EAO- congres.

Medewerkers van het bacteriologisch laboratorium, in het bijzonder beste Bas. Dank voor jullie hulp bij de coördinatie, het verwerken en opslaan van de microbiologische samples.

Lieve Danielle, dankjewel voor de kans die je mij hebt geboden om in jouw praktijk mijn eerste stappen als tandarts te zetten. Naast mijn ontwikkeling op onderzoeksgebied heb ik tegelijkertijd in de afgelopen jaren (en nog steeds) met jou als mentor op

het tandheelkundig vlak kunnen groeien. Ik hoop nog op een lange en plezierige samenwerking aan de Stadionweg.

Alle (oud)collegae van tandartspraktijk Jesse, beste Jeroen, Jetske, Liesbeth, Ilse, Moniek, Julia, Karlijn, Eline, Monica (van der Vleuten), Ka Yan, Melda, Anki, Josephine, Ellen, Nijoka, Marion, Monica (Pal), Oda, Caitlin, Puk, Laura, Beau, Laurance, Quinty. Dank voor de prettige samenwerking en gezelligheid zowel binnen als buiten de praktijk. Ik voel me vereerd om onderdeel uit te maken van een dergelijk ambitieus (vrouwelijk) team;).

Beste Reinder, beste Christopher. Dank dat ik alweer bijna 5 jaar onderdeel van het TandartsSpoedPraktijk (TSP) team in het OLVG mag uitmaken. Het is indrukwekkend om te zien hoe jullie concept in grote delen van Nederland een oplossing biedt voor zowel de tandheelkundige zorgverlener als de patiënt. Ik hoop hieraan nog lang een bijdrage te kunnen leveren!

Beste Mieke, 'tante' Lenie, Shantal, Pieter & Joyce en Inge. Dank voor jullie gastvrijheid en de mogelijkheid om door de jaren heen op woensdag bij één van jullie in Groningen te hebben kunnen overnachten.

Lieve Happy Hentmade, dank voor de warme band die we met elkaar hebben. Gelijk opgaand met de start van mijn onderzoek hebben we als familie de laatste jaren de nodige beproevingen moeten doorstaan. Het waren helaas door die momenten dat we elkaar weer in groten getale ontmoetten. Maar ondanks de meermaals verdrietige aanleiding proberen we er altijd iets positief van te maken! Juist deze voor ons allen typerende positieve levensinstelling heeft mij gesterkt gedurende mijn onderzoekstijd. Dank hiervoor! Hopelijk blijven de trieste familiereünie aanleidingen ons nog lange tijd bespaard en zien we elkaar nog vaak om het leven te vieren.

Lieve familie en vrienden, dank voor de interesse die jullie hebben getoond in mijn onderzoek en de momenten van ontspanning die jullie mij hebben geboden.

Cari nonna Maria, Monique, Piero, Charlotte, Ruben, Amalia e Arturo. La famiglia Italiana. È sempre un piacere riverderci. Grazie per far mi sentire cosi accetato nella vostra famiglia!

Lieve Michiel en Juliette, lieve broer en zus, lieve collega's. Ja dat laatste klinkt nog een beetje raar, maar sinds kort, Juul, heb ook jij ingezien wat Giel dus allang wist en ik nu ook al een tijdje: een vak in de mondzorg past ons het best. We zijn er zo allemaal op ons eigen moment achter gekomen en binnen onze levens op ons eigen moment voor gegaan. Die geheel eigen wijze typeert ons en maakt mij trots op jullie als broer en zus. Ik wil jullie danken voor onze warme band die sterk is, de afgelopen jaren versterkt is en hopelijk nog lang zo blijft.

Lieve Mam, dank voor alle steun en mogelijkheden die je mij samen met Pap hebt geboden om de promotie van vandaag te bereiken. Jouw zorgzame en warme karakter heeft de basis geboden van waaruit ik heb kunnen groeien. Jouw sterke wil om positief in het leven te staan is inspirerend en heeft mij, zeker de afgelopen jaren, geholpen om te bereiken wat ik wilde. Je bent als energieke ruggengraat van ons gezin een voorbeeld voor ons allen!

Lieve Pap, er zijn maar twee mensen die mij overtuigd hebben om de uitdaging van de afgelopen 6 jaar aan te gaan en daar was jij er één van. Ik wil je danken voor het geloof in mijn kunnen om dat gene te bewerkstelligen wat je vandaag in handen zou hebben gehad. Met de gedachte dat je in ieder geval weet dat ik er aan ben begonnen prijs ik mij gelukkig, maar wat baal ik er ervan dat je vandaag niet kan zien dat ik het ook volbracht heb. Toch geeft de gedachte aan jouw positieve, levenslustige en humoristische karakter mij iedere dag energie en een lach op mijn gezicht. Ik ben je eeuwig dankbaar!

Lieve Marlies, jij was de ander die mij overtuigde om aan dit promotietraject te beginnen! Sinds de start van mijn onderzoek heb je mij ondersteund, aangemoedigd en alle vrijheid gegeven om de uren te maken die nodig waren om tot dit eindresultaat te komen. Grazie mille! De energie en tijd die jij in jouw carrière steekt is een inspiratie voor mij. Ik geniet van jouw vrolijke, opgewekte en prachtige verschijning. Zonder jou aan mijn zijde was dit nooit gelukt. Ti amo.

CURRICULUM VITAE

Diederik Hentenaar was born on March 26th 1988 in Geldrop, the Netherlands. After finishing secondary school (Lorentz Casimir Lyceum Eindhoven) in 2006 he started studying Medicine at the University of Groningen. He obtained his bachelors degree in Medicine after which he continued to study Dentistry at the same university. During his penultimate year he applied for the Doctor of Dental Medicine/Doctor of Philosophy' ((DMD/PhD) programme for which he got assigned. This provided him the opportunity to combine his last year of dentistry with the start of his PhD research project. After his graduation in 2016, he started working as general practioner in Amsterdam at dental clinic Jesse and as emergency dentist for Tandartsspoedpraktijk at OLVG hospital. Over the last years he combined his work in Amsterdam with working on this PhD research in Groningen.

The printing, distribution and online availability of this thesis was generously supported by:

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