Complications of implant dentistry and their management

Peri-implantitis and beyond

Polymeri, A.

Publication date
2023
Document Version
Final published version

[Link to publication](#)

Citation for published version (APA):
Polymeri, A. (2023). *Complications of implant dentistry and their management: Peri-implantitis and beyond*. [Thesis, fully internal, Universiteit van Amsterdam].
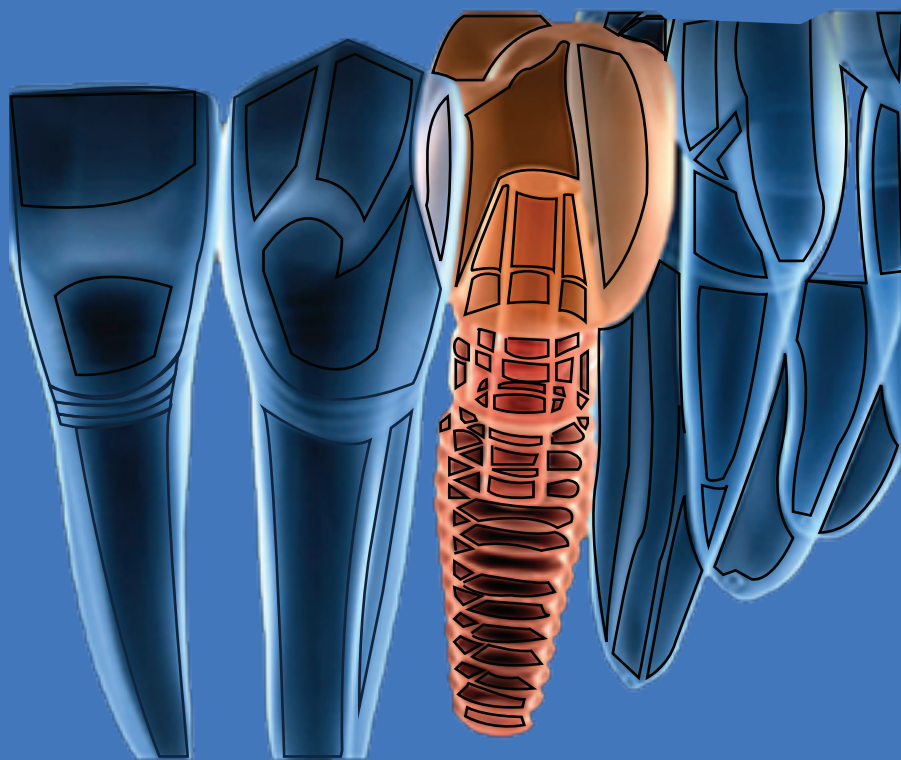
Complications of Implant Dentistry and Their Management; Peri-implantitis and Beyond

Angeliki Polymeri

Complications of Implant Dentistry
and Their Management;
Peri-implantitis and Beyond

Angeliki Polymeri

ISBN: 978-94-6419-774-7
Cover design: Markella Kompitsa
Printed by: Gildeprint B.V.
Copyright © Angeliki Polymeri, 2023

Complications of Implant Dentistry
and Their Management;
Peri-implantitis and Beyond

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit van
Amsterdam op gezag van de Rector Magnificus prof. dr. ir. P.P.C.C.
Verbeek ten overstaan van een door het College voor Promoties
ingestelde commissie, in het openbaar te verdedigen in de
Agnietenkapel
op donderdag 4 mei 2023, te 13.00 uur

door

Angeliki Polymeri

geboren te Athina

Promotiecommissie

Promotores:

Prof. dr. B.G. Loos   Universiteit van Amsterdam

Prof. dr. M.L. Laine   Vrije Universiteit

Overige leden:

Prof. dr. F. Abbas   Universiteit van Amsterdam

Prof. dr. H. De Bruyn   Radboud Universiteit

dr. M.M. Danser   Universiteit van Amsterdam

Prof. dr. W.V. Giannobile   Harvard School of Dental Medicine

dr. ir. T.J. de Vries   Universiteit van Amsterdam

Prof. dr. G.A. van der Weijden   Universiteit van Amsterdam

Prof. dr. A.J. van Winkelhoff   Rijksuniversiteit Groningen

Faculteit der Tandheelkunde

To my family

*"But you must know that only he who fights the darkness within will the
day after tomorrow have his own share in the sun."*
Odysseus Elytis

TABLE OF CONTENTS

CHAPTER 1

General introduction

1.1 A brief history of dental implants

A full set of teeth has been considered a functional and esthetic asset since ancient times. This has urged people to invent methods of replacing missing teeth. Archeologic evidence suggests that the first dental prostheses date as far back as the Neolithic period in the ancient Egypt where shells were carved in the shape of a human tooth [1]. Similar dental prostheses carved from bamboo pegs were used around 2000 BC in ancient China [2]. Other civilizations such as the Etruscans and the Phoenicians made fake teeth from animal bones or ivory and used gold wire to stabilize them [3]. Around 600 AD the Mayans used shell pieces, tapped into the mandibular bone, which is considered as the first evidence of endosseous dental implants (Fig. 1) [4]. In the 18th and 19th century, researchers used many materials including gold, silver and porcelain to make dental implants. However, these attempts failed as the human body rejected all the materials used [3].

Figure 1: In 1931 a Mayan mandible was discovered with carved shell pieces implanted to replace the three lower incisors. These "implants" were dated to 600 A.D [4].

It was in the 1950s when an orthopedic surgeon, Professor Branemark discovered accidentally the particular properties of titanium. During experiments on bone marrow healing, he placed a titanium chamber into a rabbit's femur, which later on was unable to remove since it had fused with the bone. This led him to place the first dental implant in a human volunteer in 1965, which proved to be successful [2]. Branemark coined the term "osseointegration" and defined it as the direct connection between the living bone and surface of a load-bearing

implant [5]. This discovery was a significant breakthrough in implant dentistry and set the basis for modern dental implants. Although Branemark originally proposed dental implants as a solution for the treatment of the edentulous jaw [5, 6], later on, dental implants have also been used for the replacement of missing teeth in partially edentulous patients.

1.2 Peri-implantitis; definition, risk factors and diagnostic criteria

Dental implants are a popular treatment for the prosthetic rehabilitation of partially dentate and fully edentulous patients [7]. Nevertheless, peri-implant diseases, encompassing peri-implant mucositis and peri-implantitis, are a growing concern in the dental community [8]. According to the consensus report of the 2017 World Workshop, peri-implant mucositis is characterized by inflammation confined to the soft tissues surrounding the implant, whereas the inflammatory process in peri-implantitis leads to progressive loss of supporting bone and eventually loss of the implant [9]. More and more dental professionals, from various parts around the globe with different academic backgrounds, are dealing with peri-implant diseases in their practice. Whether these differences are also reflected in the considerations, attitudes, and treatment strategies of clinicians towards peri-implant diseases, is still to be found out.

The prevalence of peri-implant mucositis and peri-implantitis ranges widely depending on the disease definition and the time of functional loading of the implant [10, 11]. In a systematic review of epidemiology of peri-implant diseases, Derks and colleagues reported a prevalence of peri-implant mucositis ranging from 19 to 65%, while the prevalence of peri-implantitis ranged from 1 to 47% at patient level [10]. The same group calculated the estimated weighted mean prevalences for peri-implant mucositis and peri-implantitis to be 43% and 22%, respectively [10]. Another systematic review concluded that the weighted mean subject-based prevalences for peri-implant mucositis and peri-implantitis were approximately 47% and 20% respectively [11]. A more

recent retrospective study of electronic health records at a U.S. dental school concluded that approximately 1 out of 3 patients and 1 out of 5 implants experienced peri-implantitis [12].

Despite potential differences in peri-implantitis prevalence, consensus exists that biofilm plays an important role in the etiology of peri-implantitis, as an initial trigger for inflammatory reactions [13, 14]. Although the microbial composition associated with peri-implantitis has been suggested to be similar to periodontitis, including pathogens such as *Fusobacterium nucleatum*, *Prevotella intermedia* and *Treponema denticola*, the peri-implant microbiome seems more complex including non-cultivable gram-negative species [15]. Furthermore, systemic, local, genetic, behavioral and iatrogenic factors have been accepted as being associated with the onset and progression of this disease [14, 16]. Diabetes mellitus is the systemic risk factor most extensively studied in relation to peri-implantitis [17]. Other systemic diseases such as osteoporosis and cardiovascular diseases, as well the treatment with oral bisphosphonates have been reported as possible risk factors; however, the evidence is weak [18, 19]. Local factors including the presence of dental plaque, lack of keratinized tissue, and implant surface roughness have also been associated with greater risk for peri-implant pathologies [16, 20-22]. Research also showed the relevance of iatrogenic factors such as improper implant position, presence of residual cement, and prosthesis design that limits oral hygiene accessibility [23, 24]. There is literature suggesting that in some cases the peri-implant marginal bone loss is associated with a foreign body immune reaction to titanium particles that leads to bone resorption by osteoclasts and eventually implant failure [25]. The view that peri-implantitis in some patients is considered a foreign body reaction, similar to an allergy, is supported by histologic data which demonstrate the presence of foreign bodies, such as titanium particles, surrounded by chronic inflammatory infiltrates [26]. In addition, occlusal overload has been associated with mechanical implant complications [27] and peri-implant bone loss [28]. However, a causal relationship as well as specific strain thresholds have not been established yet [28]. While

genetic traits may influence inflammatory responses and thus may be a risk modifier, the relationship between peri-implantitis and genetic predisposition remains unclear [29]. Patient-related factors such as smoking [30], history of periodontitis [30] and lack of maintenance care [31] have currently been associated with higher prevalence and severity of peri-implantitis.

The diagnosis of peri-implantitis is based on clinical parameters such as probing depth, bleeding and suppuration on probing, and on radiographic evidence of bone loss following initial bone remodeling [32] (Fig. 2). Monitoring the changes in the clinical and radiographic parameters following the completion of the implant-supported prosthesis is important for the proper diagnosis of peri-implantitis [9]. In the absence of previous clinical and radiographic evaluations, the diagnosis is based on the presence of a peri-implant pocket ≥6 mm accompanied by bleeding and/or purulent exudate and bone loss ≥3 mm from the implant platform [33].

Figure 2: Peri-implantitis. Left: Peri-implant soft tissue inflammation accompanied by suppuration, middle: deep peri-implant pocket of 7 mm and right: radiographic bone loss beyond initial bone remodeling (Courtesy of Dr. David Anssari Moin, ACTA).

1.3 Microbial biofilm-driven etiopathogenesis of peri-implantitis

The importance of biofilms in the etiology of peri-implant diseases has been extensively studied [15, 34]. Dysbiotic biofilms may cause tissue inflammation, which in turn alters the ecology and favors further growth of dysbiotic communities, leading to a vicious cycle, similar to periodontitis [35, 36]. Microorganisms colonize the peri-implant sulcus within 30 minutes after the surgical procedure and a complex submucosal microbiota, similar to the microbiota around natural teeth, is established within two weeks [37, 38]. Teeth and mucosal surfaces act as microbial reservoirs for the colonization of implants in partially edentulous and fully edentulous individuals, respectively [39]. If the biofilm is left undisturbed, clinical signs of inflammation in the peri-implant soft tissues start to appear, demonstrating a cause and effect relationship between biofilm and peri-implant mucositis, similar to gingivitis on natural teeth [40, 41]. Untreated peri-implant mucositis can at some point derail and progress to peri-implantitis; the interactions between bacteria and the host immune system may trigger peri-implant bone loss in susceptible individuals and therefore affect the implant long-term stability [14]. While bone loss progresses, a deep pocket is formed, and this new anaerobic environment favors the growth of Gram-negative anaerobic bacteria [42, 43]. The hypothesis that bacteria translocate from periodontally involved teeth to implant sites led to the conclusion that the composition of the peri-implant microbiota resembles the subgingival microflora of periodontitis to a great extent [15, 42]. Nevertheless, more recent evidence based on open-ended 16S rRNA gene sequencing and transcriptome sequencing methods, suggests that the periodontal and peri-implant microbiomes have some common, as well as distinct features which appear to be driven by substrate characteristics and environmental factors [15, 44-47]. Therefore, a clear understanding of the microbial profiles of the peri-implant sulcus/pocket is of great importance to understand the sequelae of ecological changes and to establish effective preventive, diagnostic and treatment strategies of peri-implant diseases.

1.4 Current therapeutic approaches for peri-implantitis

Since the role of microorganisms organized in a biofilm in peri-implant diseases as an initial trigger for inflammatory reactions is well-established, the treatment approaches proposed for their management focus on the disruption and elimination of biofilm from the implant surface [48]. The current protocols for the treatment of peri-implantitis are based on the evidence available from studies related to the treatment of periodontitis [48]. Although most periodontitis cases respond favorably to well-established non-surgical and surgical treatment and maintain long-term periodontal stability [49], this does not hold true for peri-implantitis, most probably due to structural differences in supporting tissues between implants and teeth, differences in the histopathologic features of the two lesions, and the surface characteristics of implants (Fig. 3) [50-52]. Therefore, existing therapeutic strategies are unpredictable in arresting peri-implant tissue inflammation and current evidence does not support a gold-standard treatment [53, 54].
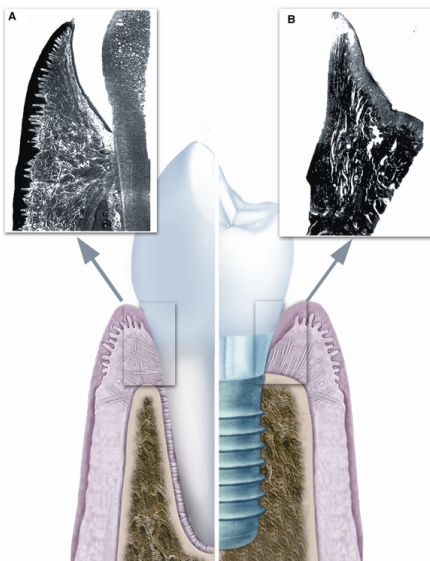
Figure 3: Comparison of healthy periodontal (A) and peri-implant tissues (B). Although the anatomical components of the supracrestal tissue attachment (formerly known as "biologic width") are present in both periodontal and peri-implant mucosa, their relative dimensions differ. (A) The epithelial attachment and connective tissue attachment of the tooth are on average 0.97 mm and 1.07 mm, respectively. (B) In peri-implant mucosa there is a longer epithelial attachment (2 mm) and 1–1.5 mm of connective tissue attachment. Furthermore, the implant lacks root cementum, periodontal ligament, and bundle bone, which makes it ankylosed to the surrounding bone [51].

The lack of effective and predictable treatments makes the management of peri-implant diseases even more challenging [48]. As that may be, the non-surgical treatment is nevertheless always the first step which may lead to improvements in bleeding tendency and in some cases to peri-implant pocket depth reduction of up to 1 mm [55]. The use of local antiseptics [56], lasers [57] and photodynamic therapy [58] have been proposed as adjunctive measures to non-surgical mechanical debridement. However, existing evidence has only shown minimal additional benefits of these adjunctive measures for improving clinical parameters [56-58]. The adjunctive use of systemic antibiotics, especially metronidazole or the combination of amoxicillin and metronidazole, in the non-surgical treatment of periodontitis has been widely investigated and in some cases has shown to improve the clinical and microbiological parameters [59, 60]. Antimicrobials have also been proposed for peri-implantitis treatment and are widely used empirically by clinicians from all over the globe, although the scientific evidence of their benefits is still limited [61]. Several studies have demonstrated that the adjunctive administration of systemic antibiotics has led to favorable results in terms of pocket depth reduction, tissue inflammation and even radiographic defect reduction [62-65]. On the other hand, two recent RCTs have shown no additional benefit to non-surgical treatment when systemic antibiotics were used adjunctively [66, 67].

In the more severe peri-implantitis cases, non-surgical treatment is insufficient to arrest the disease and to eliminate bacteria from the rough surfaces and from within the concavities of the screw threads of implants [55, 68]. Surgical therapy has proven to be more effective in the reduction of peri-implant pocket depths and bleeding on probing as well as in promoting new bone fill, possibly because it provides access to the defect area for removal of the granulation tissue and debridement/decontamination of the exposed implant threads [54, 69, 70]. Open flap debridement, resective surgery with or without implantoplasty and reconstructive approaches including the use of various bone grafts with or without the use of barrier membranes are

some of the surgical approaches reported in the literature [48, 54]. The addition of bone substitutes with or without barrier membranes has demonstrated promising results in terms of radiographic defect reduction and improvement of clinical parameters, especially in well-contained (4-wall and 3-wall) intrabony defects [71-76]. Nevertheless, complete resolution of the bony defect is still not predictable [77].

1.5 Other implant-related complications

Most implant-related complications described in the literature refer to mechanical complications of implant components, such as prosthetic screw-loosening and porcelain fracture, as well as biological complications such as peri-implant mucositis and peri-implantitis [78, 79]. The position of the implant-supported crown in relation to the adjacent natural teeth, is an esthetic parameter equally significant for implant success [80], and especially for implants placed in the anterior maxilla, yet, it is often overlooked.
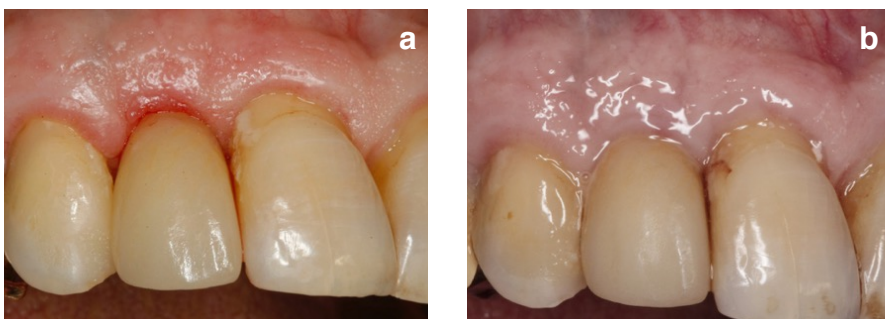
Figure 4: Clinical images illustrating a single-implant crown at the right lateral incisor position at baseline (a), and after 8 years (b) of functional loading. Note the infra-position of the implant crown at 8 years. (Courtesy of Professor Hom-Lay Wang, University of Michigan).

16

Single-tooth implants in the maxillary anterior region have the highest risk of esthetic complications from infrapositioning due to continuous maxillary growth and the continuous eruption of adjacent teeth [81] (Fig. 4). It is already well-understood that young individuals should not receive single implant therapy, since the implant, like an ankylosed tooth, fails to adapt to the maxillo-mandibular and alveolar growth as well as to the continuous eruption of the adjacent natural teeth. This results in disharmony of the occlusal plane described as infraocclusion or infrapositioning of the implant supported restoration [82, 83]. Clinical studies have shown that the placement of implants in adolescents results in infraocclusion of 0.1 to 2.2 mm at follow-up times ranging from 3 to 10 years [84, 85].

However, implant infrapositioning has also been reported in patients who receive implants during adulthood and is related to the continuous eruption of the teeth over a lifetime [86, 87]. The magnitude of implant infraposition in adult patients reported in the literature ranges from 0.10 to 1.86 mm at follow up intervals from 1 to 15 years [83, 88-92]. Sex and face anatomy were identified as significant factors for the development of infraposition of the single-implant restorations, with females and patients with long face type presenting a higher risk [87, 89, 91, 93]. Some studies have also reported an association between the amount of vertical eruption and implant location; central and lateral incisors but not canines or premolars had a significant increase of clinical crown height [89, 90].

1.6 Aims and outline of this thesis

Given the high popularity of dental implants and the increasing frequency of associated complications that many clinicians coming from different backgrounds encounter in their everyday clinical practice, this thesis first sought to assess the attitudes towards peri-implantitis of periodontists who are practicing in different parts of the world. That way, we have the opportunity to inform clinicians in one country by placing their practice in the context of the practice of clinicians in other

countries. Furthermore, this thesis sought to expand the knowledge on the microbial etiology of peri-implant mucositis and peri-implantitis, and to explore the effectiveness of a non-surgical and a surgical therapeutic approach. Last but not least, this work sheds some light on infra-positioning of dental implants, an esthetic long-term complication which has received little attention. The chapters in this thesis represent a collection of articles, which are either published or in press to several scientific journals. Although the chapters are interlinked by the objective to expand on implant-related complications, they are generally self-contained and there is no need to read all of the chapters successively.

The second chapter of this thesis (**Chapter 2**) is a cross-sectional, questionnaire-based study which examines to which degree periodontists in different parts of the world share their peri-implantitis-related considerations. The objectives of the study were to compare the responses of periodontists in the U.S. vs. Europe concerning peri-implantitis-related risk factors, diagnostic criteria and treatment approaches. The differences in the responses between the two groups can inform future educational efforts.

In a cross-sectional study **(Chapter 3)**, biofilm samples were collected from peri-implant sites of patients who visited Academic Center for Dentistry Amsterdam (ACTA) for regular maintenance of their dental implants and 16S rDNA amplicon sequencing was used in order to describe the peri-implant microbiome. Furthermore, possible associations were explored of the microbial composition with several patient- and implant-related parameters, including implant disease status, dentition status, smoking habit, sex, implant location, implant system, time of functional loading, probing pocket depth, and presence of bleeding on probing.

The subsequent chapters of this thesis **(Chapter 4 and 5)** pertain to therapeutic approaches (non-surgical and surgical) applied for the treatment of peri-implantitis. **Chapter 4** is a randomized controlled clinical trial aimed to investigate the adjunctive effect of systemic

amoxicillin and metronidazole in conjunction with non-surgical treatment of peri-implantitis, in comparison to non-surgical treatment alone. A secondary objective was to identify the factors that affected the outcome of treatment. Subsequently, a non-inferiority clinical trial **(Chapter 5)**, aimed to evaluate the reconstructive potential of two different types of xenograft (Endobon® and Bio-Oss®) when applied in contained peri-implant intra-osseous defects. It was hypothesized that the defects treated with Endobon® will not exhibit an inferior outcome as compared to Bio-Oss® in terms of radiographic defect reduction around dental implants.

Chapter 6 encompasses a retrospective cross-sectional study with the objective to evaluate the longitudinal changes in the position of single-implant prostheses adjacent to teeth in the anterior maxilla of adult patients. The secondary aim was to associate the observed changes with patient or surgery related parameters including sex, age, country of origin, implant location, implant surgical protocol and implant temporization with provisional prosthesis.

Finally, in **Chapter 7**, the main findings are summarized and discussed in order to put them in a frame of reference to the complications of implant dentistry.

References

1.   Irish J. A 5,500-year-old artificial human tooth from egypt: A historical note. Int J Oral Maxillofac Implants. 2004;19:645-7.
2.   Misch C. Contemporary implant dentistry: Mosby Elsevier; 2008.
3.   Abraham C. A brief historical perspective on dental implants, their surface coatings and treatments. Open Dent J. 2014;8:50-5.
4.   Bobbio A. The first endosseous alloplastic implant in the history of man. Bull Hist Dent. 1972;20(1):1-6.
5.   Brånemark PI, Hansson BO, Adell R, Breine U, Lindström J, Hallén O, et al. Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. Scand J Plast Reconstr Surg Hand Surg Suppl. 1977;16:1-132.
6.   Brånemark PI, Svensson B, Van Steenberghe D. Ten-year survival rates of fixed prostheses on four or six implants ad modum brånemark in full edentulism. Clin Oral Implants Res. 1995;6(4):227-31.
7.   Elani HW, Starr JR, Da Silva JD, Gallucci GO. Trends in dental implant use in the u.S., 1999-2016, and projections to 2026. J Dent Res. 2018:22034518792567.
8.   Dreyer H, Grischke J, Tiede C, Eberhard J, Schweitzer A, Toikkanen S, et al. Epidemiology and risk factors of peri-implantitis: A systematic review. J Periodontal Res. 2018;53(5):657-81.
9.   Berglundh T, Armitage G, Araujo MG, Avila-Ortiz G, Blanco J, Camargo PM, et al. Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions. J Periodontol. 2018;89 Suppl 1:S313-s8.
10.   Derks J, Tomasi C. Peri-implant health and disease. A systematic review of current epidemiology. J Clin Periodontol. 2015;42 Suppl 16:S158-71.
11.   Lee CT, Huang YW, Zhu L, Weltman R. Prevalences of peri-implantitis and peri-implant mucositis: Systematic review and meta-analysis. J Dent. 2017;62:1-12.
12.   Kordbacheh Changi K, Finkelstein J, Papapanou PN. Peri-implantitis prevalence, incidence rate, and risk factors: A study of electronic health records at a u.S. Dental school. Clin Oral Implants Res. 2019;30(4):306-14.
13.   Lindhe J, Berglundh T, Ericsson I, Liljenberg B, Marinello C. Experimental breakdown of peri-implant and periodontal tissues. A study in the beagle dog. Clin Oral Implants Res. 1992;3(1):9-16.
14.   Schwarz F, Derks J, Monje A, Wang HL. Peri-implantitis. J Periodontol. 2018;89 Suppl 1:S267-s90.

15.   Lafaurie GI, Sabogal MA, Castillo DM, Rincon MV, Gomez LA, Lesmes YA, et al. Microbiome and microbial biofilm profiles of peri-implantitis: A systematic review. J Periodontol. 2017;88(10):1066-89.

16.   Fu J-H, Wang H-L. Breaking the wave of peri-implantitis. Periodontology 2000. 2020;84(1):145-60.

17.   Monje A, Catena A, Borgnakke WS. Association between diabetes mellitus/hyperglycaemia and peri-implant diseases: Systematic review and meta-analysis. J Clin Periodontol. 2017;44(6):636-48.

18.   Guobis Z, Pacauskiene I, Astramskaite I. General diseases influence on peri-implantitis development: A systematic review. J Oral Maxillofac Res. 2016;7(3):e5.

19.   Schliephake H. The role of systemic diseases and local conditions as risk factors. Periodontology 2000. 2022;88(1):36-51.

20.   Roccuzzo M, Grasso G, Dalmasso P. Keratinized mucosa around implants in partially edentulous posterior mandible: 10-year results of a prospective comparative study. Clin Oral Implants Res. 2016;27(4):491-6.

21.   De Bruyn H, Christiaens V, Doornewaard R, Jacobsson M, Cosyn J, Jacquet W, et al. Implant surface roughness and patient factors on long-term peri-implant bone loss. Periodontol 2000. 2017;73(1):218-27.

22.   Ramanauskaite A, Schwarz F, Sader R. Influence of width of keratinized tissue on the prevalence of peri-implant diseases: A systematic review and meta-analysis. Clin Oral Implants Res. 2022;33(S23):8-31.

23.   Monje A, Insua A, Wang HL. Understanding peri-implantitis as a plaque-associated and site-specific entity: On the local predisposing factors. J Clin Med. 2019;8(2).

24.   Heitz-Mayfield L, Heitz F, Lang N. Implant disease risk assessment idra–a tool for preventing peri-implant disease. Clin Oral Impl Res. 2020;31(4):397-403.

25.   Albrektsson T, Canullo L, Cochran D, De Bruyn H. "Peri-implantitis": A complication of a foreign body or a man-made "disease". Facts and fiction. Clin Implant Dent Relat Res. 2016;18(4):840-9.

26.   Wilson TG, Valderrama P, Burbano M, Blansett J, Levine R, Kessler H, et al. Foreign bodies associated with peri-implantitis human biopsies. J Periodontol. 2015;86(1):9-15.

27.   Chrcanovic BR, Kisch J, Albrektsson T, Wennerberg A. Bruxism and dental implant treatment complications: A retrospective comparative study of 98 bruxer patients and a matched group. Clin Oral Implants Res. 2017;28(7):e1-e9.

28.   Sadowsky SJ. Occlusal overload with dental implants: A review. Int J Implant Dent. 2019;5(1):29.

29.   Laine ML, Leonhardt A, Roos-Jansaker AM, Pena AS, van Winkelhoff AJ, Winkel EG, et al. Il-1rn gene polymorphism is associated with peri-implantitis. Clin Oral Implants Res. 2006;17(4):380-5.

30.   Pimentel SP, Shiota R, Cirano FR, Casarin RCV, Pecorari VGA, Casati MZ, et al. Occurrence of peri-implant diseases and risk indicators at the patient and implant levels: A multilevel cross-sectional study. J Periodontol. 2018;89(9):1091-100.

31.   Atieh MA, Pang JK, Lian K, Wong S, Tawse-Smith A, Ma S, et al. Predicting peri-implant disease: Chi-square automatic interaction detection (chaid) decision tree analysis of risk indicators. J Periodontol. 2019;90(8):834-46.

32.   Monje A, Caballé-Serrano J, Nart J, Peñarrocha D, Wang HL, Rakic M. Diagnostic accuracy of clinical parameters to monitor peri-implant conditions: A matched case-control study. J Periodontol. 2018;89(4):407-17.

33.   Renvert S, Persson GR, Pirih FQ, Camargo PM. Peri-implant health, peri-implant mucositis, and peri-implantitis: Case definitions and diagnostic considerations. J Periodontol. 2018;89 Suppl 1:S304-s12.

34.   Charalampakis G, Belibasakis GN. Microbiome of peri-implant infections: Lessons from conventional, molecular and metagenomic analyses. Virulence. 2015;6(3):183-7.

35.   Hajishengallis G, Chavakis T, Lambris JD. Current understanding of periodontal disease pathogenesis and targets for host-modulation therapy. Periodontol 2000. 2020;84(1):14-34.

36.   Loos BG, Van Dyke TE. The role of inflammation and genetics in periodontal disease. Periodontol 2000. 2020;83(1):26-39.

37.   Quirynen M, Vogels R, Peeters W, van Steenberghe D, Naert I, Haffajee A. Dynamics of initial subgingival colonization of 'pristine' peri-implant pockets. Clin Oral Implants Res. 2006;17(1):25-37.

38.   Furst MM, Salvi GE, Lang NP, Persson GR. Bacterial colonization immediately after installation on oral titanium implants. Clin Oral Implants Res. 2007;18(4):501-8.

39.   Apse P, Ellen RP, Overall CM, Zarb GA. Microbiota and crevicular fluid collagenase activity in the osseointegrated dental implant sulcus: A comparison of sites in edentulous and partially edentulous patients. J Periodontal Res. 1989;24(2):96-105.

40.   Pontoriero R, Tonelli MP, Carnevale G, Mombelli A, Nyman SR, Lang NP. Experimentally induced peri-implant mucositis. A clinical study in humans. Clin Oral Implants Res. 1994;5(4):254-9.

41.   Schwarz F, Becker J, Civale S, Hazar D, Iglhaut T, Iglhaut G. Onset, progression and resolution of experimental peri-implant mucositis at different abutment surfaces: A randomized controlled two-centre study. J Clin Periodontol. 2018;45(4):471-83.
42.   Mombelli A, Decaillet F. The characteristics of biofilms in peri-implant disease. J Clin Periodontol. 2011;38 Suppl 11:203-13.
43.   Kroger A, Hulsmann C, Fickl S, Spinell T, Huttig F, Kaufmann F, et al. The severity of human peri-implantitis lesions correlates with the level of submucosal microbial dysbiosis. J Clin Periodontol. 2018.
44.   Dabdoub SM, Tsigarida AA, Kumar PS. Patient-specific analysis of periodontal and peri-implant microbiomes. J Dent Res. 2013;92(12 Suppl):168s-75s.
45.   Becker ST, Beck-Broichsitter BE, Graetz C, Dörfer CE, Wiltfang J, Häsler R. Peri-implantitis versus periodontitis: Functional differences indicated by transcriptome profiling. Clin Implant Dent Relat Res. 2014;16(3):401-11.
46.   Shiba T, Watanabe T, Kachi H, Koyanagi T, Maruyama N, Murase K, et al. Distinct interacting core taxa in co-occurrence networks enable discrimination of polymicrobial oral diseases with similar symptoms. Sci Rep. 2016;6:30997.
47.   Daubert DM, Weinstein BF. Biofilm as a risk factor in implant treatment. Periodontol 2000. 2019;81(1):29-40.
48.   Renvert S, Polyzois I. Treatment of pathologic peri-implant pockets. Periodontol 2000. 2018;76(1):180-90.
49.   Suvan J, Leira Y, Moreno Sancho FM, Graziani F, Derks J, Tomasi C. Subgingival instrumentation for treatment of periodontitis. A systematic review. J Clin Periodontol. 2020;47(S22):155-75.
50.   Berglundh T, Zitzmann NU, Donati M. Are peri-implantitis lesions different from periodontitis lesions? J Clin Periodontol. 2011;38(s11):188-202.
51.   Ivanovski S, Lee R. Comparison of peri-implant and periodontal marginal soft tissues in health and disease. Periodontol 2000. 2018;76(1):116-30.
52.   de Avila ED, van Oirschot BA, van den Beucken JJJP. Biomaterial-based possibilities for managing peri-implantitis. J Periodontal Res. 2020;55(2):165-73.
53.   Garaicoa-Pazmino C, Sinjab K, Wang H-L. Current protocols for the treatment of peri-implantitis. Curr Oral Health Rep. 2019;6(3):209-17.
54.   Schwarz F, Jepsen S, Obreja K, Galarraga-Vinueza ME, Ramanauskaite A. Surgical therapy of peri-implantitis. Periodontology 2000. 2022;88(1):145-81.

55.   Renvert S, Hirooka H, Polyzois I, Kelekis-Cholakis A, Wang HL. Diagnosis and non-surgical treatment of peri-implant diseases and maintenance care of patients with dental implants - consensus report of working group 3. Int Dent J. 2019;69 Suppl 2:12-7.

56.   Menezes KM, Fernandes-Costa AN, Silva-Neto RD, Calderon PS, Gurgel BC. Efficacy of 0.12% chlorhexidine gluconate for non-surgical treatment of peri-implant mucositis. J Periodontol. 2016;87(11):1305-13.

57.   Mills MP, Rosen PS, Chambrone L, Greenwell H, Kao RT, Klokkevold PR, et al. American academy of periodontology best evidence consensus statement on the efficacy of laser therapy used alone or as an adjunct to non-surgical and surgical treatment of periodontitis and peri-implant diseases. J Periodontol. 2018;89(7):737-42.

58.   Chambrone L, Wang HL, Romanos GE. Antimicrobial photodynamic therapy for the treatment of periodontitis and peri-implantitis: An american academy of periodontology best evidence review. J Periodontol. 2018;89(7):783-803.

59.   Berglundh T, Krok L, Liljenberg B, Westfelt E, Serino G, Lindhe J. The use of metronidazole and amoxicillin in the treatment of advanced periodontal disease. A prospective, controlled clinical trial. J Clin Periodontol. 1998;25(5):354-62.

60.   Teughels W, Feres M. Adjunctive effect of systemic antimicrobials in periodontitis therapy: A systematic review and meta-analysis. J Clin Periodontol. 2020;47 Suppl 22:257-81.

61.   Heitz-Mayfield L, Lang N. Antimicrobial treatment of peri-implant diseases. Int J Oral Maxillofac Implants. 2004;19 Suppl:128-39.

62.   Mombelli A, Lang NP. Antimicrobial treatment of peri-implant infections. Clin Oral Implants Res. 1992;3(4):162-8.

63.   Stein JM, Hammacher C, Michael SS. Combination of ultrasonic decontamination, soft tissue curettage, and submucosal air polishing with povidone-iodine application for non-surgical therapy of peri-implantitis: 12 month clinical outcomes. J Periodontol. 2018;89:139-47.

64.   Liñares A, Pico A, Blanco C, Blanco J. Adjunctive systemic metronidazole to nonsurgical therapy of peri-implantitis with intrabony defects: A retrospective case series study. Int J Oral Maxillofac Implants. 2019;34(5):1237-45.

65.   Nart J, Pons R, Valles C. Non-surgical therapeutic outcomes of peri-implantitis: 12-month results. 2020;24(2):675-82.

66.   Shibli JA, Ferrari DS. Microbiological and clinical effects of adjunctive systemic metronidazole and amoxicillin in the non-surgical

treatment of peri-implantitis: 1 year follow-up. Braz Oral Res. 2019;33(suppl 1):e080.

67.   De Waal YCM, Vangsted TE, Van Winkelhoff AJ. Systemic antibiotic therapy as an adjunct to non-surgical peri-implantitis treatment: A single-blind rct. J Clin Periodontol. 2021;48(7):996-1006.

68.   Persson GR, Samuelsson E, Lindahl C, Renvert S. Mechanical non-surgical treatment of peri-implantitis: A single-blinded randomized longitudinal clinical study. Ii. Microbiological results. J Clin Periodontol. 2010;37(6):563-73.

69.   Berglundh T, Wennstrom JL, Lindhe J. Long-term outcome of surgical treatment of peri-implantitis. A 2-11-year retrospective study. Clin Oral Implants Res. 2018;29(4):404-10.

70.   Sarmiento HL, Norton M, Korostoff J, Ko KI, Fiorellini JP. Surgical alternatives for treating peri-implantitis. Int J Periodontics Restorative Dent. 2018;38(5):665-71.

71.   Schwarz F, Sahm N, Bieling K, Becker J. Surgical regenerative treatment of peri-implantitis lesions using a nanocrystalline hydroxyapatite or a natural bone mineral in combination with a collagen membrane: A four-year clinical follow-up report. J Clin Periodontol. 2009;36(9):807-14.

72.   Schwarz F, Sahm N, Schwarz K, Becker J. Impact of defect configuration on the clinical outcome following surgical regenerative therapy of peri-implantitis. J Clin Periodontol. 2010;37(5):449-55.

73.   Wiltfang J, Zernial O, Behrens E, Schlegel A, Warnke PH, Becker ST. Regenerative treatment of peri-implantitis bone defects with a combination of autologous bone and a demineralized xenogenic bone graft: A series of 36 defects. Clin Implant Dent Relat Res. 2012;14(3):421-7.

74.   Roos-Jansaker AM, Persson GR, Lindahl C, Renvert S. Surgical treatment of peri-implantitis using a bone substitute with or without a resorbable membrane: A 5-year follow-up. J Clin Periodontol. 2014;41(11):1108-14.

75.   Jepsen K, Jepsen S, Laine ML, Anssari Moin D, Pilloni A, Zeza B, et al. Reconstruction of peri-implant osseous defects: A multicenter randomized trial. J Dent Res. 2016;95(1):58-66.

76.   Renvert S, Roos-Jansaker AM, Persson GR. Surgical treatment of peri-implantitis lesions with or without the use of a bone substitute-a randomized clinical trial. J Clin Periodontol. 2018;45(10):1266-74.

77.   Khoshkam V, Suarez-Lopez Del Amo F, Monje A, Lin GH, Chan HL, Wang HL. Long-term radiographic and clinical outcomes of regenerative approach for treating peri-implantitis: A systematic review and meta-analysis. Int J Oral Maxillofac Implants. 2016;31(6):1303-10.

78.   Salvi G, Brägger U. Mechanical and technical risks in implant therapy. Int J Oral Maxillofac Implants. 2009;24 Suppl:69-85.

79.   Heitz-Mayfield LJ, Needleman I, Salvi GE, Pjetursson BE. Consensus statements and clinical recommendations for prevention and management of biologic and technical implant complications. Int J Oral Maxillofac Implants. 2014;29 Suppl:346-50.

80.   Chang M, Odman P, Wennström JL, Andersson B. Esthetic outcome of implant-supported single-tooth replacements assessed by the patient and by prosthodontists. Int J Prosthodont. 1999;12(4):335-41.

81.   Zitzmann NU, Arnold D, Ball J, Brusco D, Triaca A, Verna C. Treatment strategies for infraoccluded dental implants. J Prosthet Dent. 2015;113(3):169-74.

82.   Iseri H, Solow B. Continued eruption of maxillary incisors and first molars in girls from 9 to 25 years, studied by the implant method. Eur J Orthod. 1996;18(3):245-56.

83.   Thilander B, Odman J, Jemt T. Single implants in the upper incisor region and their relationship to the adjacent teeth. An 8-year follow-up study. Clin Oral Implants Res. 1999;10(5):346-55.

84.   Thilander B, Odman J, Grondahl K, Friberg B. Osseointegrated implants in adolescents. An alternative in replacing missing teeth? Eur J Orthod. 1994;16(2):84-95.

85.   Thilander B, Odman J, Lekholm U. Orthodontic aspects of the use of oral implants in adolescents: A 10-year follow-up study. Eur J Orthod. 2001;23(6):715-31.

86.   Oesterle LJ, Cronin RJ, Jr. Adult growth, aging, and the single-tooth implant. Int J Oral Maxillofac Implants. 2000;15(2):252-60.

87.   Jemt T, Ahlberg G, Henriksson K, Bondevik O. Tooth movements adjacent to single-implant restorations after more than 15 years of follow-up. Int J Prosthodont. 2007;20(6):626-32.

88.   Bernard JP, Schatz JP, Christou P, Belser U, Kiliaridis S. Long-term vertical changes of the anterior maxillary teeth adjacent to single implants in young and mature adults. A retrospective study. J Clin Periodontol. 2004;31(11):1024-8.

89.   Jemt T, Ahlberg G, Henriksson K, Bondevik O. Changes of anterior clinical crown height in patients provided with single-implant restorations after more than 15 years of follow-up. Int J Prosthodont. 2006;19(5):455-61.

90.   Chang M, Wennstrom JL. Longitudinal changes in tooth/single-implant relationship and bone topography: An 8-year retrospective analysis. Clin Implant Dent Relat Res. 2012;14(3):388-94.

91.   Andersson B, Bergenblock S, Furst B, Jemt T. Long-term function of single-implant restorations: A 17- to 19-year follow-up study on implant infraposition related to the shape of the face and patients' satisfaction. Clin Implant Dent Relat Res. 2013;15(4):471-80.

92.   Jamilian A, Perillo L, Rosa M. Missing upper incisors: A retrospective study of orthodontic space closure versus implant. Prog Orthod. 2015;16:2.

93.   Jemt T. Single implants in the anterior maxilla after 15 years of follow-up: Comparison with central implants in the edentulous maxilla. Int J Prosthodont. 2008;21(5):400-8.

CHAPTER 2

Risk factors, diagnosis and treatment of peri-implantitis; a cross-cultural comparison of U.S. and European periodontists' considerations

ABSTRACT

Background: Peri-implantitis (PI) is a growing concern in the dental community worldwide. The study aimed to compare U.S. vs. European periodontists' considerations of risk factors, diagnostic criteria, and management of PI.

Materials and Methods: 393 periodontists from the U.S. and 100 periodontists from Europe (Germany, Greece, Netherlands) responded to anonymous surveys electronically or by mail.

Results: Compared to U.S. periodontists, European respondents were younger, more likely to be female and placed fewer implants per month (9.12 vs. 13.90;p=0.003). Poor oral hygiene, history of periodontitis, and smoking were considered as very important risk factors by both groups (rated >4 on 5-point scale). European periodontists rated poor oral hygiene (4.64 vs. 4.45;p=0.005) and history of periodontitis (4.36 vs. 4.10;p=0.006) as more important and implant surface (2.91 vs. 3.18;p=0.023), occlusion (2.80 vs. 3.75;p<0.001) and presence of keratinized tissue (3.27 vs. 3.77;p<0.001) as less important than did U.S. periodontists. Both groups rated clinical probing, radiographic bone loss, and presence of bleeding and suppuration as rather important diagnostic criteria. They rated implant exposure/mucosal recession as relatively less important with U.S. periodontists giving higher importance ratings than European periodontists (3.99 vs. 3.54;p=0.001). Both groups nearly always used patient education, plaque control and mechanical debridement when treating PI. U.S. periodontists were more likely to use antibiotics (3.88 vs. 3.07;p<0.001), lasers (2.11 vs. 1.68;p=0.005), allograft (3.39 vs. 2.14;p<0.001) and regenerative approaches (3.57 vs. 2.56;p<0.001), but less likely to use resective surgery (3.09 vs. 3.53;p<0.001) than European periodontists.

Conclusions: U.S. and European periodontists' considerations concerning risk factors, diagnosis and management of PI were evidence-based. Identified differences between the two groups can inform future educational efforts.

2.1 Introduction

With implant therapy being a significant part of dental care, peri-implantitis (PI) is becoming a growing problem encountered by dental health professionals worldwide [1]. PI is characterized by the presence of inflammation in the peri-implant soft tissues and progressive loss of supporting bone [2]. Although it is difficult to estimate the prevalence of PI and possible regional differences, studies with similar PI case definition and follow-up time showed approximately 10% higher prevalence in the U.S. as compared to Europe (26% vs 16% at patient level) [3, 4].

Despite potential differences in PI prevalence, consensus exists that biofilm plays an important role in the etiology of PI, as an initial trigger for inflammatory reactions [5]. Furthermore, systemic, local, genetic, behavioral and iatrogenic factors have been accepted as being associated with the onset and progression of this disease [2]. Diabetes mellitus is the systemic risk factor most extensively studied in relation to PI [6]. Other systemic diseases such as osteoporosis and cardiovascular diseases, as well the treatment with oral bisphosphonates have been reported as possible risk factors; however, the evidence is weak [7]. Local factors including the presence of dental plaque, lack of keratinized tissue, and implant surface roughness have also been associated with greater risk for peri-implant pathologies [2, 8, 9]. Research also showed the relevance of iatrogenic factors such as improper implant position, presence of residual cement, and poor prosthesis design that limits oral hygiene accessibility [10, 11]. In addition, occlusal overload has been associated with mechanical implant complications [12] and peri-implant bone loss [13]. However, a causal relationship as well as specific strain thresholds have not been established yet [13]. While genetic traits may influence inflammatory responses and thus may be a risk indicator, the relationship between PI and genetic predisposition remains unclear [14]. Patient-related factors such as smoking [15], history of periodontitis [15] and lack of maintenance care [16] have been associated with higher prevalence and severity of PI.

The diagnosis of PI is based on clinical parameters such as probing depth, bleeding and suppuration on probing, and on radiographic evidence of bone loss following initial bone remodeling [17]. Monitoring the changes in the clinical and radiographic parameters following the

completion of the implant-supported prosthesis is important for the diagnosis of PI [1]. In the absence of previous clinical and radiographic evaluations, the diagnosis is based on the presence of a peri-implant pocket ≥6 mm accompanied by bleeding, purulent exudate and bone loss ≥3 mm from the implant platform [18].

Although various treatment strategies for PI have been suggested, there is no consensus as to which one is the most effective intervention [19]. The non-surgical treatment is always a first option which could lead to improvements in bleeding tendency and in some cases to pocket reduction of ≤1 mm [20]. In more severe cases, non-surgical treatment alone is insufficient to arrest the disease and to eliminate bacteria from the rough surfaces of implants [20, 21]. The use of local antiseptics [22], systemic antibiotics [23], lasers [24] and photodynamic therapy [25] have been proposed as adjunctive measures to mechanical debridement. However, existing evidence has only shown minimal additional benefits of these adjunctive measures for improving clinical parameters [22-25]. Surgical therapy has proven to be more effective, resulting in reduction of probing depths and bleeding on probing and in radiographic evidence of defect fill [26]. Open flap debridement, resective surgery with or without implantoplasty and reconstructive approaches including the use of various bone grafts with or without the use of barrier membranes were some of the surgical approaches reported in the literature [27].

Given the high prevalence of PI worldwide, one question of interest is to which degree periodontists in different parts of the world share their PI-related considerations. The objectives of this study were therefore to compare the responses of periodontists in the U.S. vs. Europe concerning PI-related risk factors, diagnostic criteria and treatment approaches.

2.2 Materials and Methods

Study design and questionnaire
The research in the United States was determined to be exempt from Institutional Review Board (IRB) oversight by the Health Sciences and Behavioral Sciences IRB at the University of Michigan, Ann Arbor, MI, USA (#HUM00102795). An amendment (Ame00080866) to conduct the research in the Netherlands, Greece and Germany was approved on June 29, 2018 (# HUM00129701). The study followed the Declaration of Helsinki Ethical Principles. No written consent from the participants

was required because responding to this anonymous survey was considered as giving implicit consent.

A survey was designed based on a review of the literature and on previously validated questionnaires [28, 29]. The survey consisted of five parts. Part 1 addressed the respondents' background and educational characteristics. Part 2 asked how much eight factors could put a patient at risk for PI. Part 3 inquired how important five parameters were for diagnosing PI. Part 4 asked how frequently the respondents used 15 different treatment strategies in their professional practice. The final part consisted of six questions concerning the respondents' PI-related attitudes. The questions in Part 2 to 5 were answered on 5-point rating scales. All survey questions are provided in a supplementary document. The respondents answered the surveys anonymously either online or as a paper-pencil survey that they returned by regular mail to the research team in a provided stamped return envelope. The data were collected between June 2017 and December 2018.

Study population
A recruitment email was sent to all 4,588 active members of the American Academy of Periodontology explaining the study and providing a web link to an anonymous survey. Follow-up reminder emails were sent two weeks and two months later. The recipients could use the link to the survey only once.

The research material (invitation letter and questionnaire) was translated into Dutch, German and Greek, following the process of forward and backward translation [30]. Dental specialists who were native speakers of these three languages translated the materials into their native tongue. These materials were then back translated into English, compared with the original English version, and further adjustments were made as necessary.

The Greek survey was mailed to all 224 members of the Hellenic Society of Periodontology. In the Netherlands, a recruitment email with a link to an anonymous survey was sent to all 86 registered periodontists of the Dutch Society of Periodontology. A follow-up email was sent four months later. In Germany, a recruitment email with a link to an online survey was sent to the 311 members of the German

Society of Periodontology. Five months later, a survey was mailed to 107 periodontists for whom postal addresses were available.

Statistical analysis
The statistical analyses were performed with a commercial software package (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY, USA: IBM Corp). Descriptive statistics such as frequency distributions, means and standard deviations were calculated to provide an overview of the responses of the European vs. U.S. periodontists. Four factor analyses (Extraction Method: Principal Component Analysis; Rotation Method: Varimax Rotation with Kaiser Normalization) were computed with the four sets of questions (risk factors/indicators, diagnostic criteria, treatment modalities, Pi-related attitudes). Cronbach alpha coefficients were calculated to determine if the sets of items loading on a specific factor had sufficient reliability to allow creating an index. Cronbach alpha values above 0.7 were considered acceptable inter-item consistencies [31]. Indices were computed by averaging the responses to the items that loaded on each respective factor. Comparisons between the two groups were performed using independent sample t-tests for responses measured on rating scales and Chi-square tests for categorical variables. The level of significance was set at 5%.

2.3 Results

Response rates and participant background characteristics
 Of the 4,588 U.S. and 621 European periodontists who were invited to participate in this study, 393 (8.6%) and 100 (16.1%) respectively completed the questionnaire. The breakdown of the European response rates is as follows; 37.21% (n=32) in the Netherlands, 8.04% (n=25) in Germany, and 19.20% (n=43) in Greece. In order to assess if the sample sizes were large enough to compare the mean responses of U.S. vs European respondents, an a priori power analysis with the G3.1.3. Power Analysis Program (http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3/) was performed. Assuming a two-sided hypothesis, a medium-to-small effect size of 0.35 on the 5-point scales, a statistical significance of 0.05, a power of 0.80 and a ratio of 4:1, we would require 81 European and 323 U.S. respondents. Our actual sample sizes exceeded this requirement.

The demographic, educational and practice management characteristics of the U.S. vs. European respondents are provided in Table 1. The European sample had more female respondents (31% vs. 19.2%; p= 0.009) and was on average younger than the U.S. sample (46.34 vs. 51.49 years; p<0.001). In both groups, approximately 77% of the respondents reported working in private practice. The European periodontists worked on average seven hours more (37.72 vs 30.38 hours; p<0.001) and treated nine patients more (43.90 vs 34.63 patients; p=0.009) per week compared to U.S. periodontists. However, U.S. the periodontists performed more implant surgeries per month (13.90 vs 9.12; p=0.003) than European periodontists. Both groups reported seeing on average between three and four PI cases per month.

The European periodontists graduated from dental schools and graduate programs more recently than the U.S. periodontists (dental school graduation year: 1995.34 vs. 1991.42; p = 0.001 / graduate program graduation year: 2002.82 vs. 1995.99; p<0.001). However, the two groups did not differ in the length of the residency program nor in the percentage of time spent on implant surgeries during their residencies. The European periodontists reported being better educated during their residency about PI-related risk factors (5-point scale with 5= best education: 3.82 vs. 3.02; p<0.001), diagnostic criteria (3.85 vs. 2.99; p<0.001), and treatment approaches (3.10 vs. 2.56; p<0.001) than the periodontists in the U.S. Less than half of the respondents in both groups had treated patients with PI during their residency.

Table 1: Overview of respondents' background characteristics and professional activities. Data are presented as percentages (%) or mean ± SD.

Background characteristics	Periodontists in U.S. (n = 393)	Periodontists in EU (n = 100)	P
Gender: - male - female	81% 19%	69% 31%	0.009

Age	51.49 ± 13.671	46.34 ± 10.038	<0.001
Dental School graduation year	1991.42 ± 13.823	1995.34 ± 9.695	0.001
Graduate program graduation year	1995.99 ± 14.003	2002.82 ± 8.626	<0.001
Length of residency in years	2.92 ± 2.411	2.97 ± 1.124	0.841
Percentage of residency time spent on implant surgeries	20.90% ± 21.769	19.86% ± 18.365	0.652
Did you treat patients with PI during your residency?	Yes: 39.7%	Yes: 49%	0.092
How well were you educated about : - risk factors of PI - how to diagnose PI - treating PI	3.02 ± 1.676 2.99 ± 1.697 2.56 ± 1.563	3.82 ± 1.290 3.85 ± 1.351 3.10 ± 1.307	<0.001 <0.001 0.001
Percentage of current time at work spent: - in private practice - in a hospital setting - as a faculty member - in another setting	76.85% ± 38.129 2.04% ± 11.133 13.0% ± 28.777 3.77% ± 18.206	76.85% ± 38.129 2.04% ± 11.133 13.0% ± 28.777 3.77% ± 18.206	0.870 0.191 0.811 0.625
Number of hours per week spent at work	30.38 ± 13.288	37.72 ± 10.724	<0.001
Number of patients treated per week	34.63 ± 30.072	43.90 ± 27.356	0.009
Number of implant surgeries per month	13.90 ± 13.323	9.12 ± 13.709	0.003
Number of PI cases per month	2.71 ± 3.498	3.45 ± 4.936	0.100

Risk factors/indicators for PI

The periodontists' responses concerning the risk factors/indicators for PI are presented in Table 2. A factor analysis showed that the answers to the eight items loaded on two factors which can be described as a "patient-related" factor and an "implant-related" factor, respectively. Both groups evaluated poor oral hygiene, history of periodontitis and smoking as highly important "patient-related" risk factors and diabetes and genetic predisposition as relatively less important. However, European periodontists considered poor oral hygiene (5-point answer scale: 4.64 vs. 4.45; p = 0.005), history of periodontitis (4.36 vs. 4.10; p = 0.006) and genetic predisposition (3.77 vs. 3.53; p = 0.021) as more important than did U.S. respondents.

The "implant-related" risk indicators, namely implant surface, occlusion and presence of keratinized tissue, were overall rated as less important. However, U.S. periodontists evaluated them as more important than did European periodontists (3.18 vs. 2.91; p = 0.023, 3.75 vs. 2.80; p<0.001; 3.77 vs. 3.27; p<0.001).

In response to an open-ended question, 153 participants (31%) provided additional comments concerning risk factors. They frequently named the presence of excess cement, improper restoration, and improper implant position. Less frequently reported factors included systemic diseases, poor surgical skills, type and quality of bone, and lack of patient compliance with maintenance.

Table 2: U.S. and European respondents' considerations concerning risk factors/indicators for peri-implantitis.

Patient-related risk factors	Who	1[1] (%)	2 (%)	3 (%)	4 (%)	5 (%)	Mean ± SD	P
Poor oral hygiene	U.S.	0	3.4	10.9	23.6	62.2	4.45 ± 0.818	0.005
	EU	0	0	4.1	27.6	68.4	4.64 ± 0.561	
History of periodontitis	U.S.	0.5	4.9	17.8	38.0	38.8	4.10 ± 0.895	0.006
	EU	0	1.0	11.1	38.4	49.5	4.36 ± 0.721	
Smoking	U.S.	0	1.6	14.5	31.1	52.8	4.35 ± 0.783	0.554
	EU	0	1.0	12.1	32.3	54.5	4.40 ± 0.741	
Diabetes mellitus	U.S.	0.3	7.0	24.0	40.2	28.5	3.90 ± 0.906	0.117
	EU	0	9.1	28.3	40.4	22.2	3.76 ± 0.905	
Genetic predisposition	U.S.	3.7	12.6	29.6	35.1	19.1	3.53 ± 1.051	0.021
	EU	0	6.1	31.3	42.4	20.2	3.77 ± 0.843	
Patient related risk factor Index[2] (alpha = 0.66)	*U.S.*	*Mean = 4.07*		*SD = 0.587*			*P = 0.051*	
	EU	*Mean = 4.18*		*SD = 0.458*				

38

Implant-related risk indicators[3]	Who	1[1] (%)	2 (%)	3 (%)	4 (%)	5 (%)	Mean ± SD	P
Implant surface	U.S.	6.0	20.1	36.5	24.7	12.8	3.18 ± 1.081	0.023
	EU	11.1	17.2	45.5	22.2	4.0	2.91 ± 1.001	
Occlusion	U.S.	2.4	13.1	22.6	31.0	31.0	3.75 ± 1.102	<0.001
	EU	13.1	22.2	42.4	16.2	6.1	2.80 ± 1.059	
Presence of keratinized tissue	U.S.	2.3	7.8	24.4	41.5	24.1	3.77 ± 0.978	<0.001
	EU	7.1	17.2	32.3	28.3	15.2	3.27 ± 1.132	

Legend:
1. The answers to the question "*How much do the following factors put a patient at risk for developing peri-implantitis?*" ranged from "1" = "not at all" to "5" = "very much".
2. This index was computed by averaging the responses loading on the respective factor in the factor analysis of the responses concerning risk factors.
3. No index was computed for the knowledge responses concerning the implant related risk indicators because Cronbach alpha is 0.426.

PI diagnostic criteria

Table 3 provides an overview of the responses related to diagnostic criteria for PI. Both groups rated radiographic bone loss, clinical probing, suppuration and bleeding as the most important diagnostic factors. However, European respondents considered clinical probing as more important than U.S. respondents did (5-point scale with 5 = very important: 4.64 vs. 4.04; p<0.001). While exposure of implant surface/

recession of mucosal margin was considered less important by both groups, U.S. periodontists rated this factor as more important than European periodontists did (3.99 vs. 3.54; p = 0.001).

Table 3: U.S. vs. European respondents' considerations concerning diagnostic criteria for peri-implantitis.

Diagnostic criteria[1]	Who	1[2] (%)	2 (%)	3 (%)	4 (%)	5 (%)	Mean ± SD	P
Clinical probing	U.S.	1.8	7.8	19.8	25.8	44.8	4.04 ± 1.060	<0.001
	EU	0.0	0.0	6.1	24.2	69.7	4.64 ± 0.597	
Radiographic bone loss	U.S.	0.0	1.6	2.1	17.1	79.3	4.74 ± 0.572	0.551
	EU	0.0	0.0	1.0	20.2	78.8	4.78 ± 0.442	
Presence of bleeding	U.S.	1.0	4.9	18.9	26.4	48.7	4.17 ± 0.970	0.249
	EU	0.0	5.1	15.2	25.3	54.5	4.29 ± 0.906	
Presence of suppuration	U.S.	0.3	0.8	7.3	17.9	73.8	4.64 ± 0.674	0.172
	EU	0.0	0.0	3.0	21.2	75.8	4.73 ± 0.511	
Implant exposure and gum recession	U.S.	1.6	6.8	20.3	33.6	37.8	3.99 ± 0.997	0.001
	EU	7.1	11.1	30.3	24.2	27.3	3.54 ± 1.206	

Legend:

1. No index was computed for the diagnostic criteria responses because Cronbach alpha is 0.453.

2. The answers to the question "*How important are the following criteria to you when you make a diagnosis of peri-implantitis?*" ranged from "1" = "not at all" to "5" = "very much".

Management of PI

Table 4 summarizes the frequency of use of 15 different treatment modalities for the management of PI. Both groups reported using oral hygiene approaches, namely patient education and plaque control, nearly always. However, European periodontists used patient education even more frequently (4.95 vs. 4.86; p = 0.005) than did U.S. periodontists.

A comparison of the mean index of the responses concerning the frequency of use of three non-surgical treatment approaches and five regenerative approaches showed that U.S. periodontists utilized these techniques more frequently (3.05 vs. 2.27; p<0.001) than European periodontists. For example, mechanical debridement (4.77 vs. 4.17; p<0.001), local/systemic antibiotic therapy (3.88 vs. 3.07; p<0.001) and regeneration (3.57 vs. 2.56; p<0.001) were on average more frequently used in the U.S.

A group of five items that loaded on a third factor did not have sufficiently high inter-item consistency to justify creating an index.[31] While resective surgery (3.53 vs. 3.09; p<0.001) was used more frequently in Europe than in the U.S., the opposite held true for laser systems which were used more frequently in the U.S. than in Europe (2.11 vs. 1.68; p = 0.005).

Table 4: Percentage of use of different treatment strategies for peri-implantitis by respondent group.

Oral hygiene related treatment	Who	1[1] (%)	2 (%)	3 (%)	4 (%)	5 (%)	Mean ± SD	P
Patient education about oral hygiene	U.S.	0.0	1.3	1.8	7.0	89.9	4.86 ± 0.488	0.005
	EU	0.0	0.0	0.0	5.1	94.9	4.95 ± 0.220	
Plaque control	U.S.	0.0	1.0	1.6	7.0	90.4	4.87 ± 0.456	0.080
	EU	0.0	0.0	0.0	7.1	92.9	4.93 ± 0.258	
Oral hygiene treatment Index[2] (alpha = 0.97)	*U.S.*	*Mean = 4.86*			*SD = 0.467*			
	EU	*Mean = 4.93*			*SD = 0.228*		*P = 0.020*	
Non-surgical and Regenerative treatment	Who	1[1] (%)	2 (%)	3 (%)	4 (%)	5 (%)	Mean ± SD	P
Mechanical debridement	U.S.	0.3	0.8	3.4	13.2	82.4	4.77 ± 0.570	<0.001
	EU	7.4	4.3	12.8	14.9	60.6	4.17 ± 1.250	
Antiseptic cleansing	U.S.	2.4	5.0	13.9	22.8	56.0	4.25 ± 1.024	0.077
	EU	5.1	5.1	19.2	22.2	48.5	4.04 ± 1.160	
Local/systemic antibiotic therapy	U.S.	3.1	8.3	21.9	31.0	35.7	3.88 ± 1.085	<0.001
	EU	7.1	26.3	27.3	31.3	8.1	3.07 ± 1.090	

	Who	1[1] (%)	2 (%)	3 (%)	4 (%)	5 (%)	Mean ± SD	P
Regeneration	U.S.	4.0	10.1	30.9	35.2	19.7	3.57 ± 1.042	<0.001
	EU	15.3	33.3	33.3	16.7	1.4	2.56 ± 0.991	
Autograft	U.S.	32.3	24.7	19.9	16.3	6.7	2.40 ± 1.274	0.026
	EU	38.9	26.3	23.2	8.4	3.2	2.11 ± 1.115	
Allograft	U.S.	9.6	10.7	26.0	39.1	14.8	3.39 ± 1.150	<0.001
	EU	38.9	21.1	28.4	10.5	1.1	2.14 ± 1.088	
Xenograft	U.S.	31.7	15.9	19.8	24.4	8.2	2.61 ± 1.363	0.048
	EU	29.0	25.8	29.0	14.0	2.2	2.34 ± 1.108	
GTR with membrane	U.S.	7.7	11.6	25.1	40.7	14.8	3.43 ± 1.113	<0.001
	EU	27.7	21.3	28.7	19.1	3.2	2.49 ± 1.180	
Non-surgical and Regenerative treatment Index[2] (alpha = 0.79)	*U.S.*	*Mean = 3.05 SD = 0.848*						
	EU	*Mean = 2.27 SD = 0.824*					***P < 0.001***	
Single items	**Who**	**1[1] (%)**	**2 (%)**	**3 (%)**	**4 (%)**	**5 (%)**	**Mean ± SD**	**P**
Alloplast	U.S.	64.5	14.5	11.0	7.0	2.9	1.69 ± 1.098	0.699
	EU	56.3	21.1	15.5	5.6	1.4	1.75 ± 1.010	

Resective surgery	**U.S.**	10.6	16.4	34.1	31.2	7.7	3.09 ± 1.096	<0.001
	EU	5.2	13.5	19.8	45.8	15.6	3.53 ± 1.076	
Implantoplasty	**U.S.**	19.6	26.9	25.3	21.0	7.3	2.69 ± 1.210	0.693
	EU	25.6	20.0	17.8	26.7	10.0	2.76 ± 1.360	
Laser systems	**U.S.**	55.9	11.6	9.6	11.6	11.3	2.11 ± 1.459	0.005
	EU	72.0	8.6	7.5	3.2	8.6	1.68 ± 1.270	
Photodynamic therapy	**U.S.**	81.1	9.5	6.0	3.2	0.3	1.32 ± 0.751	0.184
	EU	75.0	13.0	5.4	3.3	3.3	1.47 ± 0.977	

Legend:

1   The answers to the question "*How often do you use the following treatment strategies when you treat a patient with peri-implantitis?*" ranged from "1" = "never" to 5 = "always".

2   Indices were computed by averaging the responses loading on the respective factor for which an index was created.

PI-related attitudes

Table 5 provides an overview of the two groups' PI-related attitudes. The factor analysis of the responses to the seven attitudinal items showed that they loaded on two factors. The first factor captures the respondents' thoughts concerning the seriousness of the problem of PI. The European periodontists considered PI an even more serious problem than did the U.S. periodontists (4.75 vs. 4.64; p = 0.042). However, the majority of periodontists in both groups agreed/agreed strongly that PI was a serious problem (U.S.: 90% vs. Europe: 94.3%)

and that it will become a more serious problem in the future (U.S.: 95% vs. Europe: 100%).

Four items loaded on a second factor that can be described as the need for better PI-related education. The majority in both groups agreed/agreed strongly that there was a great need for a standardized treatment protocol (U.S.: 87.3% vs. Europe: 96.6%), with the European periodontists agreeing on average even more strongly than did the U.S. periodontists (4.66 vs. 4.43; p = 0.002). Nearly all respondents in both groups agreed/agreed strongly that general dentists need to be better trained to diagnose PI, to refer PI cases, and to offer maintenance care for dental implants. In addition, the European periodontists agreed more strongly with the statement "I would like to attend continuing education courses about the treatment of PI" compared to the U.S. periodontists (4.47 vs. 4.17; p = 0.004).

Table 5: U.S. vs. European periodontists' attitudes related to peri-implantitis.

Attitudes towards PI	Who	1[1] (%)	2 (%)	3 (%)	4 (%)	5 (%)	Mean ± SD	P
I consider PI a serious problem currently.	U.S.	0.3	1.3	8.4	23.2	66.8	4.55 ± 0.725	0.021
	EU	0.0	0.0	5.7	17.0	77.3	4.72 ± 0.566	
PI will become a more serious issue in the future.	U.S.	0.0	1.0	3.9	15.4	79.6	4.74 ± 0.580	0.137
	EU	0.0	0.0	0.0	18.5	81.5	4.81 ± 0.391	
PI seriousness Index[2] (alpha = 0.69)	*U.S.*	*Mean = 4.64 SD = 0.573*						*P = 0.042*
	EU	*Mean = 4.75 SD = 0.433*						

Need for better education	Who	1[1] (%)	2 (%)	3 (%)	4 (%)	5 (%)	Mean ± SD	P
There is a great need for a standardized protocol for the treatment of PI	U.S.	0.8	2.9	9.1	27.2	60.1	4.43 ± 0.834	0.002
	EU	0.0	0.0	3.4	27.6	69.0	4.66 ± 0.546	
General dentists need to be better educated:	Who	1[1] (%)	2 (%)	3 (%)	4 (%)	5 (%)	Mean ± SD	P
About how to diagnose PI	US	0.0	0.3	2.1	15.9	81.7	4.79 ± 0.473	0.554
	EU	1.1	1.1	2.2	12.2	83.3	4.76 ± 0.659	
About when to refer a patient for the treatment of PI	US	0.0	0.0	1.0	10.2	88.8	4.88 ± 0.359	0.061
	EU	0.0	1.1	3.3	14.1	81.5	4.76 ± 0.562	
About how to offer maintenance care for implants	U.S.	0.3	0.5	2.9	15.1	81.2	4.77 ± 0.548	0.129
	EU	2.5	1.2	3.7	17.3	75.3	4.62 ± 0.830	
Need for better education Index[2] (alpha = 0.79)	U.S.	*Mean = 4.81 SD = 0.389*						***P = 0.186***
	EU	*Mean = 4.72 SD = 0.587*						
Single item	Who	1[1] (%)	2 (%)	3 (%)	4 (%)	5 (%)	Mean ± SD	P
I would like to attend continuing education courses about the treatment of PI.	U.S.	3.4	3.9	16.5	24.7	51.4	4.17 ± 1.058	0.004
	EU	0.0	4.5	7.9	23.6	64.0	4.47 ± 0.827	

Legend:
1.   Answers ranged from "1" = "disagree strongly" to "5" = "agree strongly".
2.   Indices were computed by averaging the responses loading on the respective factor for which an index was created.
Abbreviations: PI, peri-implantitis

2.4 Discussion

To the best of the authors' knowledge, this is the first study comparing the PI-related considerations, professional behavior and attitudes of periodontists practicing in the U.S. vs. Europe. The overall response rate in Europe was 16.1% (the Netherlands: 37.2%, Germany: 8.0%, and Greece: 19.2%). The response rates of web-based and postal mail surveys were reported to be 11% and 26%, respectively [32]. In the present study, the data in the Netherlands and most of the German data were collected with web-based surveys, while the Greek and some German data were collected via postal mail. The overall response rate for the European countries is therefore within the expected range. The response rate in the U.S. (8.6%) was slightly smaller than the percentage reported for web-based surveys [32]. Responses to web-based surveys might have decreased over the past decade due to survey fatigue [33].

The European and U.S. samples were different in terms of gender and age. Although both groups were predominantly male, the European sample included more female respondents than the U.S. sample. Even though the percentage of women in dentistry has been rising during the past decades, women are still underrepresented in specialties, academia and leadership roles [34]. Furthermore, European periodontists were younger and graduated from dental schools and specialty programs more recently than U.S. periodontists. This could be explained by the different education systems; In the U.S., it takes about eight years to become a dentist (four years of college and four years of dental school), while it takes only five to six years after high school in

Europe. The more recent graduation years might also explain why the European periodontists reported being better educated about PI-related risk factors, diagnostic criteria, and treatment approaches during their residency compared to the periodontists in the U.S.

The majority of participants in both groups worked in private practices. Although the European periodontists treated more patients per week, they placed fewer implants per month than the U.S. periodontists. The increasing prevalence of dental implants in the U.S. compared to Europe, might explain this difference [35]. According to the European Implant Market Report, the recent economic crisis in Europe limited implant treatments to some degree [36]. Furthermore, possible differences in selection criteria for implant placement in Europe versus U.S., might account for the lower number of implants placed by the European periodontists.

Poor oral hygiene, history of periodontitis and smoking were the most strongly endorsed patient-related risk factors by both groups. These results are in line with the current literature [2, 37] and are consistent with the results of previous studies [28, 38, 39]. However, in the present study, poor oral hygiene and history of periodontitis were considered as even more important by the European periodontists compared to the U.S. periodontists. Although the prevalence of periodontitis in Europe is similar to that in the U.S., and is increasing with age [40, 41], overall, the population in Europe is older [42, 43]. It is therefore possible that the European periodontists have encountered more older patients in their practices, and thus treated patients who were more prone to PI. On the other hand, while both groups considered implant-related risk indicators such as implant surface, occlusion and presence of keratinized tissue as less important, the aforementioned factors were rated more highly by the U.S. periodontists than by the European periodontists. Other studies also showed that adverse occlusal loading was a more popular risk indicator among specialists in the U.S. than among specialists in Australia and U.K. [28, 38]. When the participants were asked to provide additional comments on the risk factors for PI,

they highlighted the presence of cement, poor emergence profile of restoration, improper implant position, systemic diseases and medications, poor surgical skills, type and quality of bone and lack of patient compliance with maintenance. Recent research also identified these factors as important [9, 44, 45].

The most frequently used diagnostic criteria by both groups included radiographic bone loss, clinical probing and presence of bleeding and suppuration. Both groups evaluated implant exposure and mucosal recession as relatively less significant for the diagnosis of PI. These responses are in line with the current consensus report, which described recession of the mucosal margin as a clinical sign of PI, but did not include it in the diagnostic criteria [1]. A previous study that assessed New Zealand specialists' attitudes towards the diagnosis and treatment of PI also reported that the most frequently used diagnostic criteria were clinical probing and radiographs, while the presence of implant exposure and gingival recession were considered as less significant [29]. However, this study did not include the inflammatory parameters bleeding and suppuration upon probing [29].

The results of the present study reflect the therapeutic complexity of PI, and the lack of a standardized therapeutic protocol [19]. While both groups nearly always used patient education, plaque control and mechanical debridement, the European periodontists used patient education more frequently and mechanical debridement and antiseptics/ antibiotics less frequently than did the U.S. periodontists. The prescription of antibiotics has been higher in the U.S. than Europe, which may account for the higher preference of the U.S. periodontists towards the use of antibiotics for the treatment of PI [46]. Other adjunctive measures including lasers and photodynamic therapy were relatively less frequently used by both groups, with lasers being used more frequently in the U.S. than in Europe. This finding is in line with a report by iData Research which stated that in Europe, the use of lasers in dentistry was more limited than in the U.S. [47]. One of the reasons European dentists were more reluctant to invest in laser technologies

was the lack of government reimbursement for laser treatment in several European countries [47].

Concerning surgical treatment, U.S. periodontists, used regenerative approaches more frequently and resective surgery less frequently than European periodontists did. These results are in contrast with a survey which investigated the treatment modalities used by periodontists in the U.S. and reported that surgical debridement was selected more often than resective or regenerative approaches [38]. Another study showed that 66.7% of the periodontists in New Zealand often used surgical procedures for the treatment of PI, although no distinction was made between different surgical techniques [29]. Schmidlin et al. evaluated the management of PI in private practices of specialists vs. non-specialists in Switzerland and reported that approximately 80% of the specialists tended to use regenerative approaches [39]. However, direct comparisons among these studies cannot be made due to the heterogeneities in aims, study population, and question format. A recent systematic review on the long-term outcomes of surgical treatment concluded that the use of reconstructive approaches resulted in more successful clinical and radiographic outcomes [48]. Regarding the use of different bone fillers, it is worth noting that U.S. periodontists used allograft more frequently than did European periodontists. This preference could be attributed to the fact that in Europe, the use of allografts is very limited compared to the U.S. due to strict regulations [49].

In our research, both groups agreed that PI is a serious problem and that there is a need for better education of general practitioners about the diagnosis of PI, the referral of such cases to specialists, and the maintenance care offered to patients with dental implants. Both groups also agreed that there is a need for a standardized treatment protocol. These results are consistent with the findings of Russel et al. who assessed the attitudes towards PI of periodontists and oral and maxillofacial surgeons in New Zealand and reported that both groups of specialists considered PI a significant disease and highlighted the need

for better education of general practitioners and referral of PI cases to specialists [29].

This study has several limitations. First, we cannot necessarily assume that the three European countries are truly representative of all European countries. Future research should continue to explore PI related professional activities in different countries to allow for better understanding of the complexity of PI related professional behavior and the role of context for this behavior. Second, although combining the responses from the three European countries resulted in a sufficient sample size that allowed comparisons with the U.S. responses, subgroup analyses of the European responses were not possible. In addition, this survey did not assess whether the respondents were board certified. It only considered that the respondents were members of professional periodontology societies in their countries. Future studies should explore if board certified professionals differ from non-board certified professionals in their responses regarding PI in the U.S. or other countries. Finally, a survey consists of a limited number of questions. The fact that the respondents named some additional risk factors in their open-ended responses is important information for future research.

Conclusions

All respondents engaged in evidence-based professional behavior related to PI. Regarding PI-related risk factors/indicators, both groups rated poor oral hygiene, history of periodontitis, and smoking as very important, and, implant surface, occlusion and presence of keratinized tissue as relatively less important. However, European periodontists put a higher value on history of periodontitis and a lower value on implant surface, occlusion and presence of keratinized tissue as risk factors than did U.S periodontists. Similarly, while all periodontists assessed radiographic bone loss as the most important diagnostic factor and implant exposure/gum recession as the least important factor, U.S. and European periodontists differed in their assessment of the relative importance of clinical probing and implant exposure/gum recession.

European periodontists put a higher value on clinical probing than did their U.S. counterparts, while U.S. periodontists ranked implant exposure/gum recession as a more important diagnostic factor than did European periodontists.

For the management of PI, both groups nearly always relied on patient education, plaque control and mechanical debridement. Significant differences were found in relation to surgical treatments and the use of lasers and antibiotics. The U.S. periodontists were more likely to use antibiotics, lasers, allograft and regenerative approaches and less likely to use respective surgery than European periodontists. Both groups acknowledged that PI is an emerging, significant concern and that there is a need to educate general dentists better about identifying risk factors, diagnosing and referring PI cases for treatment to specialists.

References

1.	Berglundh T, Armitage G, Araujo MG, Avila-Ortiz G, Blanco J, Camargo PM, et al. Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions. J Periodontol. 2018;89 Suppl 1:S313-s8.
2.	Schwarz F, Derks J, Monje A, Wang HL. Peri-implantitis. J Periodontol. 2018;89 Suppl 1:S267-s90.
3.	Roos-Jansaker AM, Lindahl C, Renvert H, Renvert S. Nine- to fourteen-year follow-up of implant treatment. Part ii: Presence of peri-implant lesions. J Clin Periodontol. 2006;33:290-5.
4.	Daubert DM, Weinstein BF, Bordin S, Leroux BG, Flemming TF. Prevalence and predictive factors for peri-implant disease and implant failure: A cross-sectional analysis. J Periodontol. 2015;86(3):337-47.
5.	Lindhe J, Berglundh T, Ericsson I, Liljenberg B, Marinello C. Experimental breakdown of peri-implant and periodontal tissues. A study in the beagle dog. Clin Oral Implants Res. 1992;3(1):9-16.
6.	Monje A, Catena A, Borgnakke WS. Association between diabetes mellitus/hyperglycaemia and peri-implant diseases: Systematic review and meta-analysis. J Clin Periodontol. 2017;44(6):636-48.
7.	Guobis Z, Pacauskiene I, Astramskaite I. General diseases influence on peri-implantitis development: A systematic review. J Oral Maxillofac Res. 2016;7(3):e5.
8.	Roccuzzo M, Grasso G, Dalmasso P. Keratinized mucosa around implants in partially edentulous posterior mandible: 10-year results of a prospective comparative study. Clin Oral Implants Res. 2016;27(4):491-6.
9.	De Bruyn H, Christiaens V, Doornewaard R, Jacobsson M, Cosyn J, Jacquet W, et al. Implant surface roughness and patient factors on long-term peri-implant bone loss. Periodontol 2000. 2017;73(1):218-27.
10.	Monje A, Insua A, Wang HL. Understanding peri-implantitis as a plaque-associated and site-specific entity: On the local predisposing factors. J Clin Med. 2019;8(2).
11.	Heitz-Mayfield L, Heitz F, Lang N. Implant disease risk assessment idra–a tool for preventing peri-implant disease. Clin Oral Impl Res. 2020;31(4):397-403.
12.	Chrcanovic BR, Kisch J, Albrektsson T, Wennerberg A. Bruxism and dental implant treatment complications: A retrospective

comparative study of 98 bruxer patients and a matched group. Clin Oral Implants Res. 2017;28(7):e1-e9.

13.   Sadowsky SJ. Occlusal overload with dental implants: A review. Int J Implant Dent. 2019;5(1):29.

14.   Laine ML, Leonhardt A, Roos-Jansaker AM, Pena AS, van Winkelhoff AJ, Winkel EG, et al. Il-1rn gene polymorphism is associated with peri-implantitis. Clin Oral Implants Res. 2006;17(4):380-5.

15.   Pimentel SP, Shiota R, Cirano FR, Casarin RCV, Pecorari VGA, Casati MZ, et al. Occurrence of peri-implant diseases and risk indicators at the patient and implant levels: A multilevel cross-sectional study. J Periodontol. 2018;89(9):1091-100.

16.   Atieh MA, Pang JK, Lian K, Wong S, Tawse-Smith A, Ma S, et al. Predicting peri-implant disease: Chi-square automatic interaction detection (chaid) decision tree analysis of risk indicators. J Periodontol. 2019;90(8):834-46.

17.   Monje A, Caballé-Serrano J, Nart J, Peñarrocha D, Wang HL, Rakic M. Diagnostic accuracy of clinical parameters to monitor peri-implant conditions: A matched case-control study. J Periodontol. 2018;89(4):407-17.

18.   Renvert S, Persson GR, Pirih FQ, Camargo PM. Peri-implant health, peri-implant mucositis, and peri-implantitis: Case definitions and diagnostic considerations. J Periodontol. 2018;89 Suppl 1:S304-s12.

19.   Garaicoa-Pazmino C, Sinjab K, Wang H-L. Current protocols for the treatment of peri-implantitis. Curr Oral Health Rep. 2019;6(3):209-17.

20.   Renvert S, Hirooka H, Polyzois I, Kelekis-Cholakis A, Wang HL. Diagnosis and non-surgical treatment of peri-implant diseases and maintenance care of patients with dental implants - consensus report of working group 3. Int Dent J. 2019;69 Suppl 2:12-7.

21.   Persson GR, Samuelsson E, Lindahl C, Renvert S. Mechanical non-surgical treatment of peri-implantitis: A single-blinded randomized longitudinal clinical study. Ii. Microbiological results. J Clin Periodontol. 2010;37(6):563-73.

22.   Menezes KM, Fernandes-Costa AN, Silva-Neto RD, Calderon PS, Gurgel BC. Efficacy of 0.12% chlorhexidine gluconate for non-surgical treatment of peri-implant mucositis. J Periodontol. 2016;87(11):1305-13.

23.   Shibli JA, Ferrari DS. Microbiological and clinical effects of adjunctive systemic metronidazole and amoxicillin in the non-surgical treatment of peri-implantitis: 1 year follow-up. Braz Oral Res. 2019;33(suppl 1):e080.

24.   Mills MP, Rosen PS, Chambrone L, Greenwell H, Kao RT, Klokkevold PR, et al. American academy of periodontology best evidence consensus statement on the efficacy of laser therapy used alone or as an adjunct to non-surgical and surgical treatment of periodontitis and peri-implant diseases. J Periodontol. 2018;89(7):737-42.
25.   Chambrone L, Wang HL, Romanos GE. Antimicrobial photodynamic therapy for the treatment of periodontitis and peri-implantitis: An american academy of periodontology best evidence review. J Periodontol. 2018;89(7):783-803.
26.   Berglundh T, Wennstrom JL, Lindhe J. Long-term outcome of surgical treatment of peri-implantitis. A 2-11-year retrospective study. Clin Oral Implants Res. 2018;29(4):404-10.
27.   Renvert S, Polyzois I. Treatment of pathologic peri-implant pockets. Periodontol 2000. 2018;76(1):180-90.
28.   Mattheos N, Collier S, Walmsley AD. Specialists' management decisions and attitudes towards mucositis and peri-implantitis. Br Dent J. 2012;212(1):E1.
29.   Russell AA, Tawse-Smith A, Broadbent JM, Leichter JW. Peri-implantitis diagnosis and treatment by new zealand periodontists and oral maxillofacial surgeons. N Z Dent J. 2014;110(1):6-10.
30.   Ljungberg AK, Fossum B, Furst CJ, Hagelin CL. Translation and cultural adaptation of research instruments - guidelines and challenges: An example in famcare-2 for use in sweden. Inform Health Soc Care. 2015;40(1):67-78.
31.   DeVellis RF. Scale development: Theory and applications.: Thousand Oaks, CA: Sage; 2003.
32.   Hardigan PC, Succar CT, Fleisher JM. An analysis of response rate and economic costs between mail and web-based surveys among practicing dentists: A randomized trial. J Community Health. 2012;37(2):383-94.
33.   O'Reilly-Shah VN. Factors influencing healthcare provider respondent fatigue answering a globally administered in-app survey. PeerJ. 2017;5:e3785.
34.   McKay JC, Quinonez CR. The feminization of dentistry: Implications for the profession. 2012(1488-2159 (Electronic)).
35.   Elani HW, Starr JR, Da Silva JD, Gallucci GO. Trends in dental implant use in the u.S., 1999-2016, and projections to 2026. J Dent Res. 2018:22034518792567.
36.   Millennium Research Group I. Dental implants europe. Toronto, Ontario, Canada.: 2013.

37.   Saaby M, Karring E, Schou S, Isidor F. Factors influencing severity of peri-implantitis. Clin Oral Implants Res. 2016;27(1):7-12.

38.   Papathanasiou E, Finkelman M, Hanley J, Parashis AO. Prevalence, etiology and treatment of peri-implant mucositis and peri-implantitis: A survey of periodontists in the united states. J Periodontol. 2016;87(5):493-501.

39.   Schmidlin PR, Sahrmann P, Ramel C, Imfeld T, Muller J, Roos M, et al. Peri-implantitis prevalence and treatment in implant-oriented private practices: A cross-sectional postal and internet survey. Schweiz Monatsschr Zahnmed. 2012;122(12):1136-44.

40.   Health EPfBO. The state of oral health in europe. 2012.

41.   Eke PI, Borgnakke WS, Genco RJ. Recent epidemiologic trends in periodontitis in the USA. Periodontol 2000. 2020;82(1):257-67.

42.   Statista. Available from: https://www.statista.com/statistics/241494/median-age-of-the-us-population/.

43.   Eurostat. Available from: https://ec.europa.eu/eurostat/web/products-eurostat-news/-/DDN-20191105-1.

44.   Monje A, Galindo-Moreno P, Tozum TF, Suarez-Lopez del Amo F, Wang HL. Into the paradigm of local factors as contributors for peri-implant disease: Short communication. Int J Oral Maxillofac Implants. 2016;31(2):288-92.

45.   Mameno T, Wada M, Otsuki M, Okuno I, Ozeki K, Tahara A, et al. Risk indicators for marginal bone resorption around implants in function for at least 4 years: A retrospective longitudinal study. J Periodontol. 2020;91(1):37-45.

46.   Goossens H, Ferech M, Coenen S, Stephens P. Comparison of outpatient systemic antibacterial use in 2004 in the united states and 27 european countries. Clin Infect Dis. 2007;44(8):1091-5.

47.   Research i. U.S. Dentists lead, european dentists play it safe: U.S. Dental diode lasermarket forecasted to see significant gains. Vancouver, Canada: 2014.

48.   Di Gianfilippo R, Sirinirund B, Rodriguez MV, Chen Z, Wang H-L. Long-term prognosis of peri-implantitis treatment: A systematic review of prospective trials with more than 3 years of follow-up. Applied Sciences. 2020;10(24):9084.

49.   Haugen HJ, Lyngstadaas SP, Rossi F, Perale G. Bone grafts: Which is the ideal biomaterial? J Clin Periodontol. 2019;46 Suppl 21:92-102.

CHAPTER 3

Submucosal microbiome of peri-implant sites:
a cross-sectional study

ABSTRACT

Aim

To study the peri-implant submucosal microbiome in relation to implant disease status, dentition status, smoking habit, gender, implant location, implant system, time of functional loading, probing pocket depth (PPD), and presence of bleeding on probing (BoP).

Materials and Methods

Biofilm samples were collected from the deepest peri-implant site of 41 patients with paper points, and analyzed using 16S rRNA gene pyrosequencing.

Results

We observed differences in microbial profiles by PPD, implant disease status, and dentition status. Microbiota in deep pockets included higher proportions of the genera *Fusobacterium*, *Prevotella*, and *Anaeroglobus* compared to shallow pockets which harbored more *Rothia*, *Neisseria*, *Haemophilus* and *Streptococcus*. Peri-implantitis (PI) sites were dominated by *Fusobacterium* and *Treponema* compared to healthy implants (HI) and peri-implant mucositis (PM) which were mostly colonized by *Rothia* and *Streptococcus*. Partially edentulous (PE) individuals presented more *Fusobacterium*, *Prevotella* and *Rothia* whereas fully edentulous (FE) individuals presented more *Veillonella* and *Streptococcus*.

Conclusions

PPD, implant disease status, and dentition status may affect the submucosal ecology leading to variation in composition of the microbiome. Deep pockets, PI, and PE individuals were dominated by Gram-negative anaerobic taxa.

3.1 Introduction

Dental implants are a popular treatment for the prosthetic rehabilitation of partially dentate and fully edentulous patients [1]. Nevertheless, peri-implant diseases, encompassing peri-implant mucositis (PM) and peri-implantitis (PI), are a growing concern in the dental community [2]. PM is characterized by inflammation confined to the soft tissues surrounding the implant, whereas the inflammatory process in PI leads to progressive loss of supporting bone and eventually loss of the implant [3]. A recent study concluded that approximately 1 out of 3 patients and 1 out of 5 implants experienced PI [4]. The lack of effective and predictable treatments makes the management of peri-implant diseases even more challenging [5].

The importance of biofilms in the etiology of peri-implant diseases has been extensively studied [6, 7]. We assume that dysbiotic biofilms may lead to inflammation, which in turn alters the ecology and favors further growth of dysbiotic communities, leading to a vicious cycle, similar to periodontitis [8, 9]. Microorganisms colonize the peri-implant sulcus within 30 minutes after the surgical procedure and a complex submucosal microbiota, similar to the microbiota around natural teeth, is established within two weeks [10, 11]. Teeth and mucosal surfaces act as microbial reservoirs for the colonization of implants in partially edentulous (PE) and fully edentulous (FE) individuals, respectively [12]. If the biofilm is left undisturbed, clinical signs of inflammation in the peri-implant soft tissues start to appear, demonstrating a cause and effect relationship between biofilm and PM, similar to gingivitis on natural teeth [13, 14]. Untreated PM can at some point derail and progress to PI; the interactions between bacteria and the host immune system may trigger peri-implant bone loss in susceptible individuals and therefore affect the implant long-term stability [15]. While bone loss progresses, a deep pocket is formed, and this new anaerobic environment favors Gram-negative anaerobic bacteria [16, 17]. The hypothesis that bacteria translocate from periodontally involved teeth to implant sites

led to the conclusion that the composition of the peri-implant microbiota resembles the subgingival flora of periodontitis to a great extent [7, 16]. However, the body of evidence supporting such perceived similarities is based in older targeted approaches, such as culture and DNA-checkerboard [7, 18]. More recent evidence based on open-ended 16S rRNA gene sequencing and transcriptome sequencing methods has shown that the periodontal and peri-implant microbiomes have distinct features, which appear to be driven by substrate characteristics and environmental factors [19-22].

Since the role of microorganisms in peri-implant diseases as an initial trigger for inflammatory reactions is well-established, the treatment approaches proposed for their management focus on the elimination of biofilm from the implant surface. The current protocols for the treatment of PI are based on the evidence available from studies related to the treatment of periodontitis [5]. Although most periodontitis cases respond favorably to treatment and maintain long-term periodontal stability, this does not hold true for peri-implantitis [23]. Existing therapeutic strategies are unpredictable in arresting peri-implant tissue inflammation and current evidence does not support a gold-standard protocol to treat peri-implant diseases [24].

Therefore, a clear understanding of the microbial profiles of the peri-implant sulcus/pocket is of great importance to understand the sequelae of ecological changes and to establish effective preventive, diagnostic and treatment strategies of peri-implant diseases. The aim of the present cross-sectional study is to describe the peri-implant microbiome using 16S rDNA amplicon sequencing and explore possible associations of the microbial composition with several patient- and implant-related parameters.

3.2 Materials and Methods

Study design, ethical approval and patient recruitment

The study was designed in 2010 as a descriptive, split-mouth cross-sectional study and was approved by the ethical committee of the VU Medical Centre, Amsterdam (#2011/370). The study was conducted in accordance with the guidelines of the world Medical Association Declaration of Helsinki. The study participants were recruited consecutively from patients visiting the Academic Centre for Dentistry Amsterdam (ACTA) for regular maintenance of their dental implants. To be included in the study, patients had to be older than 18 years, systemically healthy with at least one functional dental implant. Exclusion criteria included the use of systemic antibiotics within the past 6 months, any chronic medical disease or condition, pregnancy or lactation, and presence of implant mobility. Each participant was informed about the aims, the potential risks and benefits of the study and provided written informed consent.

Clinical examination

The following parameters were recorded: age, gender, dentition status, smoking habit, implant location, implant system, time of functional loading, probing pocket depth (PPD), and presence of bleeding on probing (BoP). The clinical parameters were recorded at six sites per implant (mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual). Intra-oral peri-apical radiographs were obtained with the parallel technique, and peri-implant bone levels were evaluated. A diagnosis of implant health and disease was made according to the definitions presented at the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions [3]. Briefly, a healthy implant (HI) was diagnosed when the peri-implant crevice demonstrated no bleeding or suppuration on probing and absence of bone loss beyond the initial crestal bone remodeling. Implants with reduced bone support which presented with absence of clinical signs of inflammation were also considered healthy [25]. PM was defined by the presence of clinical signs of inflammation and absence of radiographic

bone loss, whereas PI was diagnosed on the basis of clinical inflammation, PPD ≥6 mm and radiographic bone loss of ≥3 mm from the implant shoulder [3]. For data analysis we considered shallow pockets with PPD <5 mm and deep pockets with PPD ≥5 mm.

Peri-implant biofilm sample harvesting and DNA extraction
Two implants per patient were originally sampled. In patients with >2 implants, two implants were randomly chosen using a randomization tool (http://www.randomization.com/). Submucosal biofilm samples were obtained from the deepest submucosal site of the selected implants using one sterile paper point per implant (Absorbent Points # 504; Henry Schein Inc, Melville, NY, USA). The sampling sites were isolated using cotton rolls and the supra-mucosal plaque was removed. After drying with air, a paper point was introduced into the bottom of the deepest submucosal site and removed after 10 s, then placed in an empty sterile Eppendorf tube and stored at –80°C until further analysis. DNA was extracted with the AGOWA mag Mini DNA Isolation Kit (LGC Genomics), as described previously [26].

Quantitative PCR, amplicon preparation and sequencing
Real-time qPCR, amplicon preparation, and sequencing were carried out as described previously [27]. Real-time qPCR was performed using a LC480-II light cycler (Rocher Diagnostics, Switzerland) according to the manufacturer's instructions. Barcoded amplicon libraries of the 16S rRNA gene hypervariable region V5–V7 were generated, pooled and sequenced with the 454 GS-FLX + Titanium system (Roche Molecular Diagnostics, Branford, CT, USA) [27].

Sequencing data analysis
The sequencing data were processed using the Quantitative Insights Into Microbial Ecology (QIIME) version 1.8.0 [28]. The data was demultiplexed (split_libraries.py) and barcodes, forward primers (1 mismatch allowed) and reverse primers (2 mismatches allowed) removed. In addition, no ambiguous base calls (N) were allowed and the sequences were quality-filtered using a sliding window size of 50

nucleotides with an average Phred quality score of 30, and otherwise default parameters.

Next, the reads were denoised [29] and scanned again for the presence of reverse primers, allowing 2 mismatches, and were filtered for chimeric sequences with UCHIME [30] as implemented in USEARCH version 6.1 [31] using "identify_chimeric_seqs.py" (chimera retention set to "intersection"). Thereafter, the reads were clustered into operational taxonomic units (OTUs) at a minimal sequence similarity of 97% and taxonomy was assigned using the naïve Bayesian classifier provided by the Ribosomal Database Project (RDP) [32], with a minimum confidence of 0.8, retained on the Human Oral Microbiome Database 16S rDNA sequences (HOMD v.14.51) [33]. With the use of the RDP classifier, as indicated above, many representative sequences had a species-level identification. In addition, all representative sequences were assigned a taxonomy using BLAST [34] on the HOMD website (www.homd.org) using default parameters and database HOMD 16S rRNA RefSeq Version 14.51 (Starts at position 28). Next, the 20 resulting hits were parsed and species names were assigned to the top hit only if the alignment had ≥98% coverage and ≥98.5 similarity. The taxonomies of tied hits were combined.

Earlier, we showed that the sequencing profiles of the samples could be dominated by sequences from non-oral microorganisms. The source of this "foreign" bacterial DNA was attributed to the paper points used for sample collection [35]. Therefore, we removed the OTUs detected in the unused sterile paper points. To allow for comparisons among different samples and to avoid the effect of variable sample sequencing depth on the diversity analyses, all samples were analyzed by rarefaction and the OTU table was subsampled to an equal depth of 1200 reads per sample.

Data Analysis
The demographic and clinical characteristics of the study population were expressed as mean (SD) or percentages (%). The microbiological

data were analyzed at OTU level. To compare the microbial composition between samples by disease status, dentition status, smoking habit, gender, implant location, implant system, time of functional loading, PPD, and BoP, beta-diversity measurements were performed with the principal component analysis (PCA), and one-way permutational multivariate analysis of variance (PERMANOVA) in PAST version 3.23 [36]. The data were log2-transformed for PCA analysis to normalize the distributions of OTUs. PERMANOVA was performed using the Bray-Curtis similarity index and 9999 permutations to evaluate the compositional differences between groups (with Bonferroni correction when applying to more than two groups). P values with a False Discovery Rate (FDR) of 5% or less were considered significant. Furthermore, we performed general linear model-based multivariate statistical analyses of patients' peri-implant microbiome to identify parameters associated with the microbial composition (MaAsLin, version 1.0.1, https://huttenhower.sph.harvard.edu/galaxy/). Covariates including age, gender, smoking, implant disease status, PPD, BoP, implant location, dentition status, time of functional loading and implant system were entered into the model. False discovery correction was used with a threshold of q <0.25.

Analysis of the relative abundance of the microbial communities between groups with significant differences was performed with linear discriminant analysis (LDA) Effect Size (LEfSe) in order to determine the OTUs that most likely explain the differences between the groups [37, 38]. LEfSe was performed online via the Galaxy framework, using a size-effect threshold of 4.0 on the logarithmic LDA score. OTUs, which were identified differentially abundant between the groups in LEfSe, were tested for differences in relative abundance with Mann-Whitney U test or Kruskal–Wallis test in case of more than 2 groups, in SPSS (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). The alpha diversity indices Shannon, Chao1 and observed OTUs were calculated using QIME. The level of statistical significance was set at 5%.

3.3 Results

Demographic and clinical characteristics of the study population

The study was conceived and designed in 2010 as a split-mouth cross-sectional study, aiming to compare within the same individual diseased implants (either PI or PM) to healthy implants (HI). Forty-eight patients contributing two implants each were initially enrolled from December 2011 until June 2012 (Figure 1). Two samples from one patient were lost at DNA isolation stage due to insufficient high-quality DNA. After subsampling at 1200 reads and removing the contaminants originating from the paper points [35], 27 additional samples and six patients were excluded resulting in 41 patients contributing 67 peri-implant samples. At that point, only 26 out of 41 patients had paired samples, of which 19 participants presented with the same disease status (3 with HI, 14 with PM and 2 with PI). On this premise, only one sample per patient was selected based on the higher amount of isolated DNA. Therefore, 41 implant sites in total were included in the analysis (Figure 1). Since the criteria for a split-mouth study design could not be satisfied, the current study was considered a descriptive cross-sectional study.

The demographic characteristics of the participants and the clinical features of the included implants are summarized in Table 1. The patients were on average 65.6 (8.8) years old (range: 49-83). The patient cohort comprised 13 males and 28 females, 17 partially edentulous (PE), 24 fully edentulous individuals (FE) and 5 smokers. The implants were in function for on average 7.2 (5.5) years (range: 1-18 years). Thirty out of 41 implants (73%) were located in the mandible. The mean PPD at the sampled sites was 4.2 mm (1.1) and 15 out of 41 sites (37%) presented with PPD ≥5 mm. Thirty out of 41 sites (73%) had signs of bleeding. Eleven implants (27%) were HI, 24 implants (58%) were diagnosed with PM, and 6 implants (15%) were diagnosed with PI. The implants belonged to seven different implant systems (Table 1). The characteristics of the excluded implants (n=26) are summarized in Supplementary Table S1.
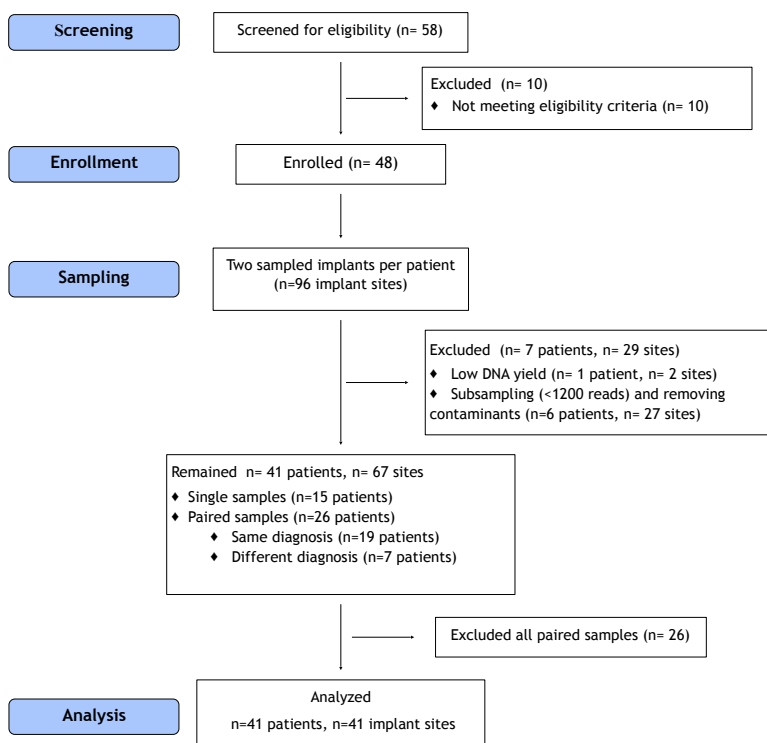
Figure 1: Consort diagram of patient distribution.

Table 1: Study population characteristics (n=41 patients). Values represent mean (SD) or frequencies (%).

Demographic and implant characteristics	
Age (SD)	65.6 (8.8)
Gender: Female / Male	28 (68%) / 13 (32%)
Smoking status: Non-smoker / Smoker	36 (88%) / 5 (12%)
Dentition status: Fully edentulous (FE) / Partially edentulous (PE)	24 (59%) / 17 (41%)
Functional loading, years (SD)	7.2 (5.5)

Demographic and implant characteristics	
Individuals with:	
Implant in function <5 years	20 (49%)
Implant in function ≥5 years	21 (51%)
Implant disease status:	
Peri-implant health (HI)	11 (27%)
Peri-implant mucositis (PM)	24 (58%)
Peri-implantitis (PI)	6 (15%)
PPD at sampled site, mm (SD)	4.2 (1.1)
Individuals with:	
PPD <5 mm at implant	26 (63%)
PPD ≥5 mm at implant	15 (37%)
Bleeding on probing at sampled site (BoP): No / Yes	11 (27%) / 30 (73%)
Implant site	
Maxilla, Anterior	3 (7%)
Maxilla, Posterior	8 (20%)
Mandible, Anterior	18 (44%)
Mandible, Posterior	12 (29%)
Implant types	
Straumann	12 (29%)
Nobel/Branemark	9 (22%)
3i	12 (29%)
Astra	5 (12%)
Other	3 (7%)

Abbreviations: SD, standard deviation; PPD, probing pocket depth

Sequencing results

After quality filtering, denoising, chimera removal and removal of contaminants 178,239 reads from 41 samples were clustered into OTUs (mean: 4,347 reads per sample, SD: 2,326, range: 1,243-10,829). The subsampled OTU table of the 41 samples (1200 reads/sample) contained 489 OTUs, with an average of 53 (SD: 22,

range: 14-114) OTUs per sample. The reads were classified using HOMD into 10 phyla: Firmicutes (41.2%), Proteobacteria (16.9%), Bacteroidetes (15.9%), Actinobacteria (13.4%), Fusobacteria (10.5%), Spirochaetes (0.9%), Synergistetes (0.3%), Saccharibacteria TM7 (0.3%), Chloroflexi (0.01%), and Gracilibacteria GN02 (0.03%). Some OTUs (0.5% of reads) could only be classified as Bacteria. The OTUs were further classified into 23 classes, 39 orders, 68 families and 124 genera.

Microbial profile analyses

PCA followed by PERMANOVA revealed significant differences in microbial profiles by PPD (F=3.931, p=0.0001), disease status (F=1.716, p=0.017), dentition status (F=1.941, p=0.020) and implant location (F=1.927, p=0.020) (Table 2, Figure 2). After FDR correction at 5%, all the aforementioned parameters remained statistically significant (Table 2). BoP, implant type, time of functional loading and gender were not significantly associated with the composition of the peri-implant microbiome (p> 0.05) (Table 2). Since only 5 out of 41 patients were smokers, analysis on smoking habit was not performed. MaAsLin did not detect any associations of a specific microbial community member with clinical metadata. The same held true, when MaAslin was repeated using only four covariates (PPD, dentition status, implant disease status and implant location).

Table 2: Parameters studied in association with the peri-implant microbiome (one-way PERMANOVA, FDR was set at 5%).

Parameter	Test value, p value	FDR corrected p value
Dentition status (PE vs FE)	F= 1.941, p= 0.020	p= 0.045
Implant disease status (HI vs PM vs PI)	F= 1.716, p= 0.017	p= 0.045

Parameter	Test value, p value	FDR corrected p value
PPD (<5 mm vs ≥5 mm)	F= 3.931, p= 0.0001	p= 0.004
BoP (presence vs absence)	F= 1.260, p= 0.183	p= 0.235
Implant location (maxilla vs mandible)	F= 1.927, p= 0.020	p= 0.045
Implant system (Straumann vs Nobel vs 3i vs Astra vs other)	F= 1.107, p= 0.245	p= 0.245
Functional loading time (<5 vs ≥5 years)	F= 1.223, p= 0.208	p= 0.235
Gender (male vs female)	F= 1.471, p= 0.095	p= 0.142

Abbreviations: PPD, probing pocket depth; PE, partially edentulous; FE, fully edentulous; HI, healthy implant; PM, peri-implant mucositis; PI, peri-implantitis; BoP, bleeding on probing

The four variables that were identified as significant from PERMANOVA, were tested for possible associations between each other using the Chi-square test. Disease status was significantly associated with PPD (p=0.009), and, implant location was significantly associated with dentition status (p<0.001), PPD (p=0.008) and implant disease status (p=0.026). Therefore, any possible impact of implant location on the composition of the peri-implant microbiome would be masked by the aforementioned associations. On this premise, implant location was excluded from further analyses.

Linear discriminant analysis Effect Size (LEfSe) was used to determine which OTUs most likely explain the observed differences between groups by PPD, implant disease status, and dentition status. From all OTUs, 9 OTUs significantly discriminated between shallow and deep pockets, 4 OTUs significantly discriminated between HI and PI, and 5

OTUs significantly discriminated between PE and FE individuals (p<0.05, LDA>4.0 for all parameters). Figure 3 illustrates the output of the LEfSe analyses.
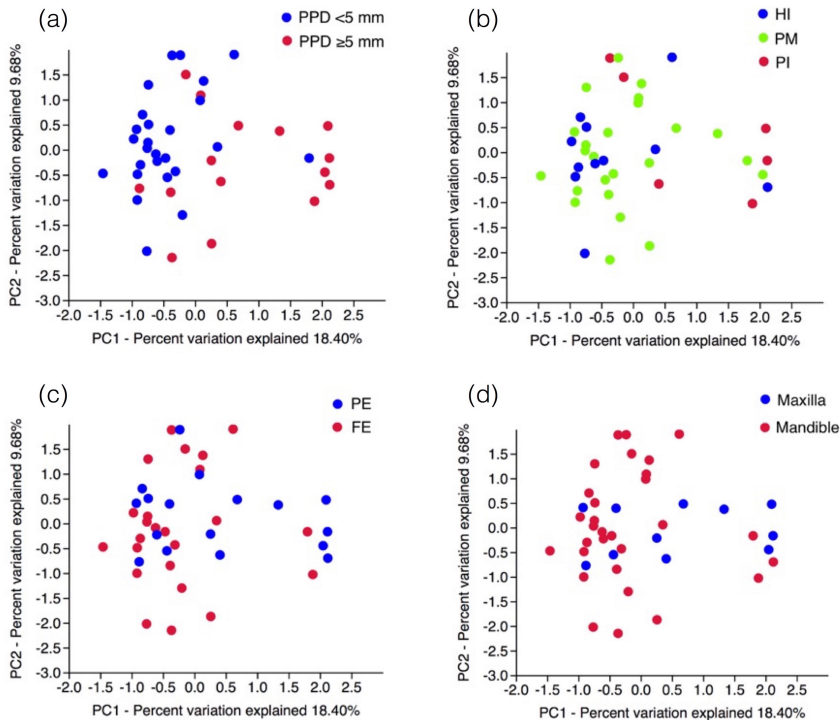

Figure 2: Principal component analysis (PCA) plots of the peri-implant microbiomes colored by: (a) PPD, (b) disease status, (c) dentition status, and, (d) implant location. OTU data were subsampled at 1200 reads per sample and log2-transformed prior to analysis.

Abbreviations: OTU, operational taxonomic unit; PPD, probing pocket depth; HI, healthy implant; PM, peri-implant mucositis; PI, peri-implantitis; PE, partially edentulous; FE, fully edentulous; PC, principal component
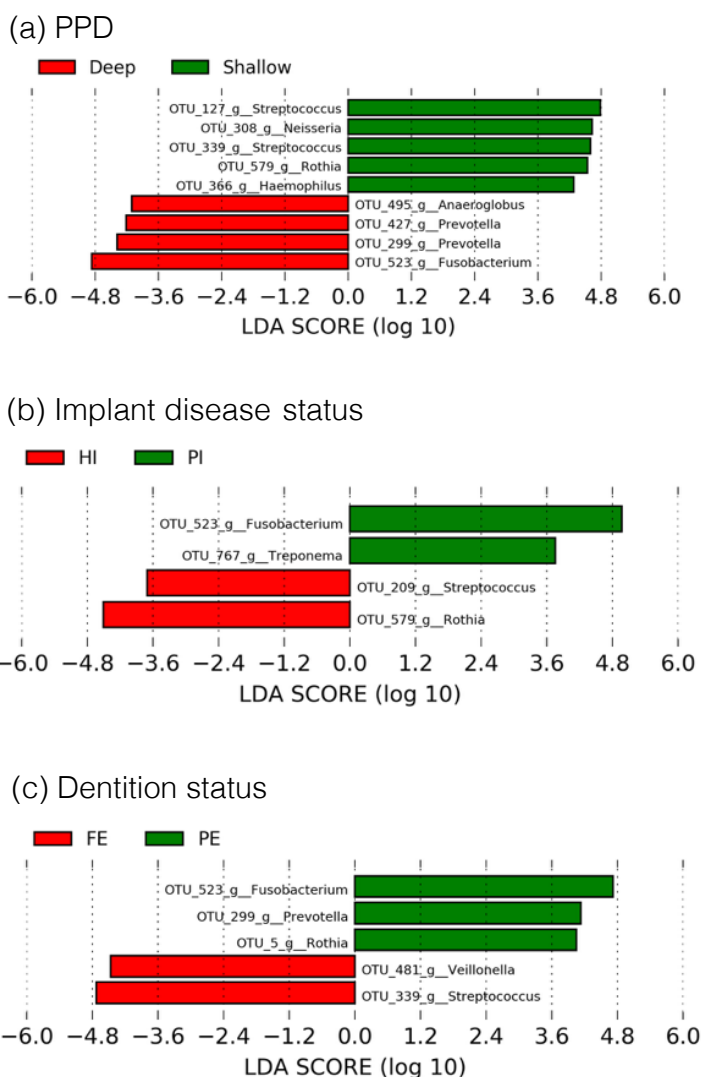
Figure 3: Histograms in (a) through (c) display the linear discriminant analysis (LDA) scores from LEfSe for differentially abundant OTUs for variables of interest, ranked by LDA score. Only OTUs meeting an LDA threshold of > 4.0 are shown.

Abbreiations: OTU, operational taxonomic unit; PPD, probing pocket depth; HI, healthy implant; PI, peri-implantitis; PE, partially edentulous; FE, fully edentulous
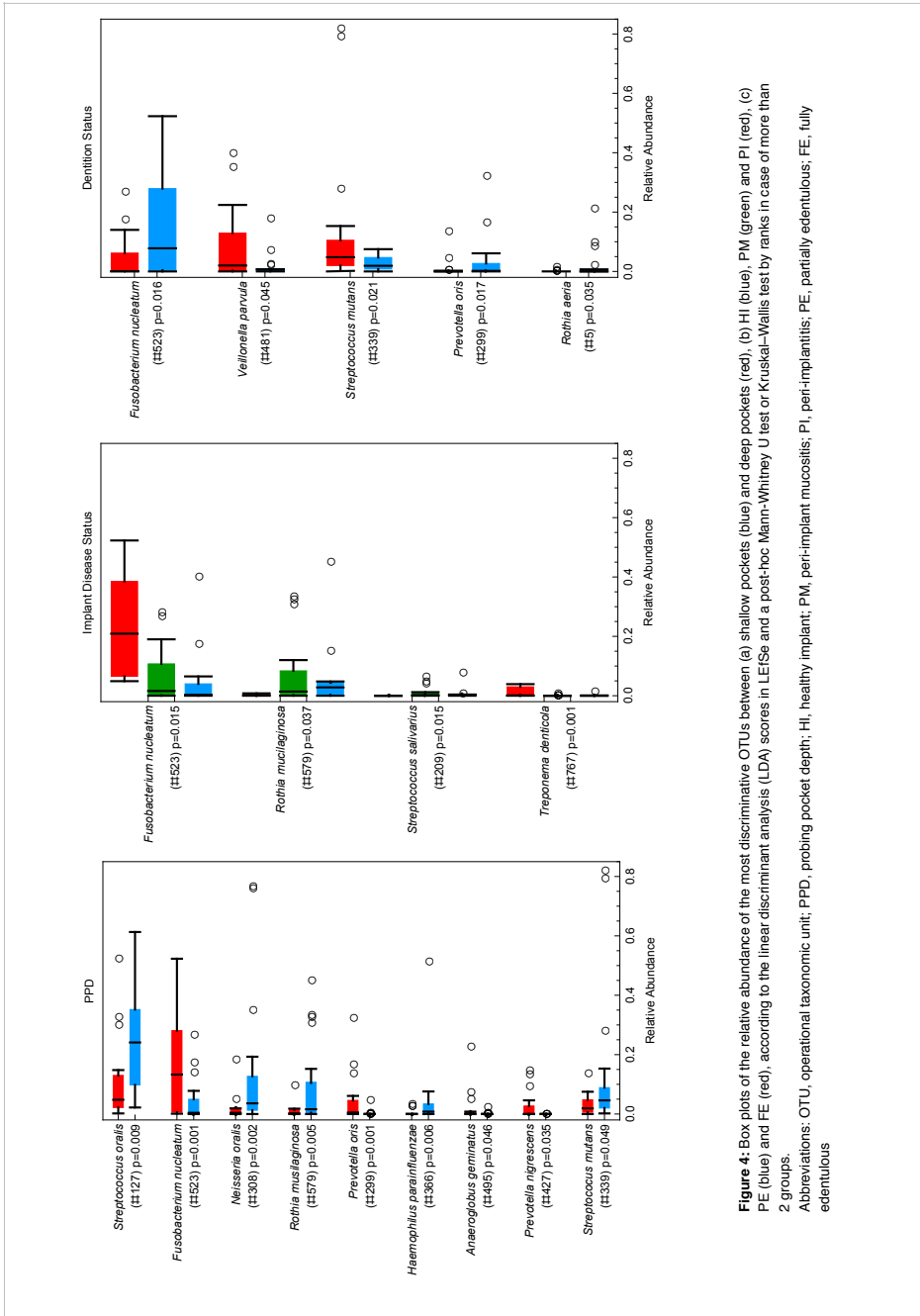
Figure 4: Box plots of the relative abundance of the most discriminative OTUs between (a) shallow pockets (blue) and deep pockets (red), (b) HI (blue), PM (green) and PI (red), (c) PE (blue) and FE (red), according to the linear discriminant analysis (LDA) scores in LEfSe and a post-hoc Mann–Whitney U test or Kruskal–Wallis test by ranks in case of more than 2 groups.

Abbreviations: OTU, operational taxonomic unit; PPD, probing pocket depth; HI, healthy implant; PM, peri-implant mucositis; PI, peri-implantitis; PE, partially edentulous; FE, fully edentulous

Figure 4 depicts the relative abundance of OTUs, which differed significantly by PPD, disease status, and dentition status, based on the LEfSE LDA scores and the Mann-Whitney U test or Kruskal–Wallis test. Deep pockets harbored significantly higher proportions of OTUs: #523 (*Fusobacterium nucleatum*) (p = 0.001), #299 (*Prevotella oris*) (p = 0.001), #427 (*Prevotella nigrescens*) (p = 0.035), and #495 (*Anaeroglobus geminatus*) (p = 0.046) compared to shallow pockets which harbored more OTUs #579 (*Rothia mucilaginosa*) (p = 0.005), #308 (*Neisseria oralis*) (p = 0.002), #366 (*Haemophilus parainfluenzae*) (p = 0.006), #127 (*Streptococcus oralis*) (p = 0.009) and #339 (*Streptococcus mutans*) (p = 0.049) (Figure 4a). Implants diagnosed with PI were colonized by higher proportions of OTUs #523 (*Fusobacterium nucleatum*) (p = 0.015) and #767 (*Treponema denticola*) (p = 0.001) compared to HI and PM which were colonized by higher proportions of #579 (*Rothia mucilaginosa*) (p = 0.037) and #209 (*Streptococcus salivarius*) (p = 0.015) (Figure 4b). Interestingly, the microbial profiles of HI and PM did not differ significantly (p > 0.05 for all 4 OTUs, data not shown). PE individuals showed significantly more OTUs #523 (*Fusobacterium nucleatum*) (p = 0.016), #299 (*Prevotella oris*) (p = 0.017) and #5 (*Rothia aeria*) (p = 0.035), whereas FE individuals presented more OTUs #481 (*Veillonella parvula*) (p = 0.045) and #339 (*Streptococcus mutans*) (p = 0.021) (Figure 4c).

The alpha diversity indices Shannon, Chao1 and observed OTUs did not present any statistically significant differences for PPD and dentition status. No statistical tests were performed to compare the alpha diversity indices between HI, PM and PI, since the patients were unevenly distributed among the health/disease categories, which did not allow to draw any meaningful conclusions (Table 3).

Table 3: Comparison of alpha diversity indices of peri-implant microbiome by PPD, disease status, and dentition status. Data are presented as median (range). Between-group differences at alpha diversity indices were assessed using Mann-Whitney U test.

Sample source	Shannon Index	Chao1 Index	No. of OTUs
PPD			
< 5 mm (n= 26)	3.1 (1.1 - 4.5)	78.5 (19.0 - 177.7)	49.5 (14 - 114)
≥ 5 mm (n= 15)	3.7 (2.2 - 4.2)	68.0 (30.2 - 184.7)	49 (29 - 105)
	p= 0.201	p= 0.445	p= 0.947
Disease status			
HI (n= 11)	3.6 (1.8 - 4.5)	91 (38 - 154.2)	59 (23 - 88)
PM (n= 24)	3.1 (1.1 - 4.5)	63.6 (19 - 177.7)	44 (14 - 114)
PI (n= 6)	4.0 (3.2 - 4.2)	70.6 (53.5 - 184.7)	58 (49 - 105)
	N/A*	N/A*	N/A*
Dentition status			
PE (n= 17)	3.3 (1.7 - 4.5)	79.0 (52.3 - 184.7)	55 (37 - 114)
FE (n= 24)	3.3 (1.1 - 4.5)	64.0 (19.0 - 127.2)	45.5 (14 - 94)
	p= 0.634	p= 0.153	p= 0.080

*No statistical tests were performed to compare the alpha diversity between HI, PM and PI, since the PI group had very small sample size relative to HI and PM, which did not allow to draw any meaningful conclusions.

Abbreviations: PPD, probing pocket depth; SD, standard deviation; OTUs, operational taxonomic units; HI, healthy implant; PM, peri-implant mucositis; PI, peri-imlantitis; PE, partially edentulous; FE, fully edentulous

3.4 Discussion

Ecological changes in the peri-implant submucosal sites may lead to shifts in the microbiome, providing favorable conditions for the overgrowth of potential pathogenic bacteria (dysbiosis), thus increasing the host's odds to develop peri-implantitis [17]. This paradigm has been proposed for periodontitis [8, 9]. To the best of our knowledge, this is the first study investigating the association of parameters such as implant disease status, dentition status, gender, implant location, implant system, time of functional loading, PPD, and BoP on the peri-implant microbiome using next generation sequencing. In the present study, we found significant associations of the bacterial communities with the following factors; PPD, implant disease status and dentition status.

Deep peri-implant pockets had a higher relative abundance compared to shallow pockets of anaerobic Gram-negative bacteria of the following genera: *Fusobacterium*, *Prevotella* and *Anaeroglobus*. *Fusobacterium* and *Prevotella* are pathogens, which have been associated with periodontitis and increased pocket depths [39-41]. In addition to the classical periodontopathogens, other microorganisms including *Anaeroglobus* have been associated with periodontitis [42]. Furthermore, the presence of *Anaeroglobus* in the oral cavity has been associated with symptomatic atherosclerosis and new-onset rheumatoid arthritis [43, 44]. In contrast, pockets <5 mm were mostly inhabited by aerobic and facultative anaerobic bacteria belonging to the genera *Rothia*, *Neisseria*, *Haemophilus*, and *Streptococcus*. A recent study that characterized the submucosal microbiome of PI at different severity levels, reported that increased PPD is associated with a shift in submucosal microbiome favoring the growth of anaerobes, which outcompeted the health-associated genera *Rothia*, *Neisseria* and *Streptococcus* [17].

The biofilm of PI sites presented a different microbial composition compared to HI or PM. We observed that the microbial characteristics

of PM were more similar to HI than to PI. PI sites presented significantly higher proportions of *Fusobacterium* and *Treponema* [41]. HI and PM sites presented higher proportions of the genera *Rothia* and *Streptococcus* when compared to PI sites. These results are in line with other studies which compared the microbiome of healthy and diseased implants using pyrosequencing and reported that species of the genus *Streptococcus* were mostly associated with peri-implant health [19, 45, 46], whereas *Fusobacterium* and *Treponema* were more abundant in disease [19, 47]. Similar studies which used open-ended techniques other than pyrosequencing corroborate these findings [48, 49] and further report on *Rothia* which was mostly associated with health [50]. It is worth noting that the aforementioned studies, detected more genera that showed statistically significant differences between healthy and diseased implants, such as *Porphyromonas*, *Filifactor*, *Veillonella*, *Fretibacterium*, *Tannerella*, *Campylobacter*, *Eubacterium*, *Chlorofexi*, *Tenericutes*, *Synergisetes*, *Desulfobulbus*, *Dialister*, and *Mitsukella*, which were mostly present in PI and *Neisseria*, *Veillonella*, *Haemophilus*, *Actinomyces*, *Atopobium*, *Gemella*, *Kingella*, *Leptotrichia*, *Propionibacter*, and *Capnocytophaga* which were mostly associated with health [45-48, 50-53]. The fact that we identified only a few genera associated with implant disease status could possibly be attributed to the fact that the aforementioned studies had higher subsampling depth, and more even sample distribution by disease status, as compared with the present study which included only six PI patients. Furthermore, in the present study we used a more stringent LDA threshold of 4.0 for the LEfSe analysis.

PE patients harbored significantly higher proportions of *Fusobacterium*, *Prevotella* and *Rothia* compared to FE patients. *Fusobacterium* and *Prevotella* are also detected in PI, whereas *Rothia* has been associated with health [41, 51]. The genera *Veillonella* and *Streptococcus*, which have been associated with health [51, 53] were detected in higher proportions in FE patients. In agreement with our results, the microbial colonization of dental implants in FE patients has been characterized by lower proportions of microorganisms and less pathogenic microbiota

76

compared with dentate patients [12, 54-56]. Other studies, however, did not find any differences in the peri-implant microbiota between PE and FE [57].

The factors BoP, implant system, time of functional loading, and gender did not seem to be associated with the composition of the peri-implant biofilms. To the best of our knowledge, there are no studies on the relationship of inflammation and the submucosal peri-implant microbiota using next-generation sequencing techniques. Two studies however, examined the relationship between clinical inflammation (presence or absence of BoP) and the subgingival microbiota in chronic periodontitis using pyrosequencing of 16S rRNA gene and reported conflicting results [58, 59]. One study reported that inflammation was not associated with a distinct microbiome, whereas the other study showed that increased inflammation was associated with more diverse microbiota and higher abundance of *Desulfobulbus*, *Eubacterium*, *Filifactor*, *Streptococcus*, *Tannerella* and *Treponema* [58, 59]. A third study, which examined the subgingival microbiome of restored and unrestored teeth, reported differences in the microbial profiles between bleeding and non-bleeding restored sites; *Prevotella* and *Treponema* were detected in higher abundance in bleeding sites, whereas *Enterococcus* was associated with non-bleeding sites [60]. In accordance with our results, the implant system is not associated with the composition of the submucosal microbiome of peri-implant sites [50]. Regarding the time of functional loading of the implant, it has been reported that the microbial complexity increased with longer loading times, but a history of periodontitis had a greater impact on the peri-implant microbiota than loading time [61]. As far as gender is concerned, it has been shown that female sex hormones affect the microbial profiles in many sites of the body, especially the gut [62]. Nevertheless, regarding oral microbiota, a review by Kumar concluded that there is no definitive evidence to indicate gender-specific differences in the subgingival microbiome [63]. Furthermore, most data on the impact of gender on the composition of oral microbiome are

based on females of reproductive age [63]. Here, the female patients were between 49 and 83 years of age (mean: 66.5 years), therefore, presenting most probably reduced levels of sex hormones.

In the present study, the microbial communities did not differ significantly in alpha diversity by PPD. These results are in contrast with a study which examined the submucosal microbiome of PI lesions at different severity levels, and reported that the alpha diversity was significantly decreased in samples with deeper pockets as compared to shallow pockets [17]. The aforementioned study, however, included only PI cases and defined shallow pockets as ≤7 mm and deep pockets as >7 mm, whereas in the present study the majority of participants was diagnosed with HI or PM and a PPD of 5 mm was used to distinguish between shallow and deep pockets [17]. Furthermore, the three alpha diversity indices did not differ by dentition status. To the best of the authors' knowledge no other studies have compared the peri-implant microbiome between PE and FE, using open-ended techniques. Regarding the implant disease status, the sample distribution in this study was too skewed to draw any meaningful conclusions. Nevertheless, previous studies which compared the microbiome of healthy and diseased peri-implant sites reported that diseased sites presented higher alpha diversity [47, 50] or lower alpha diversity compared to healthy sites [46, 64]. Yet, a study by Dabdoub et al. did not find any difference in Shannon diversity index between healthy and diseased implants [19]. The aforementioned study, however, included in diseased implants both PM and PI. Therefore, differences between studies in microbiome characterization could be attributed to differences in disease definition, the presence of confounding factors such as smoking, differences in sampling technique, different microenvironment, subject to subject variation, or even geographical variations.

Even though the sample size of the present study seems adequate compared to similar studies [17, 46-48, 51, 53, 64], some factors such

as smoking habit or implants diagnosed with PI are not evenly distributed among the study participants, which makes comparisons difficult. Furthermore, information on history of periodontal disease was not available for all patients, therefore this parameter could not be evaluated in association with peri-implant microbiome. The sampling technique employed here was based on the use of sterile paper-points. While these samples were taken and stored, we discovered later that the paper-points can harbor exogenous DNA of non-oral microorganisms and we therefore recommended the use of sterile curettes when using DNA-based techniques [35]. Although in the present study we subtracted the contaminants originating from the paper-points we can still not preclude effects of foreign DNA on microbial profiling results (Salter et al., 2014). Another limitation of the present study is the low-depth coverage which precludes the detection of rare members of the microbial community which might be highly virulent [6]. It has been reported that the accuracy of species level identification on regions of 16S rRNA gene is limited and therefore, the species names assigned to the representative OTU sequences may not be accurate [65]. This study focuses on bacterial taxonomy in relation to several patient- and implant-related parameters and no metagenome predictions tools (e.g. PICRUSt) of the functional profiles of the microbial communities were applied. Finally, although we acknowledge that multivariate analysis by linear models such as MaAsLin can be a useful tool to find associations between microbial profiles and clinical metadata, its use in the present study did not yield significant results. This could be due to the relation between the size of the study population and the number of variables. We would therefore recommend for future research a more extensive study including a few hundred patients [66, 67] and using a multivariate analysis, such as MaAsLin, to further confirm and strengthen our findings.

In conclusion, we report differences in the composition of peri-implant microbiota based on PPD, implant disease status, and dentition status. Well-recognized periodontal pathogens such as *Fusobacterium*,

Prevotella and *Treponema* were present in higher proportions in deep peri-implant pockets, PI, and PE individuals. Our results add to the knowledge that the microbiome of peri-implant sites shares common features with the periodontal microbiome.

Data availability statement
The raw sequence data and metadata that support the findings of this study are openly available in the NCBI BioProject database under accession number PRJNA694635 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA694635/).

Supplementary Table S1: Characteristics of the excluded implant sites (n=26)

Implant characteristics	
Implant disease status	
Peri-implant health (HI)	7 (27%)
Peri-implant mucositis (PM)	14 (54%)
Peri-implantitis (PI)	5 (19%)
Implant site	
Maxilla, Anterior	3 (12%)
Maxilla, Posterior	6 (23%)
Mandible, Anterior	7 (27%)
Mandible, Posterior	10 (38%)
Number of implants in partially edentulous individuals	11 (42%)
Number of implants in fully edentulous individuals	15 (58%)
Functional loading, years (SD)	8.6 (5.8)
PPD at sampled site, mm (SD)	4.5 (1.3)
Bleeding on probing at sampled site (BoP): No / Yes	7 (27%) / 19 (73%)
Implant types	
Straumann	5 (19%)
Nobel/Branemark	7 (27%)
3i	8 (31%)
Astra	3 (11%)
Implant Direct	1 (4%)
Frialit	1 (4%)
Zimmer Biomet	1 (4%)

References

1.   Elani HW, Starr JR, Da Silva JD, Gallucci GO. Trends in dental implant use in the u.S., 1999-2016, and projections to 2026. J Dent Res. 2018;97(13):1424-30.

2.   Dreyer H, Grischke J, Tiede C, Eberhard J, Schweitzer A, Toikkanen S, et al. Epidemiology and risk factors of peri-implantitis: A systematic review. J Periodontal Res. 2018;53(5):657-81.

3.   Berglundh T, Armitage G, Araujo MG, Avila-Ortiz G, Blanco J, Camargo PM, et al. Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions. J Periodontol. 2018;89 Suppl 1:S313-s8.

4.   Kordbacheh Changi K, Finkelstein J, Papapanou PN. Peri-implantitis prevalence, incidence rate, and risk factors: A study of electronic health records at a u.S. Dental school. Clin Oral Implants Res. 2019;30(4):306-14.

5.   Renvert S, Polyzois I. Treatment of pathologic peri-implant pockets. Periodontol 2000. 2018;76(1):180-90.

6.   Charalampakis G, Belibasakis GN. Microbiome of peri-implant infections: Lessons from conventional, molecular and metagenomic analyses. Virulence. 2015;6(3):183-7.

7.   Lafaurie GI, Sabogal MA, Castillo DM, Rincon MV, Gomez LA, Lesmes YA, et al. Microbiome and microbial biofilm profiles of peri-implantitis: A systematic review. J Periodontol. 2017;88(10):1066-89.

8.   Hajishengallis G, Chavakis T, Lambris JD. Current understanding of periodontal disease pathogenesis and targets for host-modulation therapy. Periodontol 2000. 2020;84(1):14-34.

9.   Loos BG, Van Dyke TE. The role of inflammation and genetics in periodontal disease. Periodontol 2000. 2020;83(1):26-39.

10.   Quirynen M, Vogels R, Peeters W, van Steenberghe D, Naert I, Haffajee A. Dynamics of initial subgingival colonization of 'pristine' peri-implant pockets. Clin Oral Implants Res. 2006;17(1):25-37.

11.   Furst MM, Salvi GE, Lang NP, Persson GR. Bacterial colonization immediately after installation on oral titanium implants. Clin Oral Implants Res. 2007;18(4):501-8.

12.   Apse P, Ellen RP, Overall CM, Zarb GA. Microbiota and crevicular fluid collagenase activity in the osseointegrated dental implant sulcus: A comparison of sites in edentulous and partially edentulous patients. J Periodontal Res. 1989;24(2):96-105.

13.	Pontoriero R, Tonelli MP, Carnevale G, Mombelli A, Nyman SR, Lang NP. Experimentally induced peri-implant mucositis. A clinical study in humans. Clin Oral Implants Res. 1994;5(4):254-9.

14.	Schwarz F, Becker J, Civale S, Hazar D, Iglhaut T, Iglhaut G. Onset, progression and resolution of experimental peri-implant mucositis at different abutment surfaces: A randomized controlled two-centre study. J Clin Periodontol. 2018;45(4):471-83.

15.	Schwarz F, Derks J, Monje A, Wang HL. Peri-implantitis. J Periodontol. 2018;89 Suppl 1:S267-s90.

16.	Mombelli A, Decaillet F. The characteristics of biofilms in peri-implant disease. J Clin Periodontol. 2011;38 Suppl 11:203-13.

17.	Kroger A, Hulsmann C, Fickl S, Spinell T, Huttig F, Kaufmann F, et al. The severity of human peri-implantitis lesions correlates with the level of submucosal microbial dysbiosis. J Clin Periodontol. 2018.

18.	Mombelli A, Marxer M, Gaberthuel T, Grunder U, Lang NP. The microbiota of osseointegrated implants in patients with a history of periodontal disease. J Clin Periodontol. 1995;22(2):124-30.

19.	Dabdoub SM, Tsigarida AA, Kumar PS. Patient-specific analysis of periodontal and peri-implant microbiomes. J Dent Res. 2013;92(12 Suppl):168s-75s.

20.	Becker ST, Beck-Broichsitter BE, Graetz C, Dörfer CE, Wiltfang J, Häsler R. Peri-implantitis versus periodontitis: Functional differences indicated by transcriptome profiling. Clin Implant Dent Relat Res. 2014;16(3):401-11.

21.	Shiba T, Watanabe T, Kachi H, Koyanagi T, Maruyama N, Murase K, et al. Distinct interacting core taxa in co-occurrence networks enable discrimination of polymicrobial oral diseases with similar symptoms. Sci Rep. 2016;6:30997.

22.	Daubert DM, Weinstein BF. Biofilm as a risk factor in implant treatment. Periodontol 2000. 2019;81(1):29-40.

23.	Lindhe J, Nyman S. Long-term maintenance of patients treated for advanced periodontal disease. J Clin Periodontol. 1984;11(8):504-14.

24.	Garaicoa-Pazmino C, Sinjab K, Wang H-L. Current protocols for the treatment of peri-implantitis. Curr Oral Health Rep. 2019;6(3):209-17.

25.	Renvert S, Persson GR, Pirih FQ, Camargo PM. Peri-implant health, peri-implant mucositis, and peri-implantitis: Case definitions and diagnostic considerations. J Periodontol. 2018;89 Suppl 1:S304-s12.

26.	Bizzarro S, Laine ML, Buijs MJ, Brandt BW, Crielaard W, Loos BG, et al. Microbial profiles at baseline and not the use of antibiotics

determine the clinical outcome of the treatment of chronic periodontitis. Sci Rep. 2016;6:20205.

27.	Kraneveld EA, Buijs MJ, Bonder MJ, Visser M, Keijser BJ, Crielaard W, et al. The relation between oral candida load and bacterial microbiome profiles in dutch older adults. PLoS One. 2012;7(8):e42770.

28.	Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. Qiime allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7(5):335-6.

29.	Reeder J, Knight R. Rapidly denoising pyrosequencing amplicon reads by exploiting rank-abundance distributions. Nat Methods. 2010;7(9):668-9.

30.	Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. Uchime improves sensitivity and speed of chimera detection. Bioinformatics. 2011;27(16):2194-200.

31.	Edgar RC. Search and clustering orders of magnitude faster than blast. Bioinformatics. 2010;26(19):2460-1.

32.	Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive bayesian classifier for rapid assignment of rrna sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007;73(16):5261-7.

33.	Chen T, Yu WH, Izard J, Baranova OV, Lakshmanan A, Dewhirst FE. The human oral microbiome database: A web accessible resource for investigating oral microbe taxonomic and genomic information. Database (Oxford). 2010;2010:baq013.

34.	Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, et al. Gapped blast and psi-blast: A new generation of protein database search programs. Nucleic Acids Res. 1997;25(17):3389-402.

35.	van der Horst J, Buijs MJ, Laine ML, Wismeijer D, Loos BG, Crielaard W, et al. Sterile paper points as a bacterial DNA-contamination source in microbiome profiles of clinical samples. J Dent. 2013;41(12):1297-301.

36.	Hammer Ø, Harper DAT, Ryan PD. Past: Paleontological statistics software package for education and data analysis. Palaeontol Electron. 2001;4(1):9pp.

37.	Goecks J, Nekrutenko A, Taylor J. Galaxy: A comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. Genome Biol. 2010;11(8):R86.

38.	Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12(6):R60.

84

39.   Wolff LF, Aeppli DM, Pihlstrom B, Anderson L, Stoltenberg J, Osborn J, et al. Natural distribution of 5 bacteria associated with periodontal disease. J Clin Periodontol. 1993;20(10):699-706.

40.   van Winkelhoff AJ, Loos BG, van der Reijden WA, van der Velden U. Porphyromonas gingivalis, bacteroides forsythus and other putative periodontal pathogens in subjects with and without periodontal destruction. J Clin Periodontol. 2002;29(11):1023-8.

41.   Socransky SS, Haffajee AD. Periodontal microbial ecology. Periodontol 2000. 2005;38:135-87.

42.   Fernandez YMM, Exterkate RAM, Buijs MJ, Beertsen W, van der Weijden GA, Zaura E, et al. A reproducible microcosm biofilm model of subgingival microbial communities. J Periodontal Res. 2017;52(6):1021-31.

43.   Scher JU, Ubeda C, Equinda M, Khanin R, Buischi Y, Viale A, et al. Periodontal disease and the oral microbiota in new-onset rheumatoid arthritis. Arthritis Rheum. 2012;64(10):3083-94.

44.   Fåk F, Tremaroli V, Bergström G, Bäckhed F. Oral microbiota in patients with atherosclerosis. Atherosclerosis. 2015;243(2):573-8.

45.   Kumar PS, Mason MR, Brooker MR, O'Brien K. Pyrosequencing reveals unique microbial signatures associated with healthy and failing dental implants. J Clin Periodontol. 2012;39(5):425-33.

46.   Daubert D, Pozhitkov A, McLean J, Kotsakis G. Titanium as a modifier of the peri-implant microbiome structure. Clin Implant Dent Relat Res. 2018;20(6):945-53.

47.   Zheng H, Xu L, Wang Z, Li L, Zhang J, Zhang Q, et al. Subgingival microbiome in patients with healthy and ailing dental implants. Sci Rep. 2015;5:10948.

48.   Koyanagi T, Sakamoto M, Takeuchi Y, Ohkuma M, Izumi Y. Analysis of microbiota associated with peri-implantitis using 16s rrna gene clone library. J Oral Microbiol. 2010;2.

49.   Koyanagi T, Sakamoto M, Takeuchi Y, Maruyama N, Ohkuma M, Izumi Y. Comprehensive microbiological findings in peri-implantitis and periodontitis. J Clin Periodontol. 2013;40(3):218-26.

50.   Sanz-Martin I, Doolittle-Hall J, Teles RP. Exploring the microbiome of healthy and diseased peri-implant sites using illumina sequencing. J Clin Periodontol. 2017;44(12):1274-84.

51.   da Silva ES, Feres M, Figueiredo LC, Shibli JA, Ramiro FS, Faveri M. Microbiological diversity of peri-implantitis biofilm by sanger sequencing. Clin Oral Implants Res. 2014;25(10):1192-9.

52.   Belibasakis GN, Mir-Mari J, Sahrmann P, Sanz-Martin I, Schmidlin PR, Jung RE. Clinical association of spirochaetes and

synergistetes with peri-implantitis. Clin Oral Implants Res. 2016;27(6):656-61.

53.   Al-Ahmad A, Muzafferiy F, Anderson AC, Wolber JP, Ratka-Kruger P, Fretwurst T, et al. Shift of microbial composition of peri-implantitis-associated oral biofilm as revealed by 16s rrna gene cloning. J Med Microbiol. 2018;67(3):332-40.

54.   Kocar M, Seme K, Hren NI. Characterization of the normal bacterial flora in peri-implant sulci of partially and completely edentulous patients. Int J Oral Maxillofac Implants. 2010;25(4):690-8.

55.   Quirynen M, Van Assche N. Rct comparing minimally with moderately rough implants. Part 2: Microbial observations. Clin Oral Implants Res. 2012;23(5):625-34.

56.   Siddiqi A, Milne T, Cullinan MP, Seymour GJ. Analysis of p. Gingivalis, t. Forsythia and s. Aureus levels in edentulous mouths prior to and 6 months after placement of one-piece zirconia and titanium implants. Clin Oral Implants Res. 2016;27(3):288-94.

57.   Hultin M, Bostrom L, Gustafsson A. Neutrophil response and microbiological findings around teeth and dental implants. J Periodontol. 1998;69(12):1413-8.

58.   Abusleme L, Dupuy AK, Dutzan N, Silva N, Burleson JA, Strausbaugh LD, et al. The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. Isme j. 2013;7(5):1016-25.

59.   Camelo-Castillo A, Novoa L, Balsa-Castro C, Blanco J, Mira A, Tomás I. Relationship between periodontitis-associated subgingival microbiota and clinical inflammation by 16s pyrosequencing. J Clin Periodontol. 2015;42(12):1074-82.

60.   Rademacher SWH, Zaura E, Kleverlaan CJ, Buijs MJ, Crielaard W, Loos BG, et al. Qualitative and quantitative differences in the subgingival microbiome of the restored and unrestored teeth. J Periodontal Res. 2019;54(4):405-12.

61.   Lee KH, Maiden MF, Tanner AC, Weber HP. Microbiota of successful osseointegrated dental implants. J Periodontol. 1999;70(2):131-8.

62.   Neuman H, Debelius JW, Knight R, Koren O. Microbial endocrinology: The interplay between the microbiota and the endocrine system. FEMS Microbiol Rev. 2015;39(4):509-21.

63.   Kumar PS. Sex and the subgingival microbiome: Do female sex steroids affect periodontal bacteria? Periodontol 2000. 2013;61(1):103-24.

64.   Apatzidou D, Lappin DF, Hamilton G, Papadopoulos CA, Konstantinidis A, Riggio MP. Microbiome of peri-implantitis affected and

healthy dental sites in patients with a history of chronic periodontitis. Arch Oral Biol. 2017;83:145-52.

65.   Edgar RC. Accuracy of taxonomy prediction for 16s rrna and fungal its sequences. PeerJ. 2018;6:e4652.

66.   Morgan XC, Kabakchiev B, Waldron L, Tyler AD, Tickle TL, Milgrom R, et al. Associations between host gene expression, the mucosal microbiome, and clinical outcome in the pelvic pouch of patients with inflammatory bowel disease. Genome Biol. 2015;16(1):67.

67.   Swarte JC, Douwes RM, Hu S, Vich Vila A, Eisenga MF, van Londen M, et al. Characteristics and dysbiosis of the gut microbiome in renal transplant recipients. J Clin Med. 2020;9(2):386.

CHAPTER 4

*Non-surgical peri-implantitis treatment
with or without systemic antibiotics;
a randomized controlled clinical trial*

ABSTRACT

Objectives
To assess the adjunctive effect of systemic amoxicillin (AMX) and metronidazole (MTZ) in patients receiving non-surgical treatment (NST) for peri-implantitis (PI).

Materials and methods
Thirty-seven patients were randomized into an experimental group treated with NST plus AMX + MTZ (N=18) and a control group treated with NST alone (N=19). Clinical parameters were evaluated at 12 weeks post-treatment. The primary outcome was the change in peri-implant pocket depth (PIPD) from baseline to 12 weeks, while secondary outcomes included bleeding on probing (BoP), suppuration on probing (SoP) and plaque. Data analysis was performed at patient level (one target site per patient).

Results
All 37 patients completed the study. Both groups showed a significant PIPD reduction after NST. The antibiotics group showed a higher mean reduction of PIPD at 12 weeks, compared with the control group (2.28 ± 1.49 mm vs 1.47 ± 1.95 mm), however this difference did not reach statistical significance. There was no significant effect of various potential confounders on PIPD reduction. Neither treatment resulted in significant improvements in BoP at follow-up; thirty out of 37 (81%) target sites still had BoP after treatment. Only two implants, one in each group, exhibited a successful outcome defined as PIPD <5 mm, and absence of BoP and SoP.

Conclusions
NST was able to reduce PIPD at implants with PI. The adjunctive use of systemic AMX and MTZ did not show statistically significant better results compared to NST alone. NST with or without antibiotics was ineffective to completely resolve inflammation around dental implants.

90

4.1 Introduction

The importance of biofilms in the etiology of peri-implantitis (PI), as an initial trigger for inflammatory reactions, has been well-established [1]. Dysbiotic biofilms may cause tissue inflammation, which alters the ecology and favors further growth of dysbiotic microbial communities, leading to a vicious cycle, similar to periodontitis [2, 3].

Although the microbial composition associated with PI is similar to periodontitis, the peri-implant microbiome is more complex including non-cultivable gram-negative species [4]. Well-recognized periodontal pathogens such as Fusobacterium nucleatum, Prevotella intermedia and Treponema denticola are present in higher proportions in deep peri-implant pockets [5]. Consequently, the current protocols for the treatment of PI are based on the evidence available from periodontal treatment and focus on resolution of inflammation and elimination of biofilm from the implant surfaces [6]. Although most periodontitis cases respond favorably to treatment and maintain long-term periodontal stability [7], this does not hold true for PI, most probably due to structural differences in supporting tissues between implants and teeth, differences in the histopathologic features of the two lesions, and the surface characteristics of implants [8, 9]. Therefore, existing therapeutic strategies are unpredictable in arresting peri-implant tissue inflammation and current evidence does not support a gold-standard treatment protocol [10].

As that may be, the non-surgical treatment (NST) is the first step in PI treatment and may lead to some reduction in the extent of inflammation and in some cases to peri-implant pocket depth (PIPD) reduction of up to 1 mm [11]. The adjunctive use of antibiotics, especially metronidazole (MTZ) or the combination of amoxicillin (AMX) and MTZ, in the non-surgical treatment of periodontitis has been widely investigated and has shown to improve the clinical and microbiological parameters [12, 13]. Antimicrobials have also been proposed for PI treatment and are widely used empirically by clinicians from all over the globe, although the scientific evidence of their benefits is still limited [14, 15]. Several studies have demonstrated that the adjunctive administration of systemic antibiotics has led to favorable results in terms of PIPD, tissue inflammation and even radiographic defect reduction [16-20]. On the other hand, two RCTs have shown no additional benefit to NST when systemic antibiotics were used

adjunctively [21, 22]. Hence, the scientific evidence for the use of systemic antibiotics in combination with NST for PI is still inconclusive. Some of the aforementioned studies used a single antimicrobial of the nitroimidazole group, most frequently metronidazole [18-20] and ornidazole [16]. On the other hand, other studies preferred the combination of amoxicillin and metronidazole [17, 21, 22]. An in vitro study, showed that the combination of metronidazole and amoxicillin was effective in lower concentration than mono-therapy, suggesting a synergistic mode of action for these agents [23]. Therefore we chose to use the combination of amoxicilin and metronidazole at low concentrations.

The purpose of the present randomized controlled clinical trial of PI treatment, was to evaluate the clinical results of the combined use of systemic AMX and MTZ in conjunction with NST, in comparison to NST alone. The null hypothesis was that there are no differences between the two treatment strategies.

4.2 Materials and Methods

Study design and ethical approval

The study was carried out as a randomized, controlled, single-blinded, clinical trial. The study protocol was approved by the ethical committee of the VU Medical Centre, Amsterdam (NL 39371.018.12), and was registered at the ISRCTN (https://www.isrctn.com/ISRCTN10896644). The study was conducted in accordance with the principles outlined in the revision of the Declaration of Helsinki (2008).

Study population

The present study is in compliance with the CONSORT guidelines. The study participants were referred to the Department of Oral Implantology and Prosthetic Dentistry or the Department of Periodontology, Academic Centre for Dentistry Amsterdam (ACTA) for treatment of PI.

Systemically healthy, adult patients (≥18 years old) with at least one dental implant were included, if the implant had been in function for more than one year, presented with PIPD ≥5 mm, bleeding and/or

suppuration on probing (BoP and/or SoP), as well as marginal bone loss ≥3 mm detected radiographically. Exclusion criteria included the use of systemic antibiotics within the past 3 months, any chronic medical disease or condition, known allergy to penicillin or metronidazole, use of anti-inflammatory prescription medications within the past 4 weeks, pregnancy or lactation, and presence of implant mobility. Each participant was informed about the aims, the potential risks and benefits of the study and provided written informed consent. The long-cone parallel technique was performed for the digital radiographic evaluation. The implant with the deepest PIPD was selected for the study (target implant). For each target implant the PIPD was evaluated at six sites and the deepest of them was defined as "target site" and was selected for analysis.

The study was conducted between 2012 and 2018. The limited availability of referral cases which fulfilled the inclusion criteria, and the fact that the individuals who were responsible for recruiting the patients, making clinical evaluations and providing the treatment (D.A.M. and J.V.D.H.) were working at ACTA part-time, delayed the completion of the study. Using a block randomization design, patients who met the inclusion criteria were assigned into one of the following treatment protocols: non-surgical treatment (NST) with systemic antibiotics (AMX and MTZ) and chlorhexidine rinses (experimental group) or NST with chlorhexidine rinses (control group) (Fig. 1).

Non-surgical treatment and follow-up
After anamneses, clinical and radiographic assessments and prophylaxis/oral hygiene instruction, the participants received one session of mechanical debridement. After local anesthesia (Ultracain-DS forte®, Sanofi, Frankfurt, Germany), the implant surfaces were treated with ultrasonic devices (EMS, Electro Medical Systems, Nyon, Switzerland) with the Polyether Ether Ketone (PEEK) fiber tip (PI instrument®, EMS, Nyon, Switzerland), and carbon-fiber reinforced

plastic hand instruments (Universal Implant Deplaquer®; Kerr Dental, Bioggio, Switzerland). The implant supported restorations were not removed during treatment. The treatment was performed by one experienced clinician (J.V.D.H.). On the day of treatment, patients started with systemic AMX 375 mg and MTZ 250 mg, 1 tablet each, every 8 hours for 7 days. All patients were instructed to start rinsing with chlorhexidine 0.12%, 2 times a day for 4 weeks. In those patients presented with periodontitis, this was treated first and more sessions were planned if necessary to complete the treatment of the whole dentition. At four weeks, an oral hygiene check was performed which included supragingival debridement, polishing with a rubber cup and a low-abrasive paste, and oral hygiene instructions as needed.

Twelve weeks after treatment, a clinical examination was performed in order to evaluate the outcome of treatment. A successful outcome was defined based on the following clinical criteria; implant survival with absence of PIPD ≥5 mm, absence of BoP and/or SoP, modified from [24]. The modification is based on the exclusion of the radiographic evaluation at 12 weeks, as it has been established that the radiographic evaluation does not permit accurate detection of minor resorptive changes in the crestal bone [25]. Treatment success was assessed at target site level and at patient level. In case of an unsuccessful outcome the patient was advised to seek further surgical treatment either at ACTA or at the referring dentist or referring oral surgeon, but this was outside the scope of the current study. In case of treatment success, the patient entered into a 3-month recall program, consisting of soft tissue examination, oral hygiene reinforcement as needed, supragingival instrumentation, and annual clinical evaluation.
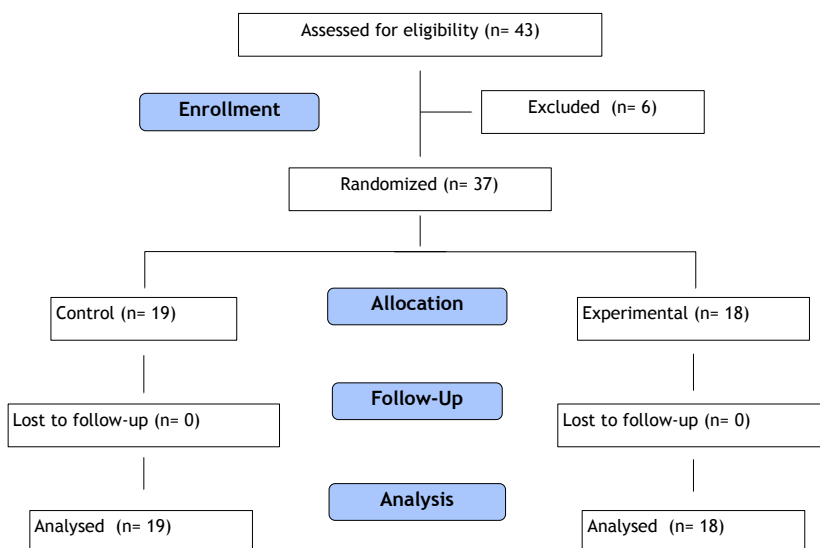
```
                    ┌─────────────────────────────────────┐
                    │   Assessed for eligibility (n= 43)   │
                    └─────────────────────────────────────┘
  ┌──────────────┐                    │
  │  Enrollment  │              ┌──────────────────────┐
  └──────────────┘              │  Excluded  (n= 6)    │
                                └──────────────────────┘
                    ┌─────────────────────────┐
                    │   Randomized (n= 37)     │
                    └─────────────────────────┘
```

Figure 1: Consort diagram of patient distribution.

Demographic data

At the beginning of the study, the following demographic data were recorded: age, sex, body mass index (BMI; expressed as kg/m²), smoking status (smoker, non-smoker), history of periodontitis (yes/no), periodontal stability (yes/no), full mouth plaque score (presence/ absence, %), implant position (maxilla/mandible and anterior/posterior), type of prosthesis connection (screw- vs. cement-retained), dental status (partially edentulous/fully edentulous), number of dental implants (≥4 vs <4), and implant brand.

A periodontitis case was determined on the basis of clinical attachment loss (CAL). When interdental CAL was detected at ≥2 non-adjacent teeth or buccal or oral CAL ≥3 mm with probing depths >3 mm detectable in ≥2 teeth, and the observed CAL could not be associated to non-periodontitis related causes, the patient was considered a

periodontitis case [26]. In cases of fully edentulous patients where previous periodontal charts or radiographs were not available, history of periodontitis was self-reported by the patient. Periodontal stability was defined as <10% bleeding sites with probing depths ≤3 mm [27].

Clinical examination

Baseline clinical measurements of the target implant included; 1) PIPD measured to the closest mm from the mucosal margin to the base of the pocket, 2) BoP (presence or absence), 3) SoP (presence or absence) and 4) plaque (presence or absence). All clinical measurements were performed at six sites. The above clinical measurements were repeated at 12 weeks. All clinical measurements were performed using a periodontal probe (PCP-UNC 15; Hu-Friedy, Chicago, IL, USA) by one calibrated examiner (D.A.M.) who was blinded to the study group allocation.

Statistical analysis

At the time of the study design, no data from RCTs were available for the non-surgical treatment of PI with the use of systemic antibiotics, therefore the power calculation to determine the sample size was based on a previous study of periodontal patients receiving non-surgical treatment alone or combined with AMX and MTZ [28]. The sample size was calculated at https://clincalc.com/stats/samplesize.aspx considering a mean difference in PIPD after treatment of 1 mm between the experimental and control group with standard deviation of 1 mm [28]. Based on these calculations, it was determined that 16 subjects per group would be sufficient to provide a power of 80% with an α of 0.05. A dropout rate of 10% was considered acceptable, therefore we aimed to recruit at least 35 patients. The Cohen's d was also calculated post-hoc for the between-group change in PIPD after treatment to evaluate the effect size. A commonly used interpretation suggested by Cohen is to categorize the effect sizes as small (d = 0.2), medium (d = 0.5), and large (d = 0.8) [29].

The primary outcome parameter was the change in PIPD from baseline to 12 weeks, while secondary outcomes included BoP, SoP and PI. Analysis was performed at one target site per patient. Descriptive statistics included mean ± SD and percentages (%) for numerical and categorical variables respectively and were reported at patient level. The Shapiro-Wilk test was used to assess the normality of data distribution. Independent samples t-test and paired t-test were used to analyze inter-group and intra-group differences respectively, for continuous data. The Chi-squared test or Fisher's exact test was used for inter-group differences in categorical variables. Intra-group comparisons of categorical variables were performed using the McNemar's chi-squared test. The SPSS version 19.00 software (SPSS Inc., Chicago, IL, USA) was used for all analyses. The level of significance was set at $p < 0.05$.

In order to explore whether the prescribed antimicrobials have an effect on PIPD reduction after controlling for potential confounding factors, we applied a linear mixed model (LMM) with random intercept and random slope including baseline PIPD and antibiotics usage (yes/no) as fixed factors (Model 0) (R 4.0.4, www.r-project.org). Age, sex (m/f), Body Mass Index (BMI), smoking (yes/no), history of periodontitis (yes/no), presence of natural teeth (yes/no), number of implants, type of prosthesis (screw-retained/cement-retained), number of sites with SoP at baseline, full mouth plaque score at baseline and implant brand (Straumann, Nobel, BioMet 3i or other) were evaluated as potential confounders. Each of the aforementioned factors was first individually screened in Model 0. Any factor that showed a p-value of <0.1 in these screening models, was to be included in the final LMM model as confounder.

4.3 Results

Patient characteristics

Of the 43 patients screened, 37 were found eligible and were randomized to the experimental (n = 18) or to the control group (n = 19) (Figure 1). All randomized patients completed the study and were included in the analysis. The characteristics of the participants are presented in Table 1. The majority of patients (65%) were female. Age ranged from 25 to 84 years, mean 59.6 ± 11.2 years. Regarding smoking habits 11 patients (30%) were smokers, and 26 patients (70%) were non-smokers at the time of the study. The majority of the participants had no history of periodontitis (n=21, 57%), however the majority of dentate patients included in the study (n=24, 83%) appeared periodontally non stable. Most implants were placed in the mandible (60%) and in the posterior region (54%). The baseline characteristics of the included implants are presented in Table 2. The two groups were comparable in terms of baseline demographic and implant characteristics.

Table 1: Study population characteristics at baseline.

Variable	NST (n=19)	NST with AMX+MTZ (n=18)	Test statistic, p value
Age, mean ± SD (range), years	60.8 ± 14.8 (25 - 84)	58.3 ± 13.9 (27 - 79)	T= 0.532, p= 0.598 †
Sex, n (%) Male Female	 6 (32%) 13 (68%)	 7 (39%) 11 (61%)	 X^2= 0.217, p= 0.737 ‡
Smoking status, n (%) Smoker Non-smoker	 3 (16%) 16 (84%)	 8 (44%) 10 (56%)	 X^2= 3.633, p= 0.056 ‡
BMI, mean ± SD (range), kg/m^2	25.3 ± 4.0 (19.6 - 34.1)	23.3 ± 2.8 (18.5 - 28.7)	T= 1.764, p= 0.087 †

Dental status, n (%)
    Fully edentulous
    Partially edentulous
	4 (21%)
	15 (79%)
		4 (22%)
		14 (78%)
			Fisher's exact test,
			p= 1.000

Number of natural teeth in dentate patients, mean ± SD (range)	21.3 ± 5.4 (10 - 28)	21.8 ± 4.9 (10 - 27)	T= 0.294, p= 0.770 †

History of periodontitis, n (%)
    Yes
    No
	7 (37%)
	12 (63%)
		9 (50%)
		9 (50%)
			X^2= 0.652,
			p= 0.515 ‡

§Periodontal stability, n (%)
    Yes
    No
	2 (13%)
	13 (87%)
		3 (21%)
		11 (79%)
			Fisher's exact test,
			p= 0.893

FMPS % mean ± SD (range)	40 ± 27.3 (0 - 100)	30.3 ± 28.1 (0 - 100)	T= 0.189, p= 0.851 †
Number of implants, n (%) ≥4 implants <4 implants	8 (42%) 11 (58%)	8 (44%) 10 (56%)	X^2= 0.021, p= 0.886 ‡

Abbreviations: NST, non-surgical treatment ; AMX, Amoxicilin ; MTZ, Metronidazole ; SD, standard deviation ; BMI, Body mass Index ; FMPS, Full mouth plaque score.

† Independent sample t-test
‡ Chi-square test
§ Periodontal stability was evaluated in dentate patients.

Table 2: Implant characteristics at baseline.

Variable	NST (n=19)	NST with AMX+MTZ (n=18)	Test statistic, p value

Implant location, n (%)
    Maxilla
    Mandible
	8 (42%)
	11 (58%)
		7 (39%)
		11 (61%)
			X^2= 0.040,
			p=1.000 ‡

Implant position, n (%)
    Anterior
    Posterior
	6 (32%)
	13 (68%)
		11 (61%)
		7 (39%)
			X^2= 3.246,
			p=0.103 ‡

Type of connection, n (%)
    Screw retained
    Cement retained
	8 (42%)
	11 (58%)
		9 (50%)
		9 (50%)
			X^2= 0.232,
			p=0.630 ‡

Implant brand, n (%)
    Nobel
    Straumann
    Biomet 3i
    Other
	5 (26%)
	5 (26%)
	5 (26%)
	4 (21%)
		9 (50%)
		4 (22%)
		1 (6%)
		4 (22%)
			X^2= 3.896,
			p=0.272 ‡

Abbreviations: NST, non-surgical treatment ; AMX, Amoxicilin ; MTZ, Metronidazole

Clinical outcomes

None of the patients reported side effects associated with the use of antibiotics or the clinical procedures performed in the study. The clinical parameters at baseline and at 12 weeks are presented in Figures 2 and 3 and in Supplementary Table S1. At baseline, all clinical parameters were comparable in both groups. At 12 weeks, both treatment modalities resulted in improvements in clinical parameters. After NST alone, the mean PIPD of the target sites changed from 8.00 ± 1.41 mm at baseline to 6.53 ± 2.59 mm at 12 weeks (p = 0.004). After NST with

the addition of antibiotics the mean PIPD of the target sites changed from 7.44 ± 1.38 mm at baseline to 5.17 ± 1.92 mm at 12 weeks (p <0.001). Regarding the secondary outcomes, intra-group analysis showed that none of the two groups achieved statistically significant reduction in BoP of target sites. Nevertheless, for both groups, the target sites showed a statistically significant reduction in SoP at 12 weeks (p <0.01). Although plaque was reduced at follow-up for both groups, only in the control group a statistically significant reduction of target sites with plaque was observed (p <0.05). At 12 weeks, none of the clinical parameters were significantly different between the two groups.
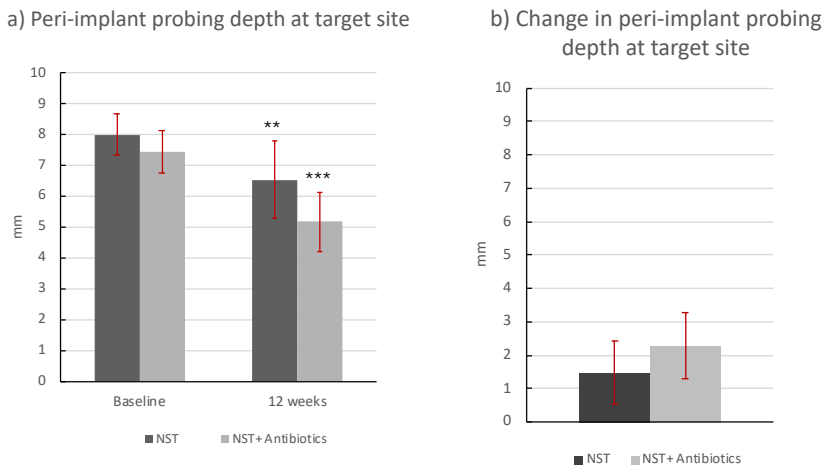

Figure 2: The histograms illustrate the peri-implant probing depth (PIPD) at target site. a) Mean PIPD at baseline and at 12 weeks, b) mean change in PIPD between baseline and 12 weeks for the control and experimental group. There were no inter-group differences. The asterisks (**; p <.01 and ***; p <.001) represent statistically significant intra-group differences from baseline to 12 weeks. Error bars: 95% confidence interval
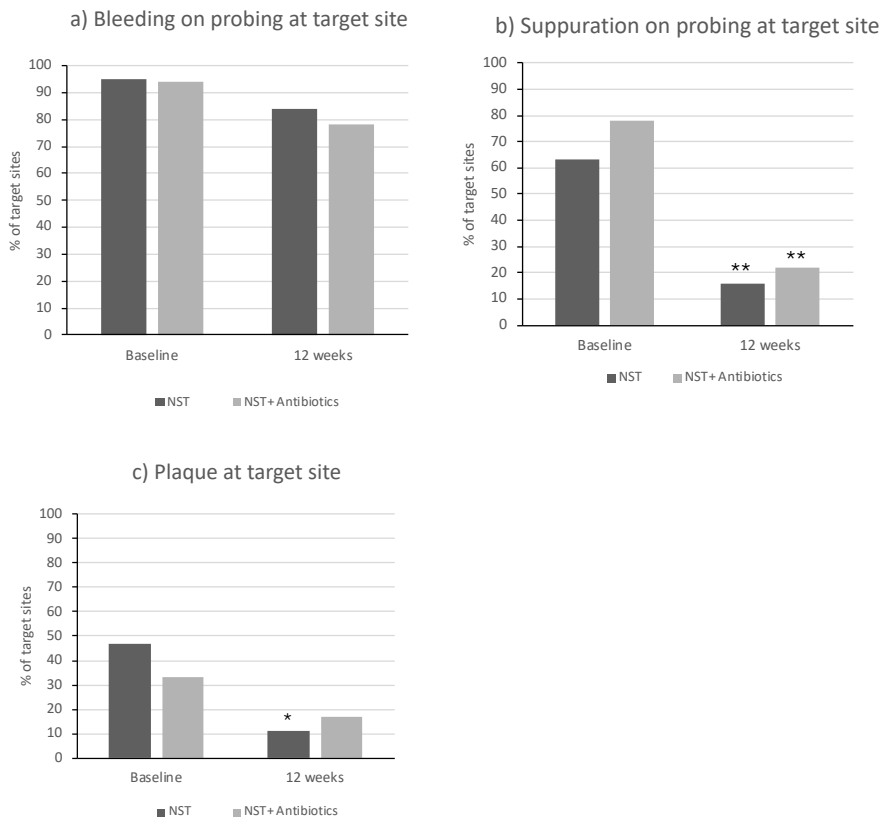
Figure 3: The bar graphs illustrate the frequencies of the secondary outcome parameters a) bleeding on probing (BoP), b) suppuration on probing (SoP), and c) plaque at target site level for the control and the experimental group at baseline and at 12 weeks. The data are expressed as percentage (%) of target sites which present BoP, SoP and plaque respectively. The asterisks (*; p <.05 and **; p <001) represent statistically significant intra-group differences from baseline to 12 weeks.

When the change (Δ) in PIPD from baseline to 12 weeks was evaluated, the experimental group showed a larger mean PIPD reduction of 2.28 ± 1.49 mm, as compared to 1.47 ± 1.95 mm in the control group. Nevertheless, the difference in mean PIPD reduction between the two groups did not reach statistical significance (p = 0.170). The Cohen's d effect size for the between-group change in PIPD was found to be 0.466, suggesting a medium effect size.

Table 3: Clinical outcomes according to baseline PIPD of all six peri-implant sites. The values represent frequency of patients (n, %) having PIPD ≥5, ≥6, ≥7, ≥8, ≥9 and ≥10 mm at baseline and at 12 weeks, for the experimental group and for the control group.

	NST (n=19)	NST with AMX + MTZ (n=18)	Between-group test statistic, p value
PIPD ≥5 mm Baseline Week 12	19 (100%) 16 (84%)	18 (100%) 14 (78%)	N/A Fisher's exact test, p= 0.693
PIPD ≥6 mm Baseline Week 12	19 (100%) 14 (74%)	16 (89%) 8 (44%) **	Fisher's exact test, p= 0.230 X^2= 3.278, p= 0.070
PIPD ≥7 mm Baseline Week 12	15 (79%) 10 (53%)	12 (67%) 4 (22%) **	Fisher's exact test, 0.476 X^2= 3.278, p= 0.057
PIPD ≥8 mm Baseline Week 12	11 (58%) 8 (42%)	9 (50%) 4 (22%)	X^2= 0.232, p= 0.630 X^2= 1.668, p= 0.197

PIPD ≥9 mm			
Baseline	9 (47%)	6 (33%)	X^2= 0.755, p= 0.385
Week 12	7 (37%)	3 (17%)	Fisher's exact test, p= 0.269
PIPD ≥10 mm			
Baseline	2 (11%)	1 (6%)	Fisher's exact test, p= 1.000
Week 12	2 (11%)	1 (6%)	Fisher's exact test, p= 1.000

Abbreviations: NST, non-surgical treatment ; AMX, Amoxicilin ; MTZ, Metronidazole ; PIPD, peri-implant pocket depth
** Significant difference between baseline and 12 weeks ($p <.01$). McNemar's Chi-Square test

Regarding treatment success at the 12-week follow-up, only three target sites in the control group and two target sites in the experimental group were treated successfully (p = 1.000), with complete absence of BoP and SoP. Considering all six sites around the target implant, two implants (e.g. two patients), one in each group, exhibited a successful outcome.

Of all the factors examined as potential confounders (including age, sex, BMI, smoking, history of periodontitis, presence of natural teeth, number of implants, type of prosthesis, number of sites with SoP, full mouth plaque score and implant brand), none was identified as significant confounder with p-value <0.1 in the initial LMM. Therefore, the final model remained Model 0 including baseline PIPD and antibiotics usage as fixed factors, without any confounders (Supplementary Table S2). From this model, the adjusted PIPD reduction in the experimental group is 0.80 mm larger than that in the control group, however, without reaching statistical significance (adjusted p value = 0.169).

4.4 Discussion

In the present study, the change in PIPD was the primary outcome. It has been demonstrated that PIPD determines the microbial ecology of the peri-implant site, with deep pockets favoring the outgrowth of Gram-negative anaerobic species, which are compatible with peri-implant disease [30]. This is based on the knowledge about the microbial communities in deep periodontitis lesions [31]. According to the results of the present study, a reduction in mean PIPD following NST plus administration of AMX and MTZ was observed after 12 weeks (mean 2.28 mm) which was greater than NST alone (mean 1.47 mm), though not reaching statistical significance. The current results are in accordance with three recent studies, one cohort and two RCTs, which evaluated the use of systemic AMX and MTZ as an adjunct in the NST of PI [17, 21, 22]. These studies reported that both NST alone and NST with antibiotics led to PIPD reduction ranging from 0.40 to 1.67 mm at 3 months [22] and 12 months [17, 21]. Taken together, from the current study and three previous studies it seems not justified to prescribe systemic AMX and MTZ in the NST of PI. On the other hand, an RCT where systemic MTZ was prescribed for 7 days, reported a mean reduction in PIPD of 2.53 mm in the experimental group vs. 1.02 mm in the placebo group (p <0.05) after 12 months [20]. That reduction in PIPD was also accompanied by a mean reduction of 2.33 mm in the intrabony component of the peri-implant defect in the experimental group, as compared with 1.13 mm in the placebo group (p <0.05). The latter study however, included recontouring of the prostheses where needed in order to facilitate oral hygiene. Furthermore, the implant-supported restorations were removed if possible during NST [20].

In the present study the success rate was very low; only three target sites in the control group and two target sites in the experimental group (or one patient in each group) showed complete resolution of the disease (PIPD <5 mm, no BoP and/or SoP). This could be attributed to the fact that 81% of the target sites still had BoP after treatment. Similar results in BoP reduction at 12 weeks after treatment were reported by

Shibli et al., even though, BoP further decreased at the one year follow-up (mean BoP 40.3% and 35.6% at control and experimental group, respectively) [21]. In any case, all previous studies and the current study agree that NST (with or without antimicrobials) is ineffective to completely resolve BoP around dental implants [17, 19, 22]. Factors that might account for the low success rates of NST for PI could be related to the inherent difficulties in removing the biofilm from the implant surfaces, to the type of instruments used to perform the debridement (ultrasonic and hand instruments vs air-abrasive devices), and to the fact that no removal and cleaning or modification of the suprastructure was performed in conjunction with NST [9, 32]. Perhaps a more strict monitoring of the patients during the study period (e.g. a biweekly hygiene check) could have resulted in more favorable outcomes in terms of inflammatory parameters [33], but practically it is not easily applicable to a regular dental office.

This study had several limitations; first, the presence of potential local etiological factors including implant positioning, excess cement, presence of keratinized attached gingiva [34], to name a few, was not evaluated. Second, 54% of the patients included in this study were presented with deep PIPD ≥8 mm. The low success rate observed in this study supports previous literature reports that in severe PI cases non-surgical treatment alone is insufficient to arrest the disease and eliminate bacteria from the rough surfaces of implants and from the concavities between implant threads [11, 35, 36]. Therefore, severe PI maybe best treated by NST first, followed by surgical therapy [37]. Third, the follow-up period was rather short, however it was not considered appropriate to delay further treatment for the cases with residual inflamed deep PIPDs. Thus, the long-term effect of the current NST modality, on implant survival and prevention of further progression of PI, for example could not be evaluated. Finally, although an a priori power analysis was performed based on a mean difference in PIPD after treatment of 1 mm between the experimental and control group with standard deviation of 1 mm, the actual difference in PIPD reduction

between the two groups, was smaller than expected (0.81 mm) and the standard deviation was 1.95 mm, almost double than the one used in the power analysis. Also, we found the effect size in change in PIPD to be moderate. This indicates that the study was underpowered and we cannot rule out that with an increased number of patients the power of the study would have increased and with that a small adjunctive, statistically significant effect of antibiotics would have been found. Nevertheless, whether such statistically significant effect would be clinically relevant needs to be seen.

The existing data regarding the benefits of use of systemic antibiotics on the microbiological parameters of the patients, are contradictory. Two recent RCTs, which evaluated the submucosal peri-implant biofilm profiles using targeted techniques after NST with or without the combination of systemically administered AMX and MTZ did not find any beneficial microbiological effects with the use of antibiotics [21, 22]. Both studies reported that at follow-up (1 year and 3 months respectively), many implants had become recolonized with periodontal pathogens, and that there were no statistically significant differences between control and experimental groups [21, 22]. On the other hand, Blanco et al. reported a significantly greater decrease in the counts of Porphyromonas gingivalis, Tannerella forsythia, and Campylobacter rectus at 12 months in patients receiving systemic MTZ compared with the control group [20]. That being said, when prescribing systemic antibiotics for the treatment of PI, we should take into consideration the potential side-effects [38], the risk of superinfection with opportunistic bacteria, yeast and viruses, which may be difficult to eradicate [39], the development of bacterial resistance [40] and the frequent need for surgery anyway to further treat residual PIPD [11, 36]. Therefore, the decision to administer adjunctive systemic antibiotics should be made with caution, and the practitioner should consider the medical history of the patient, concomitant medications, and the ultimate goal of the treatment (i.e. shallow residual pockets around the implant where PI was present).

In conclusion, the present study showed no clinical benefit from the adjunctive use of systemic AMX and MTZ in the NST of PI. We suggest that the routine use of systemic antibiotics in NST of PI is not recommended. Furthermore, neither of the tested treatment modalities achieved complete resolution of the disease. Although NST should always be the first step in PI treatment, which provides some improvement in clinical parameters and allows for oral hygiene improvement and better patient compliance, sufficient PIPD reduction in severe PI cases can only be accomplished after a surgical treatment phase.

Supplementary Table S1: Clinical parameters at baseline and at 12 weeks at target site level.

Clinical parameters	NST (n=19)	NST with AMX+MTZ (n=18)	Between-group test statistic, p value
PIPD (mm) † Baseline Week 12	 8.00 ± 1.41 6.53 ± 2.59 **	 7.44 ± 1.38 5.17 ± 1.92 ***	 T= 1.208, p= 0.235 T= 1.807, p= 0.079
Δ PIPD (mm) †	1.47 ± 1.95	2.28 ± 1.49	T=-1.402, p= 0.170
BoP ‡ Baseline Week 12	 18 (95%) 16 (84%)	 17 (94%) 14 (78%)	 Fisher's exact test, p= 1.000 Fisher's exact test, p= 0.693
SoP ‡ Baseline Week 12	 12 (63%) 3 (16%) **	 14 (78%) 4 (22%) **	 X^2= 0.946, p= 0.331 Fisher's exact test, p= 0.693
Plaque ‡ Baseline Week 12	 9 (47%) 2 (11%) *	 6 (33%) 3 (17%)	 X^2= 0.772, p= 0.380 Fisher's exact test, p= 0.658

† Values represent mean ± SD of the target site per patient.
‡ Values represent number of patients (%) with the presence of the parameters at the target site.

Abbreviations: NST, non-surgical treatment ; AMX, Amoxicilin ; MTZ, Metronidazole ; SD, standard deviation ; PIPD, peri-implant pocket depth ; BoP, bleeding on probing ; SoP, suppuration on probing.

* Significant difference between baseline and 12 weeks (p <.05)
** Significant difference between baseline and 12 weeks (p <.01)
*** Significant difference between baseline and 12 weeks (p <.001)

Supplementary Table S2: Determinants for PIPD reduction analyzed using a linear mixed effects model. Antibiotics, time and the interaction between antibiotics and time were fixed factors in the basic model. In subsequent modeling, where the potential confounders were each individually entered in this basic model, it appeared that no confounders reached a p-value of <0.10. Therefore, no confounders were included in the final model.

Effect	β-coefficient	SE	DF	t-value	p-value
Intercept	9.473	0.500	35	18.941	0.0000
Antibiotics	0.248	0.717	35	0.346	0.7310
Time	-1.473	0.399	35	-3.685	0.0008
Interaction Antibiotics x Time	-0.804	0.573	35	-1.402	0.1696

Abbreviations: SE, standard error; DF, degrees of freedom.

References

1.   Lindhe J, Berglundh T, Ericsson I, Liljenberg B, Marinello C. Experimental breakdown of peri-implant and periodontal tissues. A study in the beagle dog. Clin Oral Implants Res. 1992;3(1):9-16.

2.   Hajishengallis G, Chavakis T, Lambris JD. Current understanding of periodontal disease pathogenesis and targets for host-modulation therapy. Periodontol 2000. 2020;84(1):14-34.

3.   Loos BG, Van Dyke TE. The role of inflammation and genetics in periodontal disease. Periodontol 2000. 2020;83(1):26-39.

4.   Lafaurie GI, Sabogal MA, Castillo DM, Rincon MV, Gomez LA, Lesmes YA, et al. Microbiome and microbial biofilm profiles of peri-implantitis: A systematic review. J Periodontol. 2017;88(10):1066-89.

5.   Polymeri A, van der Horst J, Buijs MJ, Zaura E, Wismeijer D, Crielaard W, et al. Submucosal microbiome of peri-implant sites: A cross-sectional study. J Clin Periodontol. 2021;48(9):1228-39.

6.   Renvert S, Polyzois I. Treatment of pathologic peri-implant pockets. Periodontol 2000. 2018;76(1):180-90.

7.   Lindhe J, Nyman S. Long-term maintenance of patients treated for advanced periodontal disease. J Clin Periodontol. 1984;11(8):504-14.

8.   Berglundh T, Zitzmann NU, Donati M. Are peri-implantitis lesions different from periodontitis lesions? J Clin Periodontol. 2011;38(s11):188-202.

9.   de Avila ED, van Oirschot BA, van den Beucken JJJP. Biomaterial-based possibilities for managing peri-implantitis. J Periodontal Res. 2020;55(2):165-73.

10.   Garaicoa-Pazmino C, Sinjab K, Wang H-L. Current protocols for the treatment of peri-implantitis. Curr Oral Health Rep. 2019;6(3):209-17.

11.   Renvert S, Hirooka H, Polyzois I, Kelekis-Cholakis A, Wang HL. Diagnosis and non-surgical treatment of peri-implant diseases and maintenance care of patients with dental implants - consensus report of working group 3. Int Dent J. 2019;69 Suppl 2:12-7.

12.   Berglundh T, Krok L, Liljenberg B, Westfelt E, Serino G, Lindhe J. The use of metronidazole and amoxicillin in the treatment of advanced periodontal disease. A prospective, controlled clinical trial. J Clin Periodontol. 1998;25(5):354-62.

13.   Teughels W, Feres M. Adjunctive effect of systemic antimicrobials in periodontitis therapy: A systematic review and meta-analysis. J Clin Periodontol. 2020;47 Suppl 22:257-81.

14.   Heitz-Mayfield L, Lang N. Antimicrobial treatment of peri-implant diseases. Int J Oral Maxillofac Implants. 2004;19 Suppl:128-39.

15.   Polymeri A, Loos BG, Aronovich S, Steigmann L, Inglehart MR. Risk factors, diagnosis and treatment of peri-implantitis: A cross-cultural comparison of u.S. And european periodontists' considerations. J Periodontol. 2021.

16.   Mombelli A, Lang NP. Antimicrobial treatment of peri-implant infections. Clin Oral Implants Res. 1992;3(4):162-8.

17.   Stein JM, Hammacher C, Michael SS. Combination of ultrasonic decontamination, soft tissue curettage, and submucosal air polishing with povidone-iodine application for non-surgical therapy of peri-implantitis: 12 month clinical outcomes. J Periodontol. 2018;89:139-47.

18.   Liñares A, Pico A, Blanco C, Blanco J. Adjunctive systemic metronidazole to nonsurgical therapy of peri-implantitis with intrabony defects: A retrospective case series study. Int J Oral Maxillofac Implants. 2019;34(5):1237-45.

19.   Nart J, Pons R, Valles C, Estmatges A, Sanz-Martín I, Monje A. Non-surgical therapeutic outcomes of peri-implantitis: 12-month results. Clin Oral Investig. 2020;24(2):675-82.

20.   Blanco C, Pico A, Dopico J, Gándara P, Blanco J, Liñares A. Adjunctive benefits of systemic metronidazole on non-surgical treatment of peri-implantitis. A randomized placebo-controlled clinical trial. J Clin Periodontol. 2022;49(1):15-27.

21.   Shibli JA, Ferrari DS. Microbiological and clinical effects of adjunctive systemic metronidazole and amoxicillin in the non-surgical treatment of peri-implantitis: 1 year follow-up. Braz Oral Res. 2019;33(suppl 1):e080.

22.   De Waal YCM, Vangsted TE, Van Winkelhoff AJ. Systemic antibiotic therapy as an adjunct to non-surgical peri-implantitis treatment: A single-blind rct. J Clin Periodontol. 2021;48(7):996-1006.

23.   Walter C, Kulik EM, Weiger R, Zitzmann NU, Waltimo T. Additive or synergistic antimicrobial effects of amoxicillin and metronidazole on whole plaque samples: A preliminary report. J Int Acad Periodontol. 2011;13(1):11-6.

24.   Heitz-Mayfield LJA, Salvi GE, Mombelli A, Loup PJ, Heitz F, Kruger E, et al. Supportive peri-implant therapy following anti-infective surgical peri-implantitis treatment: 5-year survival and success. Clin Oral Implants Res. 2018;29(1):1-6.

25.   Ramadan A-BE, Mitchell DF. A roentgenographic study of experimental bone destruction. Oral Surg Oral Med Oral Pathol Oral Radiol. 1962;15(8):934-43.

26.　Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, et al. Periodontitis: Consensus report of workgroup 2 of the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions. J Clin Periodontol. 2018;45(S20):S162-S70.

27.　Chapple ILC, Mealey BL, Van Dyke TE, Bartold PM, Dommisch H, Eickholz P, et al. Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: Consensus report of workgroup 1 of the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions. J Clin Periodontol. 2018;45(S20):S68-S77.

28.　Silva MP, Feres M, Oliveira Sirotto TA, Silva Soares GM, Velloso Mendes JA, Faveri M, et al. Clinical and microbiological benefits of metronidazole alone or with amoxicillin as adjuncts in the treatment of chronic periodontitis: A randomized placebo-controlled clinical trial. J Clin Periodontol. 2011;38(9):828-37.

29.　Lakens D. Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and anovas. Frontiers in psychology. 2013;4:863.

30.　Mombelli A, Decaillet F. The characteristics of biofilms in peri-implant disease. J Clin Periodontol. 2011;38 Suppl 11:203-13.

31.　Hajishengallis G, Lamont RJ. Polymicrobial communities in periodontal disease: Their quasi-organismal nature and dialogue with the host. Periodontol 2000. 2021;86(1):210-30.

32.　Ronay V, Merlini A, Attin T, Schmidlin PR, Sahrmann P. In vitro cleaning potential of three implant debridement methods. Simulation of the non-surgical approach. Clin Oral Implants Res. 2017;28(2):151-5.

33.　Machtei EE, Romanos G, Kang P, Travan S, Schmidt S, Papathanasiou E, et al. Repeated delivery of chlorhexidine chips for the treatment of peri-implantitis: A multicenter, randomized, comparative clinical trial. J Periodontol. 2021;92(1):11-20.

34.　Monje A, Insua A, Wang HL. Understanding peri-implantitis as a plaque-associated and site-specific entity: On the local predisposing factors. J Clin Med. 2019;8(2):279.

35.　Persson GR, Samuelsson E, Lindahl C, Renvert S. Mechanical non-surgical treatment of peri-implantitis: A single-blinded randomized longitudinal clinical study. Ii. Microbiological results. J Clin Periodontol. 2010;37(6):563-73.

36.　Faggion Jr CM, Chambrone L, Listl S, Tu Y-K. Network meta-analysis for evaluating interventions in implant dentistry: The case of peri-implantitis treatment. Clin Implant Dent Relat Res. 2013;15(4):576-88.

37.   Renvert S, Polyzois I, Claffey N. Surgical therapy for the control of peri-implantitis. Clin Oral Implants Res. 2012;23 Suppl 6:84-94.
38.   Heta S, Robo I. The side effects of the most commonly used group of antibiotics in periodontal treatments. Medical sciences (Basel, Switzerland). 2018;6(1):6.
39.   Verdugo F. Risk of superinfection in peri-implantitis after systemic broad spectrum antibiotics. Int J Periodontics Restorative Dent. 2018;38(3):443-50.
40.   Rams TE, Degener JE, van Winkelhoff AJ. Antibiotic resistance in human peri-implantitis microbiota. Clin Oral Implants Res. 2014;25(1):82-90.
41.   Berglundh T, Wennstrom JL, Lindhe J. Long-term outcome of surgical treatment of peri-implantitis. A 2-11-year retrospective study. Clin Oral Implants Res. 2018;29(4):404-10.

CHAPTER 5

*Surgical treatment of peri-implantitis defects
with two different xenograft granules:
A randomized clinical pilot study*

ABSTRACT

Objectives
To investigate whether xenograft EB (EndoBon) is non-inferior to xenograft BO (BioOss) when used in reconstructive surgery of peri-implant osseous defects.

Materials and methods
Dental patients with one implant each, demonstrating peri-implantitis were randomized to receive surgical debridement and defect with either BO or EB. Changes in bone level (BL) and intrabony defect depth (IDD) evaluated radiographically were the primary outcomes. The secondary outcomes included changes in probing pocket depth (PPD), bleeding on probing (BoP) and suppuration on probing (SoP). All outcomes were recorded before treatment and at 6 and 12 months post-treatment.

Results
Twenty-four patients (n=11 BO, n=13 EB) completed the study. Both groups demonstrated significant within-group improvements in all clinical and radiographic parameters at 6 and 12 months ($p \leq 0.001$). At 12 months, both groups presented with IDD reductions of 2.5-3.0 mm on average. The inter-group differences were not statistically significant at all time points and for all the examined parameters ($p > 0.05$). While the radiographic defect fill in both groups exceeded >1 mm and can be considered treatment success, successful treatment outcomes as defined by Consensus Reporting (no further bone loss, PPD ≤5 mm, no BOP, and no SoP) was identified in 2/11 (18%) BO, and 0/13 (0%) EB individuals (Fisher's exact test, $p=0.199$).

Conclusions
Within the limitations of this pilot study, the application of xenograft EB showed to be non-inferior to xenograft BO when used in reconstructive surgery of peri-implant osseous defects.

116

5.1 Introduction

Peri-implantitis is a growing concern in the dental community and a public health issue associated with high economic burden [1]. The prevalence of peri-implantitis ranges from 1% to 85% depending on the disease definition [2]. A recent study reported that approximately 1 out of 3 patients and 1 out of 5 implants experienced peri-implantitis [3]. According to the 2017 World Workshop, peri-implantitis is defined as "a plaque-associated pathological condition occurring in tissues around dental implants, characterized by inflammation in the peri-implant mucosa and subsequent progressive loss of supporting bone" [4].

Various treatment protocols for peri-implantitis have been suggested, however there is no consensus as to which one is the most effective intervention [5]. Non-surgical therapy appears to be ineffective in reducing probing depths and eliminating bacteria from implant surfaces especially in more severe cases [6, 7]. Surgical therapy has proven to be more effective in the reduction of probing pocket depths and bleeding on probing as well as in promoting new bone fill, possibly because it provides access to the defect area for removal of the granulation tissue and debridement/decontamination of the exposed implant threads [8-10]. The addition of bone substitutes with or without barrier membranes has demonstrated promising results in terms of radiographic defect reduction and improvement of clinical parameters, especially in well-contained (4-wall and 3-wall) intrabony defects [11-16]. Nevertheless, complete resolution of the bony defect is still not predictable [17].

Bovine bone substitutes have been extensively used in periodontal regeneration, socket preservation, peri-implant reconstruction and alveolar bone augmentation [5, 18]. Numerous preclinical and clinical histomorphometric studies have shown that bovine xenografts are biocompatible, osteoconductive, with extremely slow degradation rate and therefore, able to maintain the volume of the augmented site in the long term [19-23].

Bio-Oss® (BO), is a well-known deproteinized sterilized cancellous bovine bone with a porosity of 75% to 80% and small granule size of 250um-1000um [24]. Due to its hydrophilic properties it facilitates the adsorption of blood cells and proteins [25]. This leads to reliable bone formation and implant osseointegration which resembles to the

osseointegration that takes place in normally healed extraction sites [26]. BO has been used extensively for the treatment of peri-implantitis showing promising results in terms of reduced radiographic defect depth and improved clinical parameters [12, 27-29].

Endobon® (EB) is a newer bovine derived hydroxyapatite ceramic with small granule size (particle size 500um-1000um) that has been fully deproteinized by a two-step, high temperature process for safety from bacteria, viruses and prions (manufacturer's information at dentalwww.zimmerbiometdental.com). This processing method leads to high crystallinity and minimal resorption of graft particles [30]. The structure of EB with the interconnecting micro and macro pores facilitates the ingress of osteogenic cells and acceleration of bone ingrowth [23, 31]. Histological and clinical data suggest that EB has similar reconstructive potential to BO when used for grafting fresh extraction sockets [32]. The use of EB in the surgical treatment of peri-implantitis has been recently reported in a clinical trial [16].

The objective of the present study is to evaluate whether the reconstructive potential of EB is non-inferior to BO when applied in peri-implant intra-osseous defects in a non-submerged technique after 6 and 12 months of healing. We hypothesize that the peri-implantitis defects treated with EB will not exhibit an inferior outcome as compared to BO in terms of radiographic defect reduction around dental implants.

5.2 Materials and Methods

Study design
The study was carried out as a randomized, controlled, single-blinded, non-inferiority clinical trial of 12 months follow-up. The study protocol was approved by the ethical committee of the VU Medical Centre, Amsterdam (NL51525.029.15), and was registered at the ISRCTN (https://www.isrctn.com/ISRCTN14347002). The study was conducted in accordance with the principles outlined in the Declaration of Helsinki (1975, revised in 2008).

Study population
The present pilot study is in compliance with the CONSORT guidelines. The study participants were recruited from patients who had been referred to the Department of Oral Implantology and Prosthodontics or the Department of Periodontology at the Academic Centre for Dentistry

118

Amsterdam (ACTA) for treatment of peri-implantitis. Before participation, each patient was given a detailed description of the procedure, its associated risks and benefits, and signed an informed consent. Patients who presented with a minimum of one osseo-integrated implant, which had been in function for more than one year, were included in the study. In patients with more than one peri-implant defect meeting the inclusion criteria, only one defect per patient was defined as the target (the most severe defect) and included in the study. All patients had received non-surgical treatment before enrollment.

Patients were screened for the following eligibility criteria; marginal bone loss ≥3 mm detected radiographically, probing pocket depth (PPD) ≥5 mm at one or more peri-implant sites, in combination with bleeding and/or suppuration on probing (BoP/SoP). The patients who met the initial eligibility criteria were assessed intra-operatively for the following defect-related inclusion criteria: intra-osseous defect component ≥3 mm at the deepest part and presence of at least three osseous walls. The exclusion criteria included diabetes mellitus (hemoglobin A1c ≥6.5%), use of corticosteroids or other anti-inflammatory prescription drugs, use of systemic antibiotics in the preceding month, pregnancy or lactation, implants previously surgically treated for peri-implantitis, and implant mobility.

Dental patients were screened for eligibility between 2015 and 2018. Using a computer-generated randomization schedule, patients who met the inclusion criteria were allocated to receive one of the two possible treatments, either BO (Bio-Oss®, Geistlich Pharma, Wolhusen, Switzerland) or EB xenograft granules (Endobon®, Zimmer Biomet, Palm Beach Gardens, FL, USA) (Fig. 1). A clinically significant difference in the effectiveness of the two graft materials was considered a difference of 1 mm in radiographic defect reduction. Therefore, a sample size calculation was performed based on the 1 mm non-inferiority limit (standard deviation 1.2 mm) in the mean radiographic defect reduction between the two groups (Roos-Jansaker, Lindahl, Persson, & Renvert, 2011; Roos-Jansåker, Renvert, Lindahl, & Renvert, 2007). The power analysis was performed using the online Sealed Envelope software (https://www.sealedenvelope.com). With a level of significance of alpha = 0.05 in a one-sided hypothesis (or equivalently with alpha = 0.10 in a two-sided hypothesis) and 80% power, 18 patients per group were required. A withdrawal/dropout rate of 10% was considered acceptable, therefore it was planned to recruit a total of 40 patients.
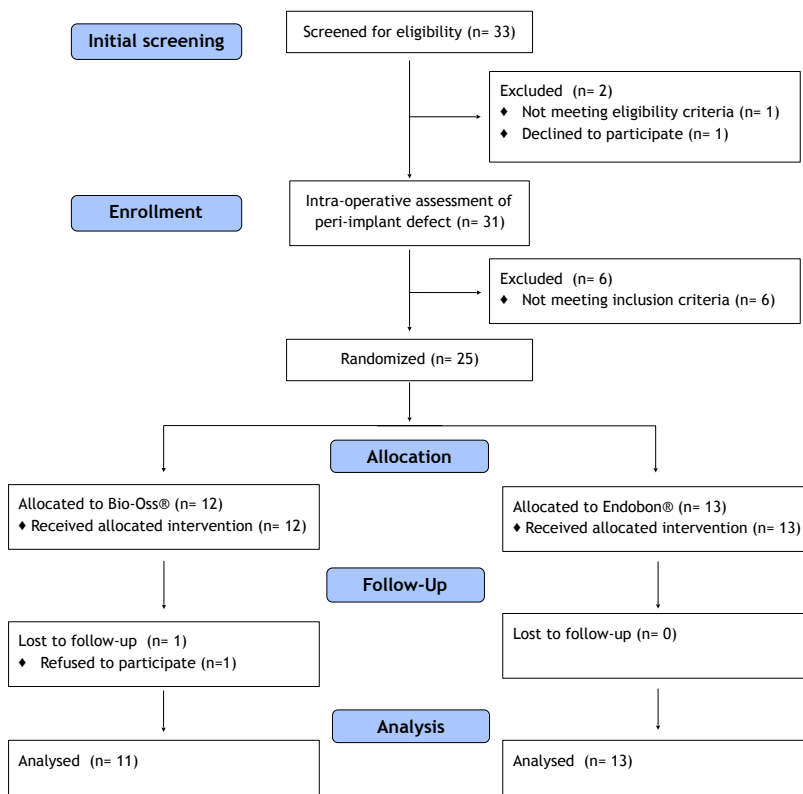
Figure 1: Consort diagram of patient distribution.

Clinical examination

The following clinical recordings were collected at baseline and at the 6 and 12-month follow-up by an experienced, calibrated examiner who was blinded to intervention assignment (D.A.M); 1. PPD to the nearest millimeter, 2. presence/absence of BoP and SoP assessed within 30 s after probing, 3. full-mouth plaque score (FMPS). All measurements were performed at six sites per implant (mesiobuccal, buccal, distobuccal, distopalatal, palatal, and mesiopalatal) using the periodontal probe XP23/UNC 15 (Hu-Friedy, Chicago, IL, USA).

Surgical treatment and post-operative care

Surgeries were performed by an experienced surgeon (D.W.). The surgical technique has been described previously [15]. Briefly, following the removal of the supra-structure whenever that was possible, intracrevicular incisions were performed around the implant. Full-thickness mucoperiosteal flaps were raised on the buccal and lingual aspects to fully access the peri-implant defect. Vertical releasing incisions into the vestibule at a distance of at least one tooth/implant from the target implant were performed as necessary for adequate access. Granulation tissue was removed with titanium curettes (HuFriedy, Chicago, IL, USA) and the exposed implant threads were carefully debrided and decontaminated with 3% H_2O_2 for 1 min, followed by rinsing with copious amounts of saline. The intrabony defect was filled with either BO or EB. Before application, both graft materials were moistened in sterile saline for 5 min. The prostheses were then reconnected and the flaps were re-approximated and sutured with monofilament non-resorbable sutures (Gore-Tex 5-0, W.L. Gore & Associates). The wound healing was performed in a non-submerged mode. In case the defect did not fill the inclusion criteria, the patient was excluded from the study and was treated with an open flap debridement procedure [9].

Detailed post-operative instructions were given to the patients. The patients were prescribed antibiotics; amoxicillin 500 mg X 3 per day and metronidazole 500 mg X 2 per day for 8 days, starting one day before the surgery. The patients were also prescribed analgesics (paracetamol 500 mg) to use as needed. During the first 4 weeks, all participants rinsed with 0.12% chlorhexidine twice daily. Patients were recalled at 6 weeks and 3, 6, 9, and 12 months after the surgery for professional oral hygiene procedures which included supragingival debridement and polishing with a rubber cup and a low-abrasive paste. Oral hygiene instructions were given to each patient as necessary. The study timeline is outlined in Supplementary Figure 1.

Radiographic evaluation

Intra-oral periapical radiographs of the target implant were taken using the parallel long-cone technique and an Eggen holder (Firma Eggen, Lillehammer, Norway) at baseline, and 6 and 12 months after surgery. The evaluation of the radiographs was performed using the software Image J, which was designed by National Institute of Health (NIH, VA, USA) for the image analysis. To compensate for the anatomic magnification and possible variation in the alignment of the films, the linear dimensions of the images were calibrated using the known length of the implant or the known distance between two implant threads.

The following radiographic measurements were recorded at the peri-implant defect (Fig. 2): (i) bone level (BL): vertical distance between the implant shoulder and the bottom of the defect, (ii) intrabony defect depth (IDD): vertical distance between the alveolar crest and the bottom of the defect. Based on these measurements, changes in bone level and vertical defect depth from baseline to 6 and 12 mo were calculated. The radiographic reduction of the intrabony component of the defect was calculated in mm based on the difference of the IDD between the baseline and the study end-points. The supracrestal component of the defect (SC) was also evaluated based on the difference between the BL and IDD values. The most coronal contact of the implant surface with bone or bone with graft material was used to define the BL and IDD. Floating graft particles or single isles of bone or bone-like material were not considered.

All radiographs were de-identified and one examiner (A.P.) who was blinded to treatment allocations made all the radiographic measurements. In order to minimize the measurement error, the radiographic measurements at baseline, 6 and 12 months of 15 randomly selected patients we repeated by the same examiner (A.P.) after one month. The intra-examiner agreement was evaluated by means of the Intra-Class Correlation coefficient (ICC).
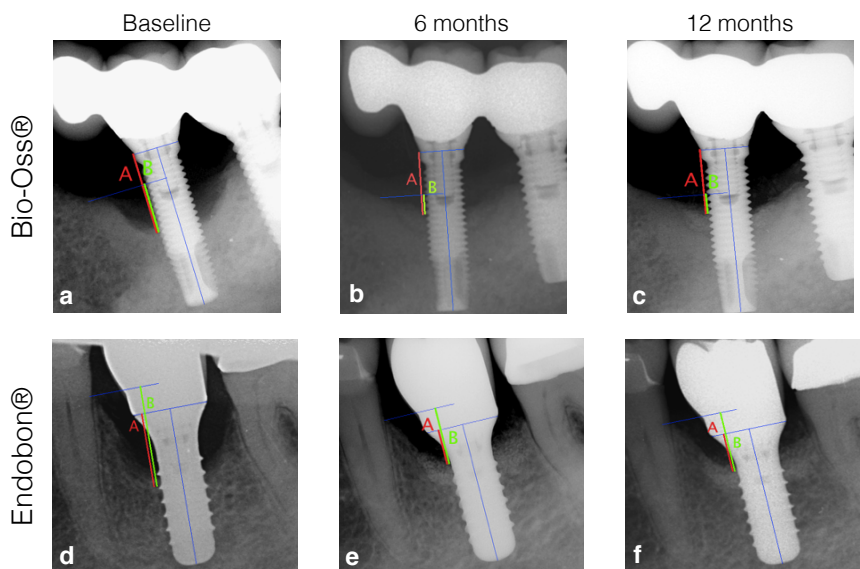
122

Figure 2: Radiographic assessment of: A) bone level (red line) and, B) intrabony defect depth (green line) at baseline, 6 and 12 months after treatment at an implant treated with BO (a-c) and EB (d-f).

Statistical analysis

The primary outcome variables were changes in the radiographic BL and IDD. Secondary outcomes included changes in PPD, BoP, and SoP. Data were expressed as mean (SD) or percentages (%). Comparisons between the two groups were performed using the independent sample t-test for quantitative variables (age, defect depth, PPD etc) and the Chi-square test or Fisher's exact test for qualitative variables (gender, smoking status, reason for placing implants, treatment success etc). A repeated measures ANOVA was performed for within group comparisons. The level of significance was set at 5%. The statistical analyses were performed with a commercial software package (SPSS inc., IBM, Armonk, New York, USA).

5.3 Results

Study population and baseline characteristics
The initial study design was to recruit a total of 40 peri-implantitis patients. However, due to the relocation outside of the Academic Center for Dentistry Amsterdam (ACTA) of the clinical examiner (D.A.M.) and the surgeon (D.W.), the screening process stopped at 33 patients. Therefore, we consider the current study as a pilot study. Figure 1 outlines the flow diagram of the patient enrollment, allocation to interventions, follow-up and data analysis. Twenty-five patients out of 33 fulfilled the inclusion criteria and were randomized to surgical treatment with either BO or EB. One patient refused to attend the follow-up examinations, therefore, 24 patients completed the study and their data were analyzed.

The demographic, dental and implant characteristics of the 24 study participants at baseline are presented in Table 1 and Supplementary Table S1. The two groups were similar in terms of age, gender, smoking status, implant location and years of functional loading. None of the participants demonstrated side effects or patient morbidity, beyond what is normally expected for similar surgical procedures.

Intra-examiner reliability
The peri-implant BL and IDD were re-assessed by the same examiner at 1 month interval. Fifteen patients were randomly selected and their baseline, 6-month and 12-month radiographs (45 radiographs in total) were re-evaluated in order to assess the reliability of the measurements. The intraclass correlation coefficient (ICC) values for the radiographic parameters at baseline, 6 months and 12 months ranged from 0.948 to 0.965, indicating high agreement between repeated measurements (Supplementary Table S2).

Table 1: Study population characteristics at baseline (n=24 patients)

Variable	BO (n=11)	EB (n=13)	Test value, p value
Age (years), mean (SD)	65.5 (11.2)	57.3 (15.1)	T= 1.479, p= 0.153 †

Gender
    Male
    Female
	5 (45%)
	6 (55%)
		8 (62%)
		5 (38%)
			X^2= 0.621,
			p=0.431 ‡

Smoking status
    Smoker
    Non-smoker
	3 (27%)
	8 (73%)
		2 (15%)
		11 (85%)
			Fisher's
			exact test,
			p= 0.630

History of periodontal treatment
    Yes
    No
    Unknown
	4 (36%)
	5 (46%)
	2 (18%)
		6 (46%)
		7 (54%)
		0 (0%)
			-

Type of prosthesis
    Single crown
    Fixed partial denture
    Overdenture
	8 (73%)
	3 (27%)
	0 (0%)
		11 (84%)
		1 (8%)
		1 (8%)
			-

Jaw
    Maxilla
    Mandible
	6 (55%)
	5 (45%)
		6 (46%)
		7 (54%)
			X^2= 0.168,
			p=0.682 ‡

Location
    Anterior
    Posterior
	2 (18%)
	9 (82%)
		2 (15%)
		11 (85%)
			Fisher's
			exact test,
			p= 1.000

Years of function mean (SD) (range)	7.0 (3.4) (3-13)	8.1 (4.9) (2-20)	T= -0.616, p= 0.544 †

Abbreviations: BO, Bio-Oss® ; EB, Endobon® ; SD, standard deviation.

† Independent sample t-test

‡ Chi-square test

Primary and secondary outcomes

The radiographic and clinical parameters at baseline and at the 6- and 12-month end-points for both groups are summarized in Tables 2 and 3 and Figures 3 and 4. Both parametric (t-test, repeated measures ANOVA) and non-parametric (chi-square, Mann-Whitney U test, Friedman) tests were used providing similar results. Here, the results of parametric tests are reported. At baseline, all radiographic and clinical parameters were similar for both groups, except the SC which was significantly different between the two groups (T=2.405, p=0.025). In the EB group most of the implants were placed subcrestally leading to a mean SC of -0.9 (1.6) (Table 2). Radiographically assessed BL and IDD presented within-group statistically significant reductions from baseline to 6 and 12 months. In the BO group the mean BL decreased from 5.3 (1.2) mm to 3.3 (1.3) mm at 6 months and to 3.1 (1.3) mm at 12 months (F= 76.890, p<0.001). In the EB group the mean BL value of 4.9 (1.1) mm at baseline, decreased to 2.5 (1.1) mm at 6 months and to 2.1 (1.3) mm at 12 months (F= 46.724, p<0.001). Regarding the IDD, the mean value recorded for the BO group was 4.9 (0.9) mm, 2.6 (0.6) mm and 2.4 (0.6) mm at baseline, 6- and 12- months respectively (F= 71.544, p<0.001). The corresponding values for the EB group were 5.9 (1.8) mm, 3.1 (1.8) mm and 2.9 (1.3) mm at baseline, 6- and 12- months respectively (F= 49.796, p<0.001) (Table 2, Fig.3). The mean changes in BL and IDD from baseline to 6 and 12 month follow-up were not statistically significant between the two groups (Table 3, Fig.4). The SC increased overall from baseline to 6 and 12 months, however the within-group differences were not statistically significant. Furthermore, the mean changes of the SC from baseline to 6 and 12 months were not significant between BO and EB (Tables 2 and 3).

All clinical parameters (secondary outcomes) improved at 6 and 12 months following surgical treatment. In the BO group the mean PPD (out of six sites per implant) decreased from 7.0 (1.8) mm to 3.5 (1.0) mm at 6 months and to 3.4 (0.6) mm at 12 months (F= 42.449, p<0.001). Similarly, in the EB group PPD decreased from 7.1 (1.2) mm to 3.4 (0.6) mm at 6 months and to 3.4 (0.5) mm at 12 months (F=

88.502, p<0.001). No statistically significant differences were found between the two study groups. The proportion of implant sites presenting with BoP was reduced by more than 50% at the 6 and 12 month post-operative evaluation in all patients. The proportion of implant sites with SoP was also reduced by more than 75% at the 6 and 12 month post-operative evaluation in all patients. There were no intergroup differences in BoP or SoP at any time-point. Full-mouth plaque scores were approximately 30% at baseline in both groups and were further reduced by 14-18% after treatment. At all time points, plaque scores did not differ by study group.

Table 2: Radiographic and clinical parameters (mean (SD)) at baseline, 6 and 12 months of the 24 peri-implant defects.

Parameter	BO	EB	Between-group comparison †
BL (mm)			
Baseline	5.3 (1.2)	4.9 (1.1)	T= 0.885, p= 0.386
6 months	3.3 (1.3)	2.5 (1.1)	T= 1.524, p= 0.142
12 months	3.1 (1.3)	2.1 (1.3)	T= 1.881, p= 0.073
Within-group comparison ‡	*F= 76.890, p<0.001*	*F= 46.724, p<0.001*	
IDD (mm)			
Baseline	4.9 (0.9)	5.9 (1.8)	T= -1.763, p= 0.094
6 months	2.6 (0.6)	3.1 (1.8)	T= -0.979, p= 0.345
12 months	2.4 (0.6)	2.9 (1.3)	T= -1.385, p= 0.183
Within-group comparison ‡	*F= 71.544, p<0.001*	*F= 49.796, p<0.001*	

SC (mm)			
Baseline	0.4 (1.2)	-0.9 (1.6)	T= 2.405, p= 0.025
6 months	0.7 (1.5)	-0.6 (1.8)	T= 1.843, p= 0.080
12 months	0.7 (1.6)	-0.6 (1.6)	T= 2.359, p= 0.028
Within-group comparison ‡	*F= 1.646, p=0.218*	*F= 0.985, p=0.389*	
PPD (mm)			
Baseline	7.0 (1.8)	7.1 (1.2)	T= -0.221, p= 0.827
6 months	3.5 (1.0)	3.4 (0.6)	T= 0.526, p= 0.604
12 months	3.4 (0.6)	3.4 (0.5)	T= 0.115, p= 0.910
Within-group comparison ‡	*F= 42.449, p<0.001*	*F= 88.502, p<0.001*	
BoP (%)			
Baseline	100 (0.0)	100 (0.0)	
6 months	47.7 (32.5)	32.7 (21.4)	T= 1.359, p= 0.188
12 months	45.5 (33.2)	50 (10.2)	T= -0.437, p= 0.670
Within-group comparison ‡	*F= 20.331, p<0.001*	*F= 93.638, p<0.001*	
SoP (%)			
Baseline	79.5 (40.0)	86.5 (33.3)	T= -0.468, p= 0.645
6 months	4.6 (15.1)	0.0 (0.0)	T= 1.000, p= 0.341
12 months	0.0 (0.0)	1.9 (6.9)	T= -0.917, p= 0.369
Within-group comparison ‡	*F= 35.552, p<0.001*	*F= 84.598, p<0.001*	

Plaque (%)			
Baseline	31.7 (13.1)	29.4 (13.0)	T= 0.390, p= 0.701
6 months	15.9 (8.0)	11.5 (6.4)	T= 1.461, p= 0.159
12 months	17.5 (11.5)	14.0 (9.3)	T= 0.776, p= 0.447
Within-group comparison ‡	*F= 12.152, p=0.001*	*F= 12.221, p<0.001*	

Abbreviations: BO, Bio-Oss® ; EB, Endobon® ; BL, bone level; IDD, intrabony defect depth; SC, supracrestal component; SD, standard deviation; PPD, probing pocket depth (mean of 6 sites per implant); BoP, bleeding on probing out of six sites per implant; PI, full mouth plaque index; SoP, suppuration on probing out of six sites per implant

† Independent sample t-test
‡ Repeated measures ANOVA

Table 3: Changes in radiographic and clinical parameters (mean (SD) at 6 and 12 months, in BO and EB treatment groups.

Parameter	BO	EB	Test value, P value †
BL (mm)			
Baseline to 6-months	2.0 (0.7)	2.4 (1.0)	T= -1.113, p= 0.278
Baseline to 12-months	2.2 (0.8)	2.8 (1.3)	T= -1.233, p= 0.231
IDD (mm)			
Baseline to 6-months	2.3 (0.9)	2.7 (1.2)	T= -1.036, p= 0.312
Baseline to 12-months	2.5 (0.8)	3.0 (1.1)	T= -1.053, p= 0.304

SC (mm)			
Baseline to 6-months	-0.3 (0.7)	-0.3 (0.8)	T= 0.117, p= 0.908
Baseline to 12-months	-0.3 (0.7)	-0.2 (0.7)	T= -0.496, p= 0.625
PPD (mm)			
Baseline to 6-months	3.5 (1.7)	3.8 (1.4)	T= -0.448, p= 0.659
Baseline to 12-months	3.6 (1.7)	3.8 (1.4)	T= -0.271, p= 0.789
BoP (%)			
Baseline to 6-months	52.3 (32.5)	67.3 (21.4)	T= -1.359, p= 0.188
Baseline to 12-months	54.5 (33.2)	50.0 (10.2)	T= 0.470, p= 0.643
SoP (%)			
Baseline to 6-months	75.0 (43.3)	86.5 (33.3)	T= -0.738, p= 0.468
Baseline to 12-months	79.5 (40.0)	84.6 (33.1)	T= -0.340, p= 0.737
Plaque (%)			
Baseline to 6-months	15.0 (12.3)	17.9 (11.6)	T= -0.555, p= 0.586
Baseline to 12-months	14.2 (8.4)	15.4 (16.3)	T= -0.190, p= 0.852

Abbreviations: BO, Bio-Oss® ; EB, Endobon® ; BL, bone level; IDD, intrabony defect depth; SC, supracrestal component; SD, standard deviation; PPD, probing pocket depth (mean of 6 sites per implant); BoP, bleeding on probing out of six sites per implant; PI, full mouth plaque index; SoP, suppuration on probing out of six sites per implant
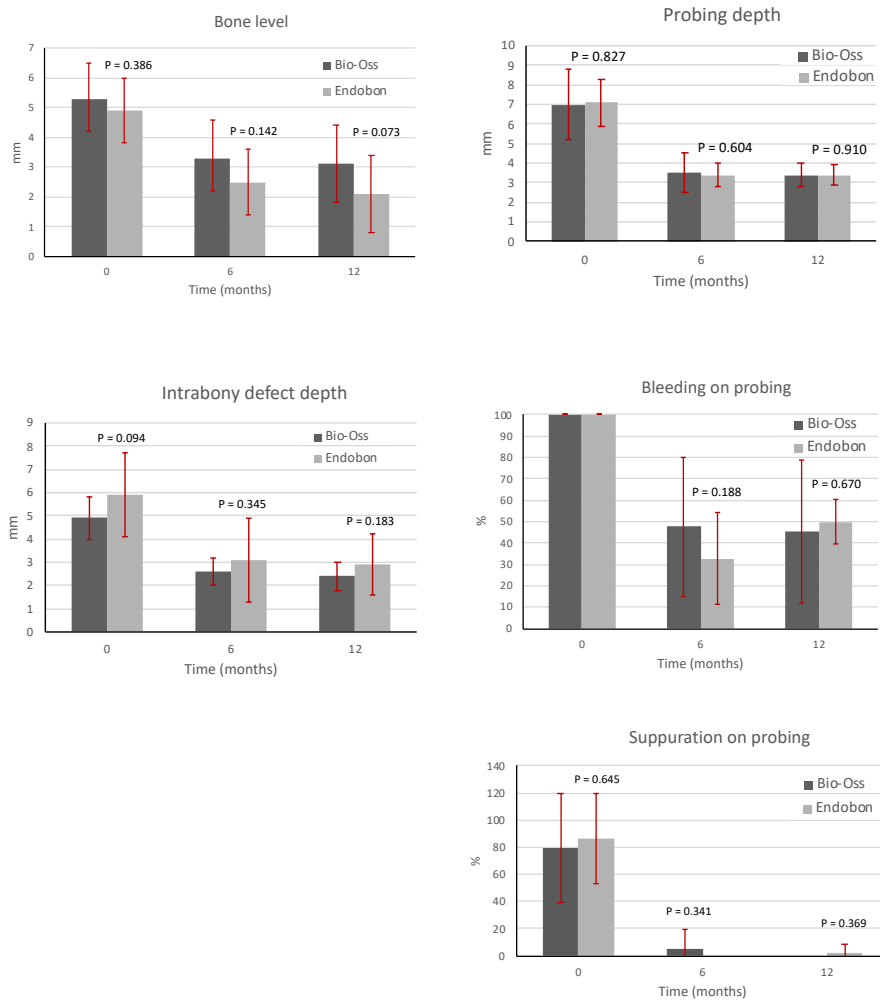† Independent sample t-test

Figure 3: Radiographic and clinical parameters around the implants at baseline, 6 months and 12 months after treatment in both groups. There were no statistically significant differences between BO and EB in any of the parameters that were examined.
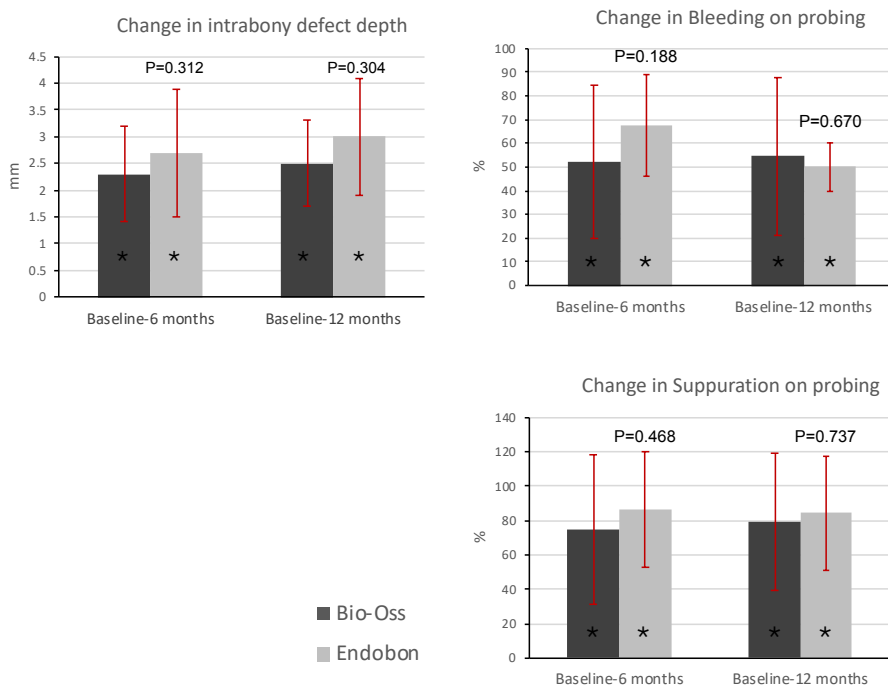
Figure 4: Changes in radiographic and clinical parameters around the implants from baseline to 6 and 12 months after treatment in both groups. No inter-group differences were found in any parameter. The asterisks (*) represent statistical significant within group differences (P<0.001) from baseline to the 6 and 12 month time-points in all parameters.

Successful treatment outcome at 12 months

Successful treatment is determined by the presence of PPD ≤5 mm, complete absence of BoP and SoP, and no further bone loss [33, 34]. Using this strict criterion, successful treatment was found in only 2 of 11 (18%) and none of 13 (0%) individuals of the BO and EB groups, respectively (Fisher's exact test, p=0.199). When less strict criteria were applied including PPD ≤5 mm, ≤ 1 site with BoP, absence of SoP, and no further bone loss [16], 2 of 11 (18%) and 1 of 13 (8%) patients treated with BO and EB, respectively, were successfully treated (Fisher's exact test, p=0.576). When it comes to regenerative therapy,

132

reduction of the radiographic defect of > 1 mm might be considered as treatment success [16]. The treatment approaches used in the present study resulted not only in no further progression of bone loss, but also in radiographic defect reduction of more than 1 mm in all patients at 12 months (Table 3).

5.4 Discussion

The aim of the present study was to compare the reconstructive potential of two different bovine-derived bone substitutes in contained, 3- or 4-wall peri-implant defects. The changes in bone level and intrabony defect depth (defect reduction) assessed radiographically were the primary outcome measures. Intra-oral radiography using the parallel technique is a quick and easy way to assess the bone level around teeth or implants and is considered a reliable tool in determining the peri-implant marginal bone level changes between different examinations [35]. This method however, has some inherent limitations; first of all, the x-ray is a two dimensional examination of three-dimensional structures and has a tendency to underestimate the amount of bone loss around implants [36]. Second, the healing of the peri-implant intra-osseous defect and re-osseointegration of the diseased implant surface can only be verified by means of histological imaging [34, 37]. Third, the interpretation of radiographic defect reduction may be affected by the fact that over time, graft material may not be distinguishable from newly formed bone [38, 39]. Regarding the histological healing following the application of bovine derived xenografts into peri-implant osseous defects, preclinical animal studies demonstrated integration of the graft particles within newly formed bone and re-osseointegration of the previously exposed implant surfaces [40, 41]. However, to the best of the authors' knowledge there is a paucity of human studies regarding the histological healing of bovine xenografts in conjunction with peri-implant related bone defects.

The present study reports no differences in the treatment outcome between the two groups. The mean radiographic defect reduction at 12 months was 2.5 (0.8) mm and 3.0 (1.1) mm for the BO and EB groups, respectively. These results are consistent with other studies where xenogenic bone grafts were used for the reconstruction of peri-implant intrabony defects [13, 28, 42]. Other studies however, reported only 1 mm reduction in bone levels after surgical treatment with bovine-derived xenografts [16, 27]. These discrepancies could be attributed to different baseline defect characteristics, as well as the use of a resorbable collagen membrane by some studies. Nevertheless, a systematic review reported that the amount of radiographic bone fill ranges from 1.46 to 3.30 mm after 3 years of healing, without achieving complete defect resolution [17]. In accordance with these results, complete defect resolution was not achieved in any of the cases of this study. In the present study, most implants in the EB group coincidentally appeared to be placed subcrestally leading to statistically significant between-group difference in the SC. Following treatment however, the SC increased slightly (approximately 0.3 mm in both groups) indicating some crestal bone resorption, which was similar between the two groups. The SC is not frequently reported in studies evaluating the reconstructive treatment of peri-implant intrabony defects. Only one study which compared the reconstructive surgery of peri-implant defects with titanium granules to open flap debridement evaluated this parameter [15]. Even though the mean values of the SC at baseline were greater than the values reported here, the mean change (i.e. crestal resorption) between baseline and 12 months for the group that received reconstructive treatment with titanium granules was similar to ours (0.15 mm with a standard deviation of 1.07 mm) [15]. Therefore, we believe that although the defect configuration was different between the two groups at baseline by coincident (due to randomization), this did not affect the changes in radiographic BL and IDD at 6 and 12 months after treatment.

With regard to the secondary outcome measures, both surgical treatment modalities resulted in improvements of the clinical conditions and there were no statistically significant differences between the two groups. At 12 months, the PPD was reduced by 3.6 (1.7) mm in the group treated with BO and by 3.8 (1.4) mm in the group treated with EB. Similar reductions in PPD have been reported by other studies which used xenografts to treat peri-implantititis [13, 16, 27-29]. Nevertheless, if we had recruited patients with peri-implantitis presenting with PPD ≥6 mm according to the new classification workshop [4], we may have had different results in PPD reductions. However, the initial planning of this study was in 2013, which prompted us to use the older definition [43]. At baseline all sites bled upon probing and at 12 months post-treatment the proportion of implant sites with BoP was reduced by approximately 50% in both groups. These results are in accordance with other studies; a systematic review which evaluated the long-term outcomes of reconstructive procedures to treat peri-implantitis reported a pooled weighted mean in the percentage of BoP reduction of 62.5% with a 95% CI of 25.2% to 89.2% [17]. Other clinical studies which evaluated the percentage of sites with BoP before and after reconstructive treatments with bovine xenografts reported a reduction in the proportion of sites with BoP in the range of 40-60% [27, 29, 44]. Furthermore, this study reported a reduction of approximately 80% in the proportion of sites with SoP in both groups. SoP is not frequently recorded; only few studies included it as an independent parameter using implants or implant sites as the unit of measurement, or reported it as part of a composite therapeutic index [13, 16, 29]. Our results are therefore comparable with the study by Aghazadeh et al. who evaluated the percentage of sites with SoP at baseline and at 1 year post-treatment and reported a mean value of 25% and 1.2% respectively [27]. We also reported that less than 2% of sites still presented SoP at 1 year, however at baseline we recorded SoP in more than 80% of sites.

The use of composite therapeutic endpoints including information on radiographic bone levels, signs of peri-implant soft tissue inflammation, and PPD has been published in multiple reports [33, 34, 39, 45]. In the present study, two different versions of the composite therapeutic index were assessed based on evidence of peri-implant tissue inflammation; (a) absolute absence of BoP and, (b) allowing one site with evidence of BoP. No differences between the two groups were found regardless of the definition used. In the case of ≤ 1 site with BoP accepted the success rate was 18% and 8% for the BO and EB group, respectively. When absolute absence of BoP was the criterion, successful treatment was found in only 18% of the individuals treated with BO and none of the individuals treated with EB. Other studies which used similar criteria reported success rates up to 60% [15, 16, 27, 29]. However, the reported success rates of reconstructive approaches in the literature range widely from as low as 14% up to 60% depending on the definition of the successful outcome, and possibly on the reconstructive approach used and the type of implant surface [39].

The low success rates reported here are associated with the fact that the treatment did not fully resolve the inflammation around the dental impants. Although there was a 50% reduction in the percentage of sites with BoP compared to baseline, at 1 year approximately 50% of sites still presented BoP. This could be attributed possibly to the fact that many implants, especially in the EB group were placed too apically in relation to the CEJ of the adjacent teeth. It has been reported that implants placed too subcrestally are not only prone to greater peri-implant bone loss, but also to a greater magnitude of peri-implant inflammation with increased accumulation of neutrophils [46-48]. Another factor that could have contributed to the lower success rate is related to the amount of keratinized tissue around the implants, which was not evaluated in this study. It has been reported that the lack of keratinized mucosa around implants impairs oral hygiene procedures, and eventually could lead to soft tissue damage, plaque accumulation and bleeding [49, 50]. According to a recent consensus report, despite

the lack of scientific evidence, the increase of non-mobile keratinized mucosa before peri-implant surgical approaches is recommended [51].

An important limitation of the present study lies in the small sample size. Even though the primary objective was to recruit a total of 40 patients, the screening process had to be terminated prematurely due to the relocation of the clinical examiner (D.A.M.) and the surgeon (D.W.). The relatively short follow-up time is another possible drawback; After 12 months, we do not know if the radiographic and clinical parameters remain stable or not.

Although this study was not designed to evaluate the effect of implant surface characteristics on the treatment outcome, this parameter cannot be ruled out [52]. An experimental study in dogs which evaluated re-osseointegration after treatment of peri-implantitis concluded that re-osseointegration took place in implants with rough (SLA) surfaces, but failed to occur in implants with smooth (turned) surfaces [53]. A clinical study in humans which compared the outcome of a reconstructive approach between two different implant surfaces reported improved clinical and radiographic parameters, as well as higher implant survival rates after 7 years in SLA surfaces compared to TPS surfaces [29]. On the other hand, Carcuac et. al reported that surgical therapy of peri-implantitis resulted in superior outcomes at implants with non-modified (turned) surfaces compared to implants with modified surfaces at 3 years [54]. The present study included numerous implant types with different surface modifications (Supplementary Table S1) and what is another limitation is that there was no control in the distribution of implant types and surfaces between the BO and EB group.

In the present study a non-submerged healing mode was applied. Although no randomized controlled trials exist comparing submerged to non-submerged healing and favoring one versus the other, a case series of twelve patients reported favorable results in terms of radiographic defect reduction and reduced PPD using a submerged

healing approach [55]. Nevertheless, these results should be interpreted with caution since no control group was included. Most recent studies evaluating reconstructive approaches in the treatment of peri-implantitis used a non-submerged healing approach and did not report any adverse events in terms of healing [15, 16, 27-29]. Despite the lack of evidence to support one mode of healing versus the other, the submerged post-operative wound closure allows healing in a protective environment and when it is feasible, it is preferred over the non-submerged healing [51].

Within the limitations of this pilot study, we demonstrated that there were no differences between BO and EB for the primary or secondary outcome measures. The treatment with bovine-derived xenografts resulted on average in radiographic defect reduction of approximately 3 mm and in PPD reduction of approximately 4 mm in both groups. Nevertheless, this study showed limited success in the resolution of inflammation. Future studies on the treatment of peri-implantitis should include histologic analysis to evaluate the healing of the peri-implant intra-osseous defect and to prove true re-osseointegration of the diseased implant surface. Longer follow-up times are necessary to confirm the stability of the treatment outcomes.

Supplementary table S1: Baseline data of the 24 included implants.

| Variable	BO (n=11)	EB (n=13)	Total (n=24)
Patient reported tooth loss			
Periodontitis	2 (18%)	2 (15%)	4 (17%)
Caries	5 (46%)	7 (53%)	12 (50%)
Endodontic failure	0 (0%)	1 (8%)	1 (4%)
Trauma	0 (0%)	1 (8%)	1 (4%)
Periodontitis and caries	1 (9%)	1 (8%)	2 (8%)
Endodontic failure and caries	2 (18%)	1 (8%)	3 (13%)
Unknown	1 (9%)	0 (0%)	1 (4%)
Total	11 (100%)	13 (100%)	24 (100%)
Type of prosthesis			
Cement retained single crown	4 (36%)	7 (54%)	11 (46%)
Cement retained FPD/Splinted crowns	4 (36%)	3 (23%)	7 (29%)
Screw retained single crown	3 (27%)	2 (15%)	5 (21%)
Over-denture	0 (0%)	1 (8%)	1 (4%)
Total	11 (100%)	13 (100%)	24 (100%)
Type of implant			
Astra	0 (0%)	1 (8%)	1 (4%)
Biohorizon	0 (0%)	1 (8%)	1 (4%)
Biomet 3i	1 (9%)	3 (23%)	4 (17%)
Camlog	0 (0%)	0 (0%)	0 (0%)
Frialit	1 (9%)	0 (0%)	1 (4%)
MIS	1 (9%)	0 (0%)	1 (4%)
Nobel/Branemark	2 (18%)	4 (31%)	6 (25%)
Straumann	3 (27%)	4 (31%)	7 (29%)
ICX	1 (9%)	0 (0%)	1 (4%)
BioComp	1 (9%)	0 (0%)	1 (4%)
Unknown	1 (9%)	0 (0%)	1 (4%)
Total	11 (100%)	13 (100%)	24 (100%)

Abbreviations: BO, BioOss® ; EB, Endobon®

Supplementary table S2: ICC values based on repeated radiographic measurements of 15 randomly selected patients.

Parameter	ICC value	95% CI	Mean difference (SD)
BL baseline	0.958***	0.876 - 0.986	0.035 (0.34)
BL 6 months	0.948***	0.835 - 0.984	0.138 (0.26)
BL 12 months	0.954***	0.808 - 0.986	0.303 (0.45)
IDD baseline	0.956***	0.824 - 0.987	0.286 (0.43)
IDD 6 months	0.962***	0.877 - 0.988	0.242 (0.51)
IDD 12 months	0.965***	0.895 - 0.988	0.008 (0.49)

Abbreviations: ICC, Intraclass correlation coefficient; BL, bone level; IDD, intrabony defect depth; CI, confidence interval; SD; standard deviation
*** Indicates statistical significance ($p < 0.001$)
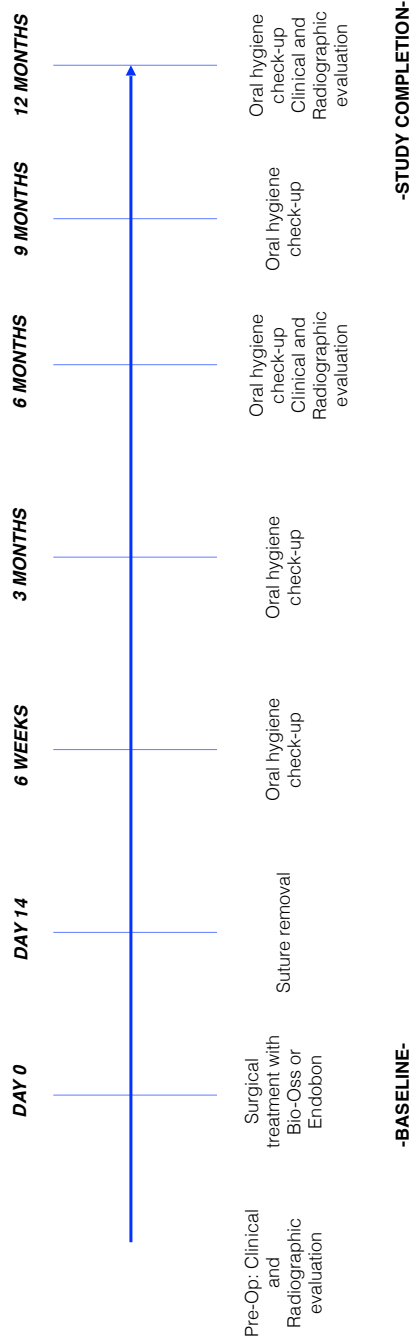
Supplementary Figure 1: Study timeline

References

1.   Schwendicke F, Tu YK, Stolpe M. Preventing and treating peri-implantitis: A cost-effectiveness analysis. J Periodontol. 2015;86(9):1020-9.
2.   Dreyer H, Grischke J, Tiede C, Eberhard J, Schweitzer A, Toikkanen S, et al. Epidemiology and risk factors of peri-implantitis: A systematic review. J Periodontal Res. 2018;53(5):657-81.
3.   Kordbacheh Changi K, Finkelstein J, Papapanou PN. Peri-implantitis prevalence, incidence rate, and risk factors: A study of electronic health records at a u.S. Dental school. Clin Oral Implants Res. 2019;30(4):306-14.
4.   Berglundh T, Armitage G, Araujo MG, Avila-Ortiz G, Blanco J, Camargo PM, et al. Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions. J Periodontol. 2018;89 Suppl 1:S313-s8.
5.   Garaicoa-Pazmino C, Sinjab K, Wang H-L. Current protocols for the treatment of peri-implantitis. Curr Oral Health Rep. 2019;6(3):209-17.
6.   Persson GR, Samuelsson E, Lindahl C, Renvert S. Mechanical non-surgical treatment of peri-implantitis: A single-blinded randomized longitudinal clinical study. Ii. Microbiological results. J Clin Periodontol. 2010;37(6):563-73.
7.   Renvert S, Hirooka H, Polyzois I, Kelekis-Cholakis A, Wang HL. Diagnosis and non-surgical treatment of peri-implant diseases and maintenance care of patients with dental implants - consensus report of working group 3. Int Dent J. 2019;69 Suppl 2:12-7.
8.   Froum SJ, Dagba AS, Shi Y, Perez-Asenjo A, Rosen PS, Wang WC. Successful surgical protocols in the treatment of peri-implantitis: A narrative review of the literature. Implant Dent. 2016;25(3):416-26.
9.   Berglundh T, Wennstrom JL, Lindhe J. Long-term outcome of surgical treatment of peri-implantitis. A 2-11-year retrospective study. Clin Oral Implants Res. 2018;29(4):404-10.
10.   Sarmiento HL, Norton M, Korostoff J, Ko KI, Fiorellini JP. Surgical alternatives for treating peri-implantitis. Int J Periodontics Restorative Dent. 2018;38(5):665-71.
11.   Schwarz F, Sahm N, Bieling K, Becker J. Surgical regenerative treatment of peri-implantitis lesions using a nanocrystalline hydroxyapatite or a natural bone mineral in combination with a collagen membrane: A four-year clinical follow-up report. J Clin Periodontol. 2009;36(9):807-14.

12.   Schwarz F, Sahm N, Schwarz K, Becker J. Impact of defect configuration on the clinical outcome following surgical regenerative therapy of peri-implantitis. J Clin Periodontol. 2010;37(5):449-55.

13.   Wiltfang J, Zernial O, Behrens E, Schlegel A, Warnke PH, Becker ST. Regenerative treatment of peri-implantitis bone defects with a combination of autologous bone and a demineralized xenogenic bone graft: A series of 36 defects. Clin Implant Dent Relat Res. 2012;14(3):421-7.

14.   Roos-Jansaker AM, Persson GR, Lindahl C, Renvert S. Surgical treatment of peri-implantitis using a bone substitute with or without a resorbable membrane: A 5-year follow-up. J Clin Periodontol. 2014;41(11):1108-14.

15.   Jepsen K, Jepsen S, Laine ML, Anssari Moin D, Pilloni A, Zeza B, et al. Reconstruction of peri-implant osseous defects: A multicenter randomized trial. J Dent Res. 2016;95(1):58-66.

16.   Renvert S, Roos-Jansaker AM, Persson GR. Surgical treatment of peri-implantitis lesions with or without the use of a bone substitute-a randomized clinical trial. J Clin Periodontol. 2018;45(10):1266-74.

17.   Khoshkam V, Suarez-Lopez Del Amo F, Monje A, Lin GH, Chan HL, Wang HL. Long-term radiographic and clinical outcomes of regenerative approach for treating peri-implantitis: A systematic review and meta-analysis. Int J Oral Maxillofac Implants. 2016;31(6):1303-10.

18.   Haugen HJ, Lyngstadaas SP, Rossi F, Perale G. Bone grafts: Which is the ideal biomaterial? J Clin Periodontol. 2019;46 Suppl 21:92-102.

19.   Artzi Z, Tal H, Dayan D. Porous bovine bone mineral in healing of human extraction sockets. Part 1: Histomorphometric evaluations at 9 months. J Periodontol. 2000;71(6):1015-23.

20.   Mellonig JT. Human histologic evaluation of a bovine-derived bone xenograft in the treatment of periodontal osseous defects. Int J Periodontics Restorative Dent. 2000;20(1):19-29.

21.   Cordaro L, Bosshardt DD, Palattella P, Rao W, Serino G, Chiapasco M. Maxillary sinus grafting with bio-oss or straumann bone ceramic: Histomorphometric results from a randomized controlled multicenter clinical trial. Clin Oral Implants Res. 2008;19(8):796-803.

22.   Spies CK, Schnurer S, Gotterbarm T, Breusch SJ. Efficacy of bone source and cementek in comparison with endobon in critical size metaphyseal defects, using a minipig model. J Appl Biomater Biomech. 2010;8(3):175-85.

23.   Ramirez-Fernandez MP, Calvo-Guirado JL, Arcesio Delgado-Ruiz R, Mate-Sanchez Del Val JE, Gomez-Moreno G, Guardia J. Experimental model of bone response to xenografts of bovine origin

(endobon): A radiological and histomorphometric study. Clin Oral Implants Res. 2011;22(7):727-34.

24.   Degidi M, Piattelli M, Scarano A, Iezzi G, Piattelli A. Maxillary sinus augmentation with a synthetic cell-binding peptide: Histological and histomorphometrical results in humans. J Oral Implantol. 2004;30(6):376-83.

25.   Jiang D, Dziak R, Lynch SE, Stephan EB. Modification of an osteoconductive anorganic bovine bone mineral matrix with growth factors. J Periodontol. 1999;70(8):834-9.

26.   Berglundh T, Lindhe J. Healing around implants placed in bone defects treated with bio-oss. An experimental study in the dog. Clin Oral Implants Res. 1997;8(2):117-24.

27.   Aghazadeh A, Rutger Persson G, Renvert S. A single-centre randomized controlled clinical trial on the adjunct treatment of intra-bony defects with autogenous bone or a xenograft: Results after 12 months. J Clin Periodontol. 2012;39(7):666-73.

28.   Matarasso S, Iorio Siciliano V, Aglietta M, Andreuccetti G, Salvi GE. Clinical and radiographic outcomes of a combined resective and regenerative approach in the treatment of peri-implantitis: A prospective case series. Clin Oral Implants Res. 2014;25(7):761-7.

29.   Roccuzzo M, Pittoni D, Roccuzzo A, Charrier L, Dalmasso P. Surgical treatment of peri-implantitis intrabony lesions by means of deproteinized bovine bone mineral with 10% collagen: 7-year-results. Clin Oral Implants Res. 2017;28(12):1577-83.

30.   Block MS. The processing of xenografts will result in different clinical responses. J Oral Maxillofac Surg. 2019;77(4):690-7.

31.   Hing KA, Best SM, Bonfield W. Characterization of porous hydroxyapatite. J Mater Sci Mater Med. 1999;10(3):135-45.

32.   Barone A, Todisco M, Ludovichetti M, Gualini F, Aggstaller H, Torres-Lagares D, et al. A prospective, randomized, controlled, multicenter evaluation of extraction socket preservation comparing two bovine xenografts: Clinical and histologic outcomes. Int J Periodontics Restorative Dent. 2013;33(6):795-802.

33.   Heitz-Mayfield LJ, Needleman I, Salvi GE, Pjetursson BE. Consensus statements and clinical recommendations for prevention and management of biologic and technical implant complications. Int J Oral Maxillofac Implants. 2014;29 Suppl:346-50.

34.   Jepsen S, Schwarz F, Cordaro L, Derks J, Hammerle CHF, Heitz-Mayfield LJ, et al. Regeneration of alveolar ridge defects. Consensus report of group 4 of the 15th european workshop on periodontology on bone regeneration. J Clin Periodontol. 2019;46 Suppl 21:277-86.

35.   Cassetta M, Di Giorgio R, Barbato E. Are intraoral radiographs reliable in determining peri-implant marginal bone level changes? The correlation between open surgical measurements and peri-apical radiographs. Int J Oral Maxillofac Surg. 2018;47(10):1358-64.

36.   Serino G, Sato H, Holmes P, Turri A. Intra-surgical vs. Radiographic bone level assessments in measuring peri-implant bone loss. Clin Oral Implants Res. 2017;28(11):1396-400.

37.   Ritter L, Elger MC, Rothamel D, Fienitz T, Zinser M, Schwarz F, et al. Accuracy of peri-implant bone evaluation using cone beam ct, digital intra-oral radiographs and histology. Dentomaxillofac Radiol. 2014;43(6):20130088.

38.   Beaman FD, Bancroft LW, Peterson JJ, Kransdorf MJ, Menke DM, DeOrio JK. Imaging characteristics of bone graft materials. Radiographics. 2006;26(2):373-88.

39.   Tomasi C, Regidor E, Ortiz-Vigon A, Derks J. Efficacy of reconstructive surgical therapy at peri-implantitis-related bone defects. A systematic review and meta-analysis. J Clin Periodontol. 2019;46 Suppl 21:340-56.

40.   Hammerle CH, Chiantella GC, Karring T, Lang NP. The effect of a deproteinized bovine bone mineral on bone regeneration around titanium dental implants. Clin Oral Implants Res. 1998;9(3):151-62.

41.   Benic GI, Thoma DS, Munoz F, Sanz Martin I, Jung RE, Hammerle CH. Guided bone regeneration of peri-implant defects with particulated and block xenogenic bone substitutes. Clin Oral Implants Res. 2016;27(5):567-76.

42.   Roccuzzo M, Bonino F, Bonino L, Dalmasso P. Surgical therapy of peri-implantitis lesions by means of a bovine-derived xenograft: Comparative results of a prospective study on two different implant surfaces. J Clin Periodontol. 2011;38(8):738-45.

43.   Zitzmann NU, Berglundh T. Definition and prevalence of peri-implant diseases. J Clin Periodontol. 2008;35(8 Suppl):286-91.

44.   Schwarz F, Sculean A, Bieling K, Ferrari D, Rothamel D, Becker J. Two-year clinical results following treatment of peri-implantitis lesions using a nanocrystalline hydroxyapatite or a natural bone mineral in combination with a collagen membrane. J Clin Periodontol. 2008;35(1):80-7.

45.   Sanz M, Chapple IL. Clinical research on peri-implant diseases: Consensus report of working group 4. J Clin Periodontol. 2012;39 Suppl 12:202-6.

46.   Broggini N, McManus LM, Hermann JS, Medina R, Schenk RK, Buser D, et al. Peri-implant inflammation defined by the implant-abutment interface. J Dent Res. 2006;85(5):473-8.

47.   Mailoa J, Fu JH, Chan HL, Khoshkam V, Li J, Wang HL. The effect of vertical implant position in relation to adjacent teeth on marginal bone loss in posterior arches: A retrospective study. Int J Oral Maxillofac Implants. 2015;30(4):931-6.

48.   Gatti C, Gatti F, Silvestri M, Mintrone F, Rossi R, Tridondani G, et al. A prospective multicenter study on radiographic crestal bone changes around dental implants placed at crestal or subcrestal level: One-year findings. Int J Oral Maxillofac Implants. 2018;33(4):913-8.

49.   Roccuzzo M, Grasso G, Dalmasso P. Keratinized mucosa around implants in partially edentulous posterior mandible: 10-year results of a prospective comparative study. Clin Oral Implants Res. 2016;27(4):491-6.

50.   Monje A, Insua A, Wang HL. Understanding peri-implantitis as a plaque-associated and site-specific entity: On the local predisposing factors. J Clin Med. 2019;8(2):279.

51.   Khoury F, Keeve PL, Ramanauskaite A, Schwarz F, Koo KT, Sculean A, et al. Surgical treatment of peri-implantitis - consensus report of working group 4. Int Dent J. 2019;69 Suppl 2:18-22.

52.   Albouy JP, Abrahamsson I, Persson LG, Berglundh T. Implant surface characteristics influence the outcome of treatment of peri-implantitis: An experimental study in dogs. J Clin Periodontol. 2011;38(1):58-64.

53.   Persson LG, Berglundh T, Lindhe J, Sennerby L. Re-osseointegration after treatment of peri-implantitis at different implant surfaces. An experimental study in the dog. Clin Oral Implants Res. 2001;12(6):595-603.

54.   Carcuac O, Derks J, Abrahamsson I, Wennström J, Petzold M, Berglundh T. Surgical treatment of peri-implantitis: 3-year results from a randomized controlled clinical trial. J Clin Periodontol. 2017;44(12):1294-303.

55.   Roos-Jansaker AM, Renvert H, Lindahl C, Renvert S. Submerged healing following surgical treatment of peri-implantitis: A case series. J Clin Periodontol. 2007;34(8):723-7.

CHAPTER 6

Occlusal migration of teeth adjacent to implant prostheses in adults; A long-term study

ABSTRACT

Purpose

To evaluate the effect of continuous tooth eruption on the outcomes of single-implant–supported restorations in the anterior maxilla of adults.

Materials and Methods

Seventy-six patients (age: 21 to 78 years) treated with single-implant–supported restorations in the esthetic zone were included. Radiographs obtained at crown placement and follow-up examinations from 1 to 15 years postloading were analyzed with regard to vertical incisal plane changes of the implant-supported crown relative to adjacent teeth.

Results

Infraocclusion increased over time by 0.08 ± 0.02 mm/year. Infraocclusion was more pronounced ($P = .04$) for delayed (0.09 mm/year) versus immediate implant placement (0.06 mm/year) and for younger versus older adults (0.0013 mm/year per additional year of age; $P = .014$). No statistically significant association between infraocclusion and sex, ethnicity, implant site, timing of implant temporization, surgical protocol, and type of restoration was found.

Conclusion

Infraocclusion of single-implant–supported maxillary anterior restorations may result in esthetic concerns over time. Greater infraocclusion occurs in delayed implant placement and in younger individuals.

6.1 Introduction

Implant-supported restorations are considered the most biomimetic design for the replacement of single missing teeth. The good long-term success and high patient satisfaction in terms of esthetic outcomes have made single implants a routine procedure [1-3]. However, the most challenging area for single-tooth implant use is the anterior maxilla due to high esthetic demands [4].

It has been well-understood that growing individuals are not good candidates for single implant therapy, since the implant, like an ankylosed tooth, fails to adapt to the maxillo-mandibular and alveolar growth as well as to the continuous eruption of the adjacent natural teeth. This results in disharmony of the occlusal plane described as infra-occlusion or infra-positioning of the implant supported restoration [5, 6]. Clinical studies have shown that the placement of implants in adolescents results in infra occlusion of 0.1 to 2.2 mm at follow-up times ranging from 3 to 10 years [7, 8].

The assumption that adults cease to grow is no longer valid; although more subtle and slowly progressing over decades, changes in adults do occur even after the age of 40 years, and have a long-term effect on single implant supported restorations adjacent to natural teeth [9]. These changes are more pronounced in the vertical dimension with a continuous increase in the lower anterior facial height which has been attributed to an increase in the anterior dentoalveolar height, as well as to the continuous eruptive movement of the teeth [10-12]. An average increase of 1.6 mm in lower anterior facial height has been reported in patients from 25 to 45 years of age; 0.95 mm of it was attributed to the continuous eruption of maxillary incisors [11]. Following the active growth phase, the incisal vertical change progresses at a mean rate of 0.1 mm per year [13].

The continuous eruption of teeth in adults results in infra-occlusal positioning of dental implants (Fig. 1) [5, 6, 14-17]. The magnitude of im-

plant infra-position in adult patients reported in the literature ranges from 0.10 to 1.86 mm at follow up intervals from 1 to 15 years [4, 6, 14, 15, 18, 19]. Gender and face anatomy were identified as significant factors for the development of infra-position of the single-implant restorations, with females and patients with long face type presenting a higher risk [4, 15-17]. Some studies have also reported an association between the amount of vertical eruption and implant location. Some studies have reported that central and lateral incisors but not canines or premolars had a significant increase of clinical crown height [15, 18].

Figure 1: Clinical images illustrating a single-implant crown at the right lateral incisor position at baseline (a), after 2 years (b), after 5 years (c) and after 8 years (d) of functional loading. Note the infra-position of the implant crown which is more pronounced at 8 years.

Even small amounts of infra-occlusion of single implant-supported restorations in the anterior maxilla can cause esthetic concerns. Changing the incisors' proportions affects the relative smile attractiveness, which in turn affects the perception of facial attractiveness [20]. It has been reported that even though small changes of up to 1mm in the width of the incisors are still accepted as esthetic by the patients, any changes in the length of the incisors are not well tolerated [21]. Psychosocial research suggests that there is a link between beauty and health; adults with excellent dental esthetics have more favorable oral-

hygiene attitudes and preventive behaviors (toothbrushing and dental visits), than those with adverse dental esthetics [22, 23].

The primary aim of the present study was to evaluate the longitudinal changes in the position of single-implant prostheses adjacent to teeth in the anterior maxilla of adult patients. The secondary aim was to associate the observed changes with patient or surgery related parameters including: 1) gender, 2) age, 3) country of origin, 4) implant location, 5) implant surgical protocol: 5.a) immediate versus delayed implant placement, 5.b) one versus two-stage implants, 5.c) the performance of guided bone regeneration at the time of implant placement and 6) implant temporization with provisional prosthesis (immediate versus delayed).

6.2 Materials and Methods

Subjects

The subjects for this study were selected retrospectively from the pool of patients who were treated with single implant-supported restorations in the anterior maxilla at the Department of Periodontics and Oral Medicine, University of Michigan, Ann Arbor, USA, and at the Center of Digital Dentistry, Peking University School and Hospital of Stomatology, Beijing, China.

To be included in the study, the patients had to be older than 20 years at the time of implant surgery with available radiographs of the implant site at crown delivery (baseline) and at least one follow-up within the range of 1 to 15 years post-loading. The adjacent natural teeth were periodontally healthy or periodontally stable according to the following criteria: absence of pockets >4 mm, absence of mobility, absence of clinical and radiographic signs of trauma from occlusion, and no radiographic evidence of progressive alveolar bone loss. The exclusion criteria included: metabolic bone diseases (e.g. Paget's disease, hypercalcemia, vitamin D3 abnormalities, osteoporosis), history of bisphos-

phonate use, anterior open bite and/or cross-bite, presence of peri-implantitis (bleeding/suppuration on probing and >2 mm bone loss) [24], and use of orthodontic appliances. In order to confirm eligibility for the study, all dental charts and intra-oral radiographs were screened by two calibrated examiners (AP, QL). The study was approved by the Institutional Review Board (IRB) of the University of Michigan (HUM00119425) and the ethical committee of School and Hospital of Stomatology, Peking University (PKUSSIRB-201839159).

Radiographic measurements

The existing peri-apical intra-oral radiographs, taken with the 'parallel technique' (Rinn holder, Dentsply) were used to assess implant infra-occlusion over time. The radiographic images were evaluated using an open source image processing program (ImageJ/Fiji 1.46, U.S. National Institutes of Health) [25]. Images were calibrated on Image J by measuring the radiographic length of the implant (L) and comparing it to the known implant length.

The implant crown infra-occlusion was measured as follows; (a) selection of an easily identifiable point of reference located on the implant-abutment junction (A), (b) selection of a point of reference located on the adjacent mesial tooth (B). The point of reference on the tooth was either the cementoenamel junction (CEJ), the margin of a restoration, or, the intersection between the incisal and distal border of the mesial tooth. If no points of reference could be identified on the adjacent mesial tooth, the distal tooth was used instead. Subsequently, (c) the vertical distance between the two reference points (AB) was measured in mm at baseline and at follow-up examinations (Fig. 2). The amount of infra-occlusion was calculated based on the difference of the above mentioned measurements.
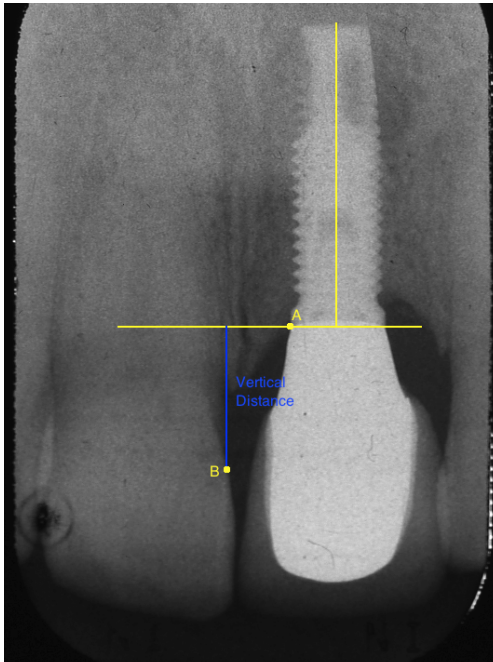
Figure 2: Radiographic assessment of implant infra-occlusion over time. The vertical distance was measured in mm between A (implant-abutment junction) and B (CEJ of the adjacent mesial tooth). The same measurement was performed on the radiograph taken at each follow-up examination. The amount of infra-occlusion (in mm) was calculated based on the difference between the baseline and follow-up measurements.

Data Analysis

All radiographic measurements were performed independently by two examiners (AP, QL). Each examiner was blinded to the measurements made by the other examiner. The inter-rater reliability was calculated based on an intercept-only linear mixed model with infra-occlusion as the outcome and random intercepts for the rater and for each combination of subject and time. A value of 0.88, with a 95% confidence interval (CI) ranging from 0.81 to 0.93, showed good agreement between the examiners.

Infra-occlusion was modeled in terms of time after the delivery of the permanent implant-supported crown in a linear mixed model, with intercept set to 0. Also, it was investigated how the rate of infraocclusion varied based on country of origin (China vs USA), gender, age, implant site (central incisors, lateral incisors, canines), type of restoration (ce-

ment retained vs screw retained), time of implant surgery (delayed vs immediate), implant surgical protocol (two stage vs one stage), performance of guided bone regeneration (yes vs no), and implant temporization with provisional restoration (delayed vs immediate). The statistical analysis used the open source software package R (version 3.3.2, 2016); linear mixed models were created with the lme4 package lme4 and p-values were determined by the lmerTest package. Statistical significance was set at 5%.

6.3 Results

Seventy-six patients (40 patients from the US and 36 patients from China) were included in the study. The sample included 46 males and 30 females. The mean age of the patients at implant surgery was 45 years ranging from 21 to 78 years. The patients were treated with 77 single implant-supported crowns in the anterior maxilla including 48 central incisors, 24 lateral incisors, and 5 canines. The baseline demographic characteristics of all study participants as well as the distribution of factors with a potential effect on the rate of infra-occlusion are displayed in Table 1.

Table 1: Patient demographics and distribution of factors with potential relationship to the presence of implant infra-occlusion. Data are presented separately for patients treated in the USA and China, along with the total number of patients studied.

	USA	China	Total
Number of patients	40	36	76
Gender (Male/Female)	28/12	18/18	46/30

Mean age (Range)	56 (21-78)	36 (25-48)	45 (21-78)
Implant sites			
Central incisors	26	22	48
Lateral incisors	13	11	24
Canines	1	4	5
Total	40	37	77
Implant prosthesis			
Cement retained	25	34	59
Screw retained	9	1	10
Not-known	6	2	8
Implant surgical protocol			
Immediate/Delayed	20/20	8/29	28/49
One stage/Two stage	12/28	10/26*	22/54*
Provisional restoration			
Immediate temporization	8	7	15
Delayed temporization	30	30	60
Not-known	2	0	2
Intra-operative guided bone re-generation (Yes/No)	12/28	17/20	29/48
Intra-operative use of bone graft			
None	12	6	18
Autograft	0	5	5
Allograft	21	0	21
Xenograft	2	19	21
Mixture with autograft	4	7	11
Polymer-based	1	0	1

Implant brand			
Zimmer	17	0	17
Nobel/Brånemark	13	24	37
ITI/Straumann	1	7	8
Biohorizons	6	0	6
NeoBiotech	1	0	1
3i	0	6	6
Sultzer	1	0	1
Sybron	1	0	1
Years of follow-up (range)	1-11	1-15	1-15

* Missing data for one implant site

The vertical tooth movement in adults resulted in infra-occlusion of single implant-supported restorations in the anterior maxilla which reached a maximum of 1.67 mm for a period of up to 15 years after the permanent prosthesis delivery. Infra-occlusion progressed over time by 0.08 mm/year, with a 95% CI ranging from 0.06 to 0.1 mm/year. The rate of infra-occlusion showed a significant change over time ($p < 0.001$) (Fig. 3). In some cases the longitudinal vertical change in the position of the teeth relative to the single implant-supported restorations was minimal (less than 0.5 mm) even at longer follow-up times, whereas in other cases the infra-occlusion progressed at a faster rate (Fig. 3).

Interestingly, infra-occlusion was more pronounced for delayed versus immediate implant placement (mean rate 0.09 mm/yr vs 0.06 mm/yr, respectively, p=0.04), (Fig. 4). Another factor which was found to be significantly associated with infra-occlusion was age. Infra-occlusion progressed faster for younger subjects than for older subjects. It was calculated that the rate of infra-occlusion decreased by 0.0013 mm/yr per additional year of age at implant placement (p=0.014), (Fig. 5).
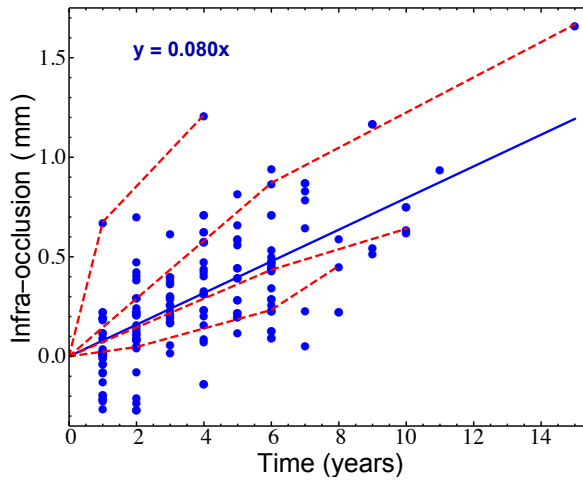
Figure 3: Infra-occlusion (mm) by years after implantation with an overall trend line derived from the linear mixed model results (blue solid line). While infra-occlusion progressed at a rate of 0.08 mm/year on average, individual variations existed. The red dashed lines show the progression of infra-occlusion in four different patients over time, exhibiting larger or smaller progression rates.

A more pronounced vertical change was observed in implants placed at central incisor location compared to lateral incisors, as well as in implants which were temporized with a provisional prosthesis using a delayed protocol compared to implants which received immediate temporization. None of these differences however was statistically significant (Table 2).
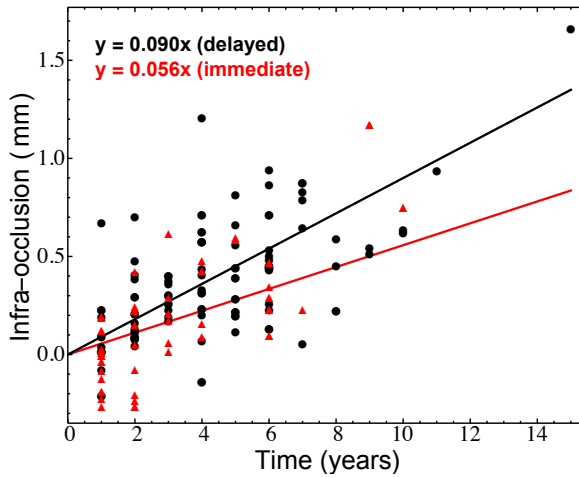
Figure 4: Infra-occlusion (mm) for delayed (black dots) and immediate implant placement (red triangles). A trend line derived from the linear mixed model results for each group is shown. The rate of infra-occlusion is higher for delayed implant placement than for immediate implant placement at a statistically significant level (p=0.04).

No association between infra-occlusion and gender, country of origin (USA vs China), implant site, surgical protocol (one vs two stage), application of guided bone regeneration, and type of permanent prosthesis (screw vs cement retained) was found (Table 2). Moreover, no differences existed in the amount of infra-occlusion for various implant brands, as well as for the types of bone grafts used (data not shown).
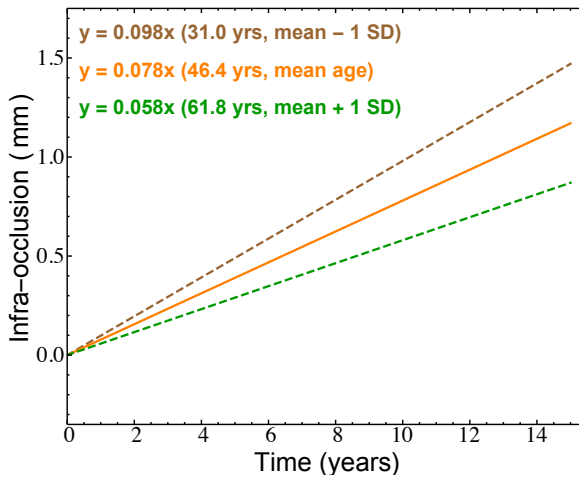
Figure 5: The predicted infra-occlusion over time for patients that are 61.8 and 31 years old. These correspond to ages that are one standard deviation above and below the mean age of the patients at implant placement. Based on our analysis, infra-occlusion is expected to progress faster for younger subjects than for older subjects.

Table 2: Differences in rates of infra-occlusion according to various patient and surgery related parameters. Results are derived using a linear mixed model. The timing of implant placement (immediate vs delayed) and the age of the patients at implant surgery showed statistically significant association with infra-occlusion.

Variable or comparison	Estimated difference in the rate of infraocclusion (mm/year)	95% confidence interval for the difference in the rate of infraocclusion (mm/year)	P-value
China vs U.S.	0.018	[-0.012, 0.049]	0.24
Female vs male	0.018	[-0.012, 0.048]	0.25
Age	-0.0013/additional year of age	[-0.0023, -0.00029] / additional year of age	0.014
Central incisors vs lateral incisors	0.028	[-0.0062, 0.062]	0.12
Canines vs lateral incisors	0.02	[-0.039, 0.080]	0.51
Cement retained vs screw retained prosthesis	0.0071	[-0.045, 0.058]	0.79
Delayed vs immediate implant placement	0.034	[0.0024, 0.067]	0.040

Implant surgical protocol: two stage vs one stage	-0.0036	[-0.038, 0.031]	0.84
Provisional: delayed vs immediate temporization	0.030	[-0.012, 0.072]	0.16
Guided bone regeneration: no vs yes	0.0045	[-0.026, 0.035]	0.77

6.4 Discussion

Besides adolescents and young adults [6-8, 19], infra-occlusion of the single implant crown in relation to the adjacent teeth has also been observed in mature adults [14]. Similar to an ankylosed tooth, the implant does not follow the facial bone growth and the continuous eruption of adjacent teeth, resulting in a discrepancy of the occlusal plane which might eventually raise esthetic concerns [26, 27]. This phenomenon is more pronounced during the active growth phase when the teeth erupt at a rate of 1.2 to 1.5 mm per year. After the growth spurt, the eruption of teeth continues at a slower rate of 0.1-0.2 mm per year [5, 13]. Kawanami and coworkers evaluated the infra-position of ankylosed incisors and reported an infra-position rate of 0.07 mm per year in adult patients [28].

In the present study, we found that infra-occlusion of single implant-supported restorations in the anterior maxilla of adult patients ranged from no obvious changes to 1.67 mm for a period of 1 to 15 years after the permanent prosthesis delivery. Infra-occlusion of a single implant-supported crown was measured as eruptive movement of neighboring

natural teeth. We observed an average infra-occlusion rate of 0.08 mm per year. These results are consistent with previous studies; Bernard and coworkers reported infra-occlusion from 0.12 to 1.86 mm in mature adults aged from 40 to 55 years who were followed for a mean period of 4 years (range from 1 to 9 years) [14]. Chang and coworkers reported mean vertical changes of teeth adjacent to single implants in the maxilla of 0.1 mm (range: 0.03 - 0.19 mm) at 1 year, 0.3 mm (range 0.18 - 0.41 mm) at 5 years, and 0.4 mm (range: 0.15 - 0.60 mm) at 8 years in 31 adult patients with a mean age of 40 years [18]. In a prospective 3 year study Vilhjalmsson and colleagues measured the continuous eruption of teeth adjacent to single implants in the anterior maxilla of 50 adult patients aged 35 years (range 20 to 56 years) and reported a mean value of 0.67 mm, ranging from 0.13 to 1.75 mm [27]. In a group of 10 young adults (mean age: 20 ± 1.4 years) treated with single implant supported crowns to replace congenitally missing maxillary central and/or lateral incisors, Jamilian and coworkers reported that all cases had significant infra-occlusion of more than 1 mm at 5 years [19].

Even though infra-occlusion increased over time, inter-individual variations existed. Most patients remained relatively stable throughout time presenting small signs of infra-occlusion (less than 0.5 mm), while a few cases showed more severe infra-occlusion of more than 1 mm. Similar variations among individuals have also been reported by other researchers. Andersson and colleagues followed-up 57 adult patients (age range 15-57 years) for 17 to 19 years and found that half of the patients remained relatively stable with less than 0.5 mm of infra-occlusion. Severe infra-occlusion of more than 1 mm was evident in 35% of the patients [4]. Jemt and coworkers evaluated clinically 25 patients (mean age 25 ± 10 years) restored with 28 single implant crowns in the anterior maxilla. After 15-17 years, the majority of patients showed insignificant infra-position of less than half a millimeter. Only 11% of the patients showed clinical infra-position from 0.5 to 1 mm and 14% of the patients showed severe infra-position of more than 1 mm [16].

In the present study, age was identified as a significant factor for the development of implant infra-occlusion. It was estimated that the rate of infra-occlusion decreased by 0.0013 mm per year per additional year of age at implantation. We concluded therefore, that infra-occlusion developed faster in younger than in older adults. Other studies have also reported an association between age and the development of infra-occlusion, however these studies included only adolescents and young adults up to 20 years of age [6-8, 13, 19]. On the other hand, Bernard and coworkers compared "young adults" (age range 15.5 to 21 years) and "mature adults" (age range 40 to 55 years) and found no differences in the amount of infra-occlusion between the two groups [14]. Another two studies which included only adult patients failed to identify age as a risk factor for the development of infra-occlusion [18, 27]. These differences between the results of the present study and previous studies might be partially explained by the variations in skeletal maturation, tooth eruption and continuous growth patterns which exist among individuals [7]. Furthermore, tooth wear is a common occurrence in adults and increases with age [29-31]. In cases where a single implant crown is adjacent to natural teeth which are subject to incisal wear, this may counteract the changes in incisor plane in the esthetic zone up to an extent.

Gender and implant site have also been studied as possible factors associated with implant infra-occlusion. Female patients have been reported to be at higher risk of implant infra-position [4, 15, 16] even though this has not been confirmed by other investigators [14, 18, 27]. Our results indicated that infra-occlusal positioning of dental implants was not associated with gender. Regarding implant location as a risk factor for infra-occlusion, Chang and coworkers reported more pronounced vertical changes for the maxillary incisors compared to maxillary premolars [18]. Nevertheless, studies which included only the anterior maxillary teeth (canine to canine) failed to show any differences in the magnitude of infra-occlusion of the implant-supported crown at various locations of the implant (central incisors, lateral incisors, or canines) [14, 27]. Here, we report a trend for implants located in central

incisor position to show more infra-occlusion compared to lateral incisors and canines although the differences did not reach statistical significance. This warrants further investigation with larger sample size. A "long face" type has also been reported as a possible risk factor for infra-occlusion [4, 16, 26], however in the present study face anatomy was not evaluated due to lack of relevant data in patients' records.

The literature regarding surgery-related and prosthesis-related parameters as possible risk factors for implant infra-occlusion has been very scarce so far. Vilhjalmsson and coworkers in a prospective 3-year study found no effect of preoperative bone augmentation and type of implant on the development of infra-occlusion over time [27]. In the present study, infra-occlusion progressed faster for implants placed using a delayed protocol than for implants placed immediately after dental extraction. To the best of the authors' knowledge this is the first study that reports an association between the timing of implant placement (immediate vs delayed) and the rate of progression of infra-occlusion. Moreover, a more pronounced vertical change was observed in implants that received delayed temporization with provisional prosthesis compared to implants which were temporized immediately, even though this difference did not reach statistical significance. On the other hand, the implant brand, implant surgical protocol (one versus two staged approach), performance of intra-operative guided bone regeneration, type of bone graft and type of permanent implant prosthesis (screw retained versus cement retained) appeared to have no effect on the development of infra-occlusion. The variables implant brand and type of bone graft included multiple sub-categories (see Table 1) with very few data in most of them. Our analysis did not yield anything of statistical significance, however, more research with larger sample sizes is needed in order to further elaborate on these associations.

The use of radiographs to assess vertical tooth movement in relation to single implant crowns has some inherent limitations. Since the radiographic evaluation was performed retrospectively, the existing periapical radiographs were not fully standardized (i.e. with the use of cus-

tomized acrylic stents), therefore the radiographic measurements were subject to errors. In an effort to overcome these limitations we included radiographs with no obvious distortion, where the implant threads could be clearly identified on the mesial as well as on the distal aspect of the implant. Also, the radiographs were calibrated using the known implant length and the linear measurements were performed by two calibrated independent examiners.

For future prospective studies we recommend a larger sample size with standardized, age, gender, surgical conditions, and implant brands, as well as the use of fully standardized, digital radiographic technique which allows for more accurate measurements. Since tooth wear, may, to some extent, counteract the the changes caused by the continuous eruption of teeth it would be useful to use a tooth wear index at baseline and at the follow-up examinations in order to estimate the amount of infra-occlusion more precisely. Given our findings, which were solely based on radiographic evaluation, it would be interesting for future studies to correlate the radiographic measurements with a clinical assessment of infra-occlusion, as well as the patient's assessment of the aesthetic result.

Conclusions

The present study confirms that adult patients with implant-supported restorations in the esthetic zone display long-term changes which may jeopardize the implant aesthetics. However, the magnitude of infra-occlusion was small, showing in most situations differences in incisor plane between teeth and implant restorations of less than 1 mm. This means that in most cases a replacement of the implant supported crown will address the esthetic concerns. In more severe cases, restorative adjustments of the adjacent natural teeth might be necessary in conjunction with the replacement of the implant-supported prosthesis [32]. Age and implant placement using a delayed approach were the only variables with a significant effect on the rate of implant-infra-occlusion.

Acknowledgements

References

1.   Henry PJ, Laney WR, Jemt T, Harris D, Krogh PH, Polizzi G, et al. Osseointegrated implants for single-tooth replacement: A prospective 5-year multicenter study. Int J Oral Maxillofac Implants. 1996;11(4):450-5.

2.   Mozzati M, Gallesio G, Del Fabbro M. Long-term (9-12 years) outcomes of titanium implants with an oxidized surface: A retrospective investigation on 209 implants. J Oral Implantol. 2015;41(4):437-43.

3.   Angkaew C, Serichetaphongse P, Krisdapong S, Dart MM, Pimkhaokham A. Oral health-related quality of life and esthetic outcome in single anterior maxillary implants. Clin Oral Implants Res. 2017;28(9):1089-96.

4.   Andersson B, Bergenblock S, Furst B, Jemt T. Long-term function of single-implant restorations: A 17- to 19-year follow-up study on implant infraposition related to the shape of the face and patients' satisfaction. Clin Implant Dent Relat Res. 2013;15(4):471-80.

5.   Iseri H, Solow B. Continued eruption of maxillary incisors and first molars in girls from 9 to 25 years, studied by the implant method. Eur J Orthod. 1996;18(3):245-56.

6.   Thilander B, Odman J, Jemt T. Single implants in the upper incisor region and their relationship to the adjacent teeth. An 8-year follow-up study. Clin Oral Implants Res. 1999;10(5):346-55.

7.   Thilander B, Odman J, Grondahl K, Friberg B. Osseointegrated implants in adolescents. An alternative in replacing missing teeth? Eur J Orthod. 1994;16(2):84-95.

8.   Thilander B, Odman J, Lekholm U. Orthodontic aspects of the use of oral implants in adolescents: A 10-year follow-up study. Eur J Orthod. 2001;23(6):715-31.

9.   Oesterle LJ, Cronin RJ, Jr. Adult growth, aging, and the single-tooth implant. Int J Oral Maxillofac Implants. 2000;15(2):252-60.

10.   Sarnas KV, Solow B. Early adult changes in the skeletal and soft-tissue profile. Eur J Orthod. 1980;2(1):1-12.

11.   Forsberg CM, Eliasson S, Westergren H. Face height and tooth eruption in adults--a 20-year follow-up investigation. Eur J Orthod. 1991;13(4):249-54.

12.   Tallgren A, Solow B. Age differences in adult dentoalveolar heights. Eur J Orthod. 1991;13(2):149-56.

13.   Op Heij DG, Opdebeeck H, van Steenberghe D, Quirynen M. Age as compromising factor for implant insertion. Periodontol 2000. 2003;33:172-84.

14.   Bernard JP, Schatz JP, Christou P, Belser U, Kiliaridis S. Long-term vertical changes of the anterior maxillary teeth adjacent to single implants in young and mature adults. A retrospective study. J Clin Periodontol. 2004;31(11):1024-8.

15.   Jemt T, Ahlberg G, Henriksson K, Bondevik O. Changes of anterior clinical crown height in patients provided with single-implant restorations after more than 15 years of follow-up. Int J Prosthodont. 2006;19(5):455-61.

16.   Jemt T, Ahlberg G, Henriksson K, Bondevik O. Tooth movements adjacent to single-implant restorations after more than 15 years of follow-up. Int J Prosthodont. 2007;20(6):626-32.

17.   Jemt T. Single implants in the anterior maxilla after 15 years of follow-up: Comparison with central implants in the edentulous maxilla. Int J Prosthodont. 2008;21(5):400-8.

18.   Chang M, Wennstrom JL. Longitudinal changes in tooth/single-implant relationship and bone topography: An 8-year retrospective analysis. Clin Implant Dent Relat Res. 2012;14(3):388-94.

19.   Jamilian A, Perillo L, Rosa M. Missing upper incisors: A retrospective study of orthodontic space closure versus implant. Prog Orthod. 2015;16:2.

20.   Machado AW. 10 commandments of smile esthetics. Dental Press J Orthod. 2014;19(4):136-57.

21.   Alsulaimani FF, Batwa W. Incisors' proportions in smile esthetics. J Orthod Sci. 2013;2(3):109-12.

22.   Klages U, Bruckner A, Guld Y, Zentner A. Dental esthetics, orthodontic treatment, and oral-health attitudes in young adults. Am J Orthod Dentofacial Orthop. 2005;128(4):442-9.

23.   Dumitrescu AL, Dogaru BC, Dogaru CD. Self-control and self-confidence: Their relationship to self-rated oral health status and behaviours. Oral Health Prev Dent. 2009;7(2):155-62.

24.   Derks J, Schaller D, Hakansson J, Wennstrom JL, Tomasi C. Peri-implantitis - onset and pattern of progression. J Clin Periodontol. 2016;43(4):383-8.

25.   Mailoa J, Fu JH, Chan HL, Khoshkam V, Li J, Wang HL. The effect of vertical implant position in relation to adjacent teeth on marginal bone loss in posterior arches: A retrospective study. Int J Oral Maxillofac Implants. 2015;30(4):931-6.

26.   Heij DG, Opdebeeck H, van Steenberghe D, Kokich VG, Belser U, Quirynen M. Facial development, continuous tooth eruption, and mesial drift as compromising factors for implant placement. Int J Oral Maxillofac Implants. 2006;21(6):867-78.

27.   Vilhjalmsson VH, Klock KS, Storksen K, Bardsen A. Radiological evaluation of single implants in maxillary anterior sites with special emphasis on their relation to adjacent teeth: A 3-year follow-up study. Dent Traumatol. 2013;29(1):66-72.

28.   Kawanami M, Andreasen JO, Borum MK, Schou S, Hjorting-Hansen E, Kato H. Infraposition of ankylosed permanent maxillary incisors after replantation related to age and sex. Endod Dent Traumatol. 1999;15(2):50-6.

29.   Kaifu Y, Kasai K, Townsend GC, Richards LC. Tooth wear and the "design" of the human dentition: A perspective from evolutionary medicine. Am J Phys Anthropol. 2003;Suppl 37:47-61.

30.   Van't Spijker A, Rodriguez JM, Kreulen CM, Bronkhorst EM, Bartlett DW, Creugers NH. Prevalence of tooth wear in adults. Int J Prosthodont. 2009;22(1):35-42.

31.   Margvelashvili A, Zollikofer CP, Lordkipanidze D, Peltomaki T, Ponce de Leon MS. Tooth wear and dentoalveolar remodeling are key factors of morphological variation in the dmanisi mandibles. Proc Natl Acad Sci U S A. 2013;110(43):17278-83.

32.   Zitzmann NU, Arnold D, Ball J, Brusco D, Triaca A, Verna C. Treatment strategies for infraoccluded dental implants. J Prosthet Dent. 2015;113(3):169-74.

CHAPTER 7

General discussion

7.1 General discussion

Implant-supported restorations are considered the most biomimetic design for the replacement of missing teeth, and the high success rates reported in the literature have made them a popular treatment option [1]. However, the increasing use of dental implants has led to increasing frequency of implant-associated complications [2, 3]. Peri-implantitis is the most challenging biologic complication which can eventually lead to implant loss [4]. According to the 2017 World Workshop, peri-implantitis is defined as "a plaque-associated pathological condition occurring in tissues around dental implants, characterized by inflammation in the peri-implant mucosa and subsequent progressive loss of supporting bone" [5, 6]. Although the prevalence of peri-implantitis ranges widely in the literature, from 1% to 85%, depending on the disease definition [7, 8], a recent study concluded that approximately 1 out of 3 patients and 1 out of 5 implants experienced peri-implantitis [9]. Furthermore, studies with similar peri-implantitis case definition and follow-up time showed that there could be regional differences in the prevalence of peri-implantitis, with approximately 10% higher prevalence in the U.S. as compared to Europe (26% vs 16% at patient level) [10, 11].

Given the possible regional differences in the prevalence of peri-implantitis, as well as the increasing frequency of peri-implantitis that many clinicians coming from different professional backgrounds encounter in their everyday clinical practice [3, 8, 12], one important question is to which degree periodontists in different parts of the world have common peri-implantitis-related diagnostic and therapeutic attitudes. Therefore, a transatlantic comparison of professional behavior related to peri-implantitis was initiated. The objective of **chapter 2** was to compare the responses of periodontists in the U.S. vs. Europe concerning peri-implantitis-related risk factors, diagnostic criteria and treatment approaches. Interestingly, the considerations and attitudes towards peri-implantitis of all respondents were evidence-based and in line with the current literature. Although both groups gave overall similar answers concerning peri-implantitis-related risk factors and diagnostic criteria,

there were some differences in the management of peri-implantitis. The U.S. periodontists were more likely to use antibiotics, lasers, allograft and regenerative approaches and less likely to use resective surgery than European periodontists. These discrepancies in the management of peri-implantitis between European and U.S. periodontists could have been expected, since the existing literature has not yet identified a "gold standard" treatment protocol [13, 14]. Nevertheless, both groups acknowledged that peri-implantitis is an emerging, significant concern and that there is a need to educate general dentists better about identifying risk factors, diagnosing and referring peri-implantitis cases for treatment to specialists. The knowledge of the discrepancies in attitudes towards the treatment of peri-implantitis between European and U.S. dental specialists is useful for the planners and attendees of consensus meetings. Therefore, the results of this study could be useful not only for shaping future educational efforts, but also show the need to organize a World Workshop on the guidelines or steps in the treatment of peri-implantitis, similar to the approach used recently for the treatment of periodontitis [15, 16]. One aspect that came forward from the work in **chapter 2** and needs special attention in treatment guidelines for peri-implantitis, is the possible superfluous use of antibiotics; according to FDI, dentists are responsible for 10% of all antibiotics prescriptions [17]. Therefore, the worldwide increases in antibiotic resistance should be of particular concern to the dental community [17]. Clear guidelines are essential to tackle the overuse of antibiotics and to encourage dentists to optimize antibiotic prescription attitudes. Antibiotic stewardship aims at measuring and improving how antibiotics are prescribed by clinicians and used by patients, playing therefore a pivotal role in the efforts to tackle antibiotic resistance [18, 19]. In this thesis this aspect is also addressed in **chapter 4**.

Although numerous factors have been associated with the onset and progression of peri-implantitis, including systemic, local, genetic, behavioral and iatrogenic factors, consensus exists that biofilm (environment) plays an important role in the etiology of peri-implantitis, as an initial trigger for inflammatory reactions [4, 20, 21]. Dysbiotic biofilms in the

submucosal region may cause tissue inflammation, which alters the ecology and favors further expansion of dysbiotic microbial communities, leading to a vicious cycle, similar to periodontitis [22, 23]. Common opinion states that the pathogenic bacteria in peri-implant sulcus/pocket are very comparable to those traditionally identified in periodontitis, with a dominance of cultivable and non-cultivable Gram-negative anaerobic bacterial species [20]. Current more advanced molecular techniques than the traditional culturing, microscopic or immunofluorescence techniques can lead to a better understanding of the microbial profiles of the peri-implant sulcus/pocket. This is of great importance to gain insight in the types of dysbiosis encountered, and to compare from these new perspectives with the existing knowledge on gingivitis and periodontitis associated dysbiotic biofilms. Moreover, new knowledge on the peri-implant sulcus/pocket associated microbiomes (healthy and diseased) may be helpful to better motivate the need of certain antibiotics in the treatment of peri-implantitis. In fact, one may question the need for such pharmacological support. Therefore, a cross-sectional study (**chapter 3**) aimed to describe the peri-implant microbiome at healthy implants, at implants with peri-implant mucositis and at implants with peri-implantitis using 16S rDNA amplicon sequencing. With the results, we explored possible associations of the microbial composition with several patient- and implant-related parameters. Significant associations of specific bacterial communities were found with the following factors; probing depth (<5 mm vs ≥5 mm), implant disease status (healthy implant / peri-implant mucositis / peri-implantitis) and dentition status (partially vs fully edentulous patients). The factors bleeding on probing, implant system, time of functional loading, and sex were not associated with the composition of the peri-implant biofilms. For clarity and proper interpretation of the results of the study in **chapter 3**, it needs to be re-iterated that the diagnosis of implant health and disease was made according to the definitions presented at the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions [5, 6]. Briefly, a healthy implant was diagnosed when the peri-implant crevice demonstrated no bleeding or suppuration on probing and absence of bone loss beyond the initial crestal bone

remodeling. Implants with reduced bone support which presented with absence of clinical signs of inflammation were also considered healthy [5, 6]. Peri-implant mucositis was defined by the presence of clinical signs of inflammation and absence of radiographic bone loss, whereas peri-implantitis was diagnosed on the basis of clinical inflammation, PPD ≥6 mm and radiographic bone loss of ≥3 mm from the implant shoulder [5, 6]. For data analysis we considered shallow pockets with PPD <5 mm and deep pockets with PPD ≥5 mm. The selection of the cut-off point of 5 mm to distinguish shallow from deep peri-implant pockets was based on similar studies of periodontal pockets [24-26].

To further elaborate on the results of the study in **chapter 3**, irrespective of the diagnosis of peri-implant status, the shallow peri-implant pockets with PPD <5 mm were compared with deep peri-implant pockets of ≥5 mm. Deep peri-implant pockets had a higher relative abundance compared to shallow pockets of anaerobic Gram-negative bacteria of the following genera: *Fusobacterium*, *Prevotella* and *Anaeroglobus*. In contrast, pockets <5 mm were mostly inhabited by aerobic and facultative anaerobic bacteria belonging to the genera *Rothia*, *Neisseria*, *Haemophilus* and *Streptococcus*. These results are in line with a recent study that also characterized the submucosal microbiome around implants and reported that increased probing depth is associated with a shift in submucosal microbiome favoring the growth of anaerobes, which outcompeted the health-associated genera *Rothia*, *Neisseria* and *Streptococcus* [27]. Regarding disease status, the study in **chapter 3** confirmed that the biofilm of peri-implantitis sites presented a different microbial composition compared to healthy implant sites or peri-implant mucositis sites. It was also observed that the microbial characteristics of peri-implant mucositis sites were more similar to healthy implant sites than to peri-implantitis sites. Peri-implantitis sites presented significantly higher proportions of the genera *Fusobacterium* and *Treponema*. Healthy implants and peri-implant mucositis sites presented higher proportions of the genera *Rothia* and *Streptococcus* when compared to peri-implantitis sites. Similar to other studies, the microbial colonization of dental implants in edentulous patients overall

has been characterized by lower proportions of microorganisms and less pathogenic microbiota compared with dentate patients [28-31]. Dentate patients harbored significantly higher proportions of *Fusobacterium*, *Prevotella* and *Rothia* compared to edentulous patients. On the other hand, *Veillonella* and *Streptococcus*, were detected in higher proportions in edentulous patients.

Overall, the study in **chapter 3** reported differences in the composition of peri-implant microbiota based on probing depth, implant disease status and dentition status. Nevertheless, these results should be interpreted with caution since the disease status was not evenly distributed among the study participants; in the present study the majority of participants was diagnosed with healthy implants (n= 11) or peri-implant mucositis (n= 24) and only six patients were diagnosed with peri-implantitis. In any case, these results add to the knowledge that the microbiome of peri-implant sites shares common features with the periodontal microbiome [20, 32]. One can argue that the ecological niche of peri-implantitis pockets is different from periodontal pockets; in peri-implantitis there is the titanium (moderately rough) surface with threads (concavities), while in periodontitis there is a cementum and/or dentin surface where each surface may favor the growth of different microbes [33-35]. If indeed the microbiome of peri-implantitis is more or less the same as in periodontitis, it is justified to use the same treatment steps and procedures and if antibiotics are considered, those that have been proven effective for periodontitis can be chosen. Nevertheless, antibiotic resistance has become one of the bigger threats in global public health; therefore, activities that monitor the appropriate use of systemic antibiotics in dentistry   (antibiotic stewardship) are a top priority (see above discussion related to **chapter 2**).

The following part of this thesis concerns two RCTs, which aimed to evaluate a non-surgical and a surgical therapeutic approach (**chapters 4 and 5,** respectively) in the treatment of peri-implantitis. It is worth mentioning one more time, that before the 2017 World Workshop [5], there was a lack of a uniform definition of peri-implantitis, which

prompted different studies to use different thresholds to define peri-implantitis [7, 36]. In this thesis, the initial planning of the two RCTs (**chapters 4 and 5**) took place in early 2010s; therefore the study protocols and patient recruitment were based on an older definition of peri-implantitis [37]. Peri-implantitis patients were recruited based on probing depth ≥5 mm at one or more peri-implant sites, in combination with bleeding and/or suppuration on probing, and marginal bone loss ≥3 mm detected radiographically [37]. If the design and patient recruitment of the clinical trials were carried out in the present time, the current case definition of peri-implantitis based on clinical inflammation, probing depth ≥6 mm and radiographic bone loss of ≥3 mm from the implant shoulder would be applied [5, 6]. Nevertheless, in both RCTs the most severe peri-implantitis cases were selected and included in the analysis, therefore, the small deviation in the definition in terms of the peri-implant probing depth, most probably has not affected the treatment outcomes.

As already indicated in **chapter 2** of this thesis and the discussion above, there were notable differences in the management of peri-implantitis between European and U.S. periodontists. This is not surprising since a plethora of treatment strategies for peri-implantitis has been described in the literature, however there is a lack of standardized treatment protocol based on scientific evidence [13, 14]. Although existing therapeutic strategies are unpredictable in arresting peri-implant tissue inflammation, it has been recommended that the non-surgical treatment is the first step in peri-implantitis treatment and that it may lead to some reduction in the extent of inflammation and in some cases to peri-implant pocket depth reduction of up to 1 mm [38]. Systemic antibiotics have been proposed as an adjunct to non-surgical mechanical debridement, and are prescribed empirically for the treatment of peri-implantitis although the scientific evidence of their benefits is still limited and inconclusive [39, 40]. Therefore, a randomized controlled clinical trial was initiated (**chapter 4**), which aimed to assess the adjunctive effect of systemic antibiotics amoxicillin and metronidazole in patients receiving non-surgical treatment for peri-implantitis. The primary out-

come of the study was the change in peri-implant pocket depth. As also discussed above, probing depth determines to a great extent the microbial ecology of the peri-implant site, with deep pockets favoring the outgrowth of Gram-negative anaerobic species, which are characteristic of peri-implant disease [41]. According to the results of the study in **chapter 4**, non-surgical treatment with or without antibiotics was able to reduce peri-implant pocket depth at implants with peri-implantitis. At a 12-week follow-up time point, the mean peri-implant probing depth reduction in the antibiotics group and in the control group was 2.28 ± 1.49 mm and 1.47 ± 1.95 mm, respectively. Although the reductions per group seem different, the between-group difference did not reach statistical significance. Perhaps more importantly, non-surgical treatment with or without antibiotics was ineffective to completely resolve inflammation around dental implants, and there was not a complete elimination of peri-implant pockets of ≥5 mm. Therefore, the adjunctive use of systemic amoxicillin and metronidazole did not show statistically significant better results compared to non-surgical treatment alone. The success defined as functional implant with absence of probing depth ≥5 mm, absence of bleeding and/or suppuration on probing (modified from [42]) was very low; only one patient in each group showed complete resolution of the disease. A possible explanation for this could be related to the fact that very severe peri-implantitis cases were included; 54% of the patients presented with peri-implant pocket depth ≥8 mm at baseline. Other factors that might account for the low success in complete resolution of peri-implantitis in this study could be related to the inherent difficulties in removing the biofilm from the implant surfaces, to the type of instruments used to perform the debridement (ultrasonic and hand instruments vs air-abrasive devices), and to the fact that no removal and cleaning or modification of the suprastructure was performed [35, 43, 44]. However, the low success rate reported here, is in line with other studies [38, 40, 45-47] and supports existing evidence that in severe peri-implantitis cases, non-surgical treatment alone is insufficient to arrest the disease and eliminate bacteria from the rough surfaces of implants and from the concavities between implant threads. One of the limitations of this study is that the follow-up period was rather short,

however it was not considered appropriate to delay further treatment for the cases with residual inflamed deep pockets. Since existing evidence, as mentioned above, suggests that in most cases peri-implantitis cannot be treated with non-surgical treatment alone, longer follow-up times are not deemed necessary. We could therefore consider the non-surgical treatment as a preparatory phase which aims at healthier soft peri-implant tissues, and improved oral hygiene performed by the patient before the surgical treatment phase. Therefore, it can be concluded that the routine use of systemic antibiotics in non-surgical treatment of peri-implantitis is not the "solution" and although non-surgical treatment should always be the first step in peri-implantitis treatment, in severe peri-implantitis cases, disease resolution can only be accomplished after a surgical treatment phase, following the initial non-surgical phase.

Since non-surgical approaches alone have shown a limited efficacy in treating most cases of peri-implantitis (**chapter 4**), the next step would be to apply one of the surgical treatment options that are available, which in general have shown more promising results in terms of reduction of probing depth and bleeding on probing and radiographic evidence of bony defect fill [14, 48]. Open flap debridement, resective surgery with or without implantoplasty and reconstructive approaches including the use of various bone grafts with or without the use of barrier membranes are some of the surgical approaches reported in the literature [14, 49]. Reconstructive approaches may result in approximately 2 mm of radiographic defect fill, and in some cases in improvement of peri-implant clinical parameters [50-52]. Nevertheless, there is not enough evidence to support the use of any specific grafting material or membrane that would be the most advantageous [14, 52]. In the last decade new materials and the same type of materials from different companies have flooded the dental arena [53]. Examples include allografts, xenografts of porcine or bovine bone, synthetic materials such as calcium phosphate or bioactive glass, and even titanium particles [53, 54]. There are many different processing procedures of these bone grafts depending on the manufacturer, which could eventually result in different clinical responses [55]. Bovine bone xenografts are very popu-

lar in Europe, due to strict regulations which have limited the use of alternatives (i.e. allograft) and have been extensively used in periodontal regeneration, socket preservation, peri-implant reconstruction and alveolar bone augmentation [13, 53]. Therefore, the purpose of the study presented in **chapter 5** was to evaluate the reconstructive potential of two different bovine xenografts. More specifically, it was hypothesized that peri-implantitis defects treated with Endobon® will not exhibit an inferior outcome as compared to Bio-Oss® in terms of radiographic defect reduction around dental implants. The results of the study in **chapter 5** showed that at 12 months post-treatment, both groups presented with comparable radiographic intrabony defect depth reductions of 2.5-3.0 mm on average. Furthermore, both treatment modalities resulted in improvement of the clinical parameters probing depth and bleeding on probing. In both groups, the probing depths reduced by approximately 3.5 mm, and bleeding on probing reduced by 50% compared to baseline. There were no statistically significant differences between the two different xenografts in any of the radiographic or clinical parameters. These results further back up the results of the study presented in **chapter 4**, that the peri-implant pocket depth reduction cannot be accomplished with non-surgical treatment alone; the pocket depth reduction is accomplished on defects that had received first a non-surgical treatment phase, followed by surgical treatment. Although both reconstructive approaches were effective in radiographic defect reduction and probing depth reduction, neither resulted in complete resolution of peri-implant tissue inflammation, i.e. with complete absence of bleeding. The lack of complete success may be related to factors that have initially contributed to the disease, such as not optimal implant placement [56], the amount of keratinized tissue [57], and implant surface characteristics [58]. Furthermore, it has been suggested that the selection of the healing approach (e.g. submerged vs non-submerged) may affect the outcome of the reconstructive surgery [59]. In the study presented in **chapter 5**, as in other similar studies [54, 58, 60], a non-submerged healing mode was applied, i.e. the suprastructure was not removed before the surgical procedure, thus the implant fixture was not completely submerged during the healing period. De-

spite the lack of evidence to support one mode of healing versus the other, the submerged post-operative wound closure, where the implant suprastructure is removed and a primary wound closure and coverage of the grafted area is obtained, is preferred over the non-submerged healing when it is feasible [59, 61]. It has been suggested that the removal of the implant-supported crown can provide increased access for better detoxification of the infected implant surface, and the complete coverage of the grafted area may allow an undisturbed wound healing, creating a more favorable environment for bone formation [59].

Last but not least, this thesis investigated another implant complication next to peri-implant mucositis and peri-implantitis. After long term function there may be infra-positioning of dental implants, an esthetic long-term complication which has received little attention. In **chapter 6**, a retrospective long-term study evaluated the effect of continuous tooth eruption on the outcomes of single-implant–supported restorations in the anterior maxilla of adults (e.g. patients who were older than 20 years at the time of implant surgery). The mean age of the patients was 45 years (range: 21 - 78) and it was shown that infra-occlusion increased over time by 0.08 ± 0.02 mm/year. These results confirmed that also middle-aged adult patients with implant-supported restorations in the esthetic zone display long-term changes, mostly due to the continuous eruption of the neighboring natural teeth, which may jeopardize the implant esthetics. However, the magnitude of infra-occlusion was small, showing in most situations differences in the incisor planes between teeth and implant restorations of less than 1 mm. This means that in most cases a replacement of the implant-supported crown can address the esthetic concerns if present. In more severe cases, restorative adjustments of the adjacent natural teeth have been proposed in conjunction with the replacement of the implant-supported prosthesis [62]. Severe cases could be defined as the ones where infra occlusion exceeds 1 mm [63]. In the study of **chapter 6**, the infra-occlusion of single implant-supported restorations in the anterior maxilla reached a maximum of 1.67 mm for a period of up to 15 years after the permanent prosthesis delivery. Similarly, another study reported that

infra-occlusion reached a maximum of 1.86 mm in adults aged from 40 to 55 years who were followed for a mean period of 4 years (range from 1 to 9 years) [64].

Furthermore, **chapter 6** sought to associate the observed infra-occlusion with patient- or surgery-related parameters including: 1) gender, 2) age, 3) country of origin, 4) implant location, 5) implant surgical protocol: 5.a) immediate vs delayed implant placement, 5.b) one vs two-stage implants, 5.c) the performance of guided bone regeneration at the time of implant placement and 6) implant temporization with provisional prosthesis (immediate vs delayed). Age and implant placement using a delayed approach (after 6 months or later from the extraction of the tooth) were the only variables with a significant effect on the rate of implant infra-occlusion; it was shown that infra-occlusion was more pronounced for delayed (0.09 mm/year) versus immediate implant placement (0.06 mm/year) and for younger versus older adults (0.0013 mm/year per additional year of age). The literature regarding surgery-related and prosthesis-related parameters as possible risk factors for implant infra-occlusion has been very scarce so far. To the best of the authors' knowledge the study presented in **chapter 6** is the first study that reports an association between the timing of implant placement (immediate vs delayed) and the rate of progression of infra-occlusion. Definitely, more research with larger sample sizes is needed in order to further elaborate on these associations.

Finally, it has to be acknowledged that infra-occlusion of the implant-supported prosthesis is more of an esthetic concern than a health issue, which nevertheless, can affect the patient's oral health-related quality of life [65]. It has been reported that any changes in the length of the maxillary central incisors are not well-tolerated by the patients [66]. Therefore, even small amounts of infra-occlusion of less than 1 mm, of a single implant-supported restoration in the anterior maxilla can cause esthetic concerns since they affect the relative smile attractiveness, which in turn affects the perception of facial attractiveness [67]. On this premise, it is highly recommended that future studies take into consid-

eration the patient's perspective and include patient-reported outcomes [68]. The results of this study could guide clinical decision making and encourage clinicians to better explain to their patients this long-term esthetic complication. Clinicians should inform in advance the patients who are candidates for implant-supported restorations in the anterior maxilla, especially in the central incisor location, of these long-term changes which may jeopardize the implant esthetics. The patients should be aware that if this esthetic problem occurs in the future, a replacement of the implant supported crown possibly in conjunction with restorative adjustments of the adjacent natural teeth might be necessary to address the esthetic concerns. Furthermore, the results of the study in **chapter 6** (i.e. that infra-occlusion developed faster in younger than in older adults, as well as in implants placed using a delayed protocol than in implants placed immediately after dental extraction), could guide clinicians to opt for an immediate implant placement in younger patients when this is possible.

7.2 Conclusions and recommendations for further research

In conclusion, this thesis illustrated that peri-implantitis is a growing concern in the dental community that perplexes many clinicians around the globe. A major etiologic factor is related to microbial biofilm which acts as an initial trigger for inflammatory reactions. This thesis has shown that the peri-implant microbiome shares common features with the periodontal microbiome, which explains why many treatment approaches based on periodontal treatment have been proposed for peri-implant diseases and have been used empirically, however there is still a lack of a standardized evidence-based treatment. Furthermore, as it was shown in this thesis, the treatment of peri-implantitis is quite challenging due to the difficulty to access and clean properly the contaminated implant surface. Therefore, considering the complexities and the unpredictive nature of the existing therapies for peri-implantitis, the focus of the dental community should shift towards preventive measures in order to minimize the occurrence of the disease.

184

In addition to microbial biofilms, there are numerous factors that increase a patient's risk for developing peri-implantitis. These include patient related factors such as poor immune fitness (i.e. poor lifestyle, smoking, poor diet, stress) and local factors such as lack of keratinized tissue around the implant, iatrogenic factors such as improper implant position and prosthesis design, as well as factors that are related to the implant per se, such as implant design and implant surface to name a few. Future studies, with large sample sizes and adequate power are necessary in order to evaluate and control the many factors, that play a role in successful implant dentistry and in the development of implant-related complications. The dental professionals should work towards the development of standardized treatments for the implant-related complications, such as the treatment of peri-implant mucositis and peri-implantitis, which are the most prevalent complications. Single-center RCT's cannot ensure large sample sizes which provide adequate power and have adequate generalizability, therefore future studies could aim at multiple academic centers (multi-center), but also at private practices which can enlarge the sample sizes significantly. Nevertheless, such large studies may come with many logistical and analytical challenges, and costs. On the other hand, the generation of large data sets will be beneficial for numerous research questions and modern techniques available from a wide variety of scientific and social science fields can be applied, such as bioinformatics and artificial intelligence.

Besides the well-studied biologic complications, such as peri-implantitis, esthetic complications may also arise over time. Thus, in this thesis one esthetic aspect was also studied. That was the infra-occlusion of implant-supported crowns in the anterior maxilla, related to the adjacent natural teeth, a long-term esthetic complication which has received little attention so far from the dental community. Infra-occlusion may negatively affect the patient's perception of smile attractiveness. In the end, implant dentistry is performed for the restoration of function and esthetics in a dental patient due to tooth loss. In this context, patients' perceptions regarding the functional and esthetic aspect of oral health-related

quality of life are becoming more and more significant in evaluating treatment outcomes in implant dentistry, and should be taken into consideration in future studies.

References

1. Moraschini V, Poubel LA, Ferreira V, Barboza E. Evaluation of survival and success rates of dental implants reported in longitudinal studies with a follow-up period of at least 10 years: A systematic review. Int J Oral Maxillofac Sur. 2014;44(3):377-388.
2. Berglundh T, Persson L, Klinge B. A systematic review of the incidence of biological and technical complications in implant dentistry reported in prospective longitudinal studies of at least 5 years. J Clin Periodontol. 2002;29 Suppl 3:197-212; discussion 32-3.
3. Elani HW, Starr JR, Da Silva JD, Gallucci GO. Trends in dental implant use in the U.S., 1999-2016, and projections to 2026. J Dent Res. 2018;97(13):1424-30.
4. Schwarz F, Derks J, Monje A, Wang HL. Peri-implantitis. J Periodontol. 2018;89 Suppl 1:S267-s90.
5. Berglundh T, Armitage G, Araujo MG, Avila-Ortiz G, Blanco J, Camargo PM, et al. Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions. J Periodontol. 2018;89 Suppl 1:S313-s8.
6. Renvert S, Persson GR, Pirih FQ, Camargo PM. Peri-implant health, peri-implant mucositis, and peri-implantitis: Case definitions and diagnostic considerations. J Periodontol. 2018;89 Suppl 1:S304-s12.
7. Doornewaard R, Jacquet W, Cosyn J, De Bruyn H. How do peri-implant biologic parameters correspond with implant survival and peri-implantitis? A critical review. Clin Oral Implants Res. 2018;29 Suppl 18:100-23.
8. Dreyer H, Grischke J, Tiede C, Eberhard J, Schweitzer A, Toikkanen S, et al. Epidemiology and risk factors of peri-implantitis: A systematic review. J Periodontal Res. 2018;53(5):657-81.
9. Kordbacheh Changi K, Finkelstein J, Papapanou PN. Peri-implantitis prevalence, incidence rate, and risk factors: A study of electronic health records at a U.S. Dental school. Clin Oral Implants Res. 2019;30(4):306-14.
10. Roos-Jansaker AM, Lindahl C, Renvert H, Renvert S. Nine- to fourteen-year follow-up of implant treatment. Part ii: Presence of peri-implant lesions. J Clin Periodontol. 2006;33(4):290-5.
11. Daubert DM, Weinstein BF, Bordin S, Leroux BG, Flemmig TF. Prevalence and predictive factors for peri-implant disease and implant failure: A cross-sectional analysis. J Periodontol. 2015;86(3):337-47.

12.   Louropoulou A, van der Weijden F. [the dutch guidelines for prevention and treatment of periodontal and peri-implant diseases]. Nederlands tijdschrift voor tandheelkunde. 2021;128(12):585-92.

13.   Garaicoa-Pazmino C, Sinjab K, Wang H-L. Current protocols for the treatment of peri-implantitis. Curr Oral Health Rep. 2019;6(3):209-17.

14.   Schwarz F, Jepsen S, Obreja K, Galarraga-Vinueza ME, Ramanauskaite A. Surgical therapy of peri-implantitis. Periodontol 2000. 2022;88(1):145-81.

15.   Sanz M, Herrera D, Kebschull M, Chapple I, Jepsen S, Berglundh T, et al. Treatment of stage I–III periodontitis—the EFP S3 level clinical practice guideline. J Clin Periodontol. 2020;47(S22):4-60.

16.   Herrera D, Sanz M, Kebschull M, Jepsen S, Sculean A, Berglundh T, et al. Treatment of stage IV periodontitis: The EFP S3 level clinical practice guideline. J Clin Periodontol. 2022;49(S24):4-71.

17.   Antibiotic resistance in dentistry. Available from: https://www.fdiworlddental.org/antibiotic-resistance-dentistry.

18.   Fluent MT, Jacobsen PL, Hicks LA. Considerations for responsible antibiotic use in dentistry. J Am Dent Assoc. 2016;147(8):683-6.

19.   Teoh L, Thompson W, Suda K. Antimicrobial stewardship in dental practice. J Am Dent Assoc. 2020;151(8):589-95.

20.   Lafaurie GI, Sabogal MA, Castillo DM, Rincon MV, Gomez LA, Lesmes YA, et al. Microbiome and microbial biofilm profiles of peri-implantitis: A systematic review. J Periodontol. 2017;88(10):1066-89.

21.   Fu J-H, Wang H-L. Breaking the wave of peri-implantitis. Periodontol 2000. 2020;84(1):145-60.

22.   Hajishengallis G, Chavakis T, Lambris JD. Current understanding of periodontal disease pathogenesis and targets for host-modulation therapy. Periodontol 2000. 2020;84(1):14-34.

23.   Loos BG, Van Dyke TE. The role of inflammation and genetics in periodontal disease. Periodontol 2000. 2020;83(1):26-39.

24.   Rabbani GM, Ash MM, Caffesse RG. The effectiveness of subgingival scaling and root planing in calculus removal. J Periodontol. 1981;52(3):119-23.

25.   Ge X, Rodriguez R, Trinh M, Gunsolley J, Xu P. Oral microbiome of deep and shallow dental pockets in chronic periodontitis. PLoS One. 2013;8(6):e65520.

26.   Cobb CM, Sottosanti JS. A re-evaluation of scaling and root planing. J Periodontol. 2021;92(10):1370-8.

27.   Kroger A, Hulsmann C, Fickl S, Spinell T, Huttig F, Kaufmann F, et al. The severity of human peri-implantitis lesions correlates with the

level of submucosal microbial dysbiosis. J Clin Periodontol. 2018;45(12):1498-509.

28.   Apse P, Ellen RP, Overall CM, Zarb GA. Microbiota and crevicular fluid collagenase activity in the osseointegrated dental implant sulcus: A comparison of sites in edentulous and partially edentulous patients. J Periodontal Res. 1989;24(2):96-105.

29.   Kocar M, Seme K, Hren NI. Characterization of the normal bacterial flora in peri-implant sulci of partially and completely edentulous patients. Int J Oral Maxillofac Implants. 2010;25(4):690-8.

30.   Quirynen M, Van Assche N. RCT comparing minimally with moderately rough implants. Part 2: Microbial observations. Clin Oral Implants Res. 2012;23(5):625-34.

31.   Siddiqi A, Milne T, Cullinan MP, Seymour GJ. Analysis of P. gingivalis, T. forsythia and S. aureus levels in edentulous mouths prior to and 6 months after placement of one-piece zirconia and titanium implants. Clin Oral Implants Res. 2016;27(3):288-94.

32.   Charalampakis G, Belibasakis GN. Microbiome of peri-implant infections: Lessons from conventional, molecular and metagenomic analyses. Virulence. 2015;6(3):183-7.

33.   Albouy JP, Abrahamsson I, Persson LG, Berglundh T. Implant surface characteristics influence the outcome of treatment of peri-implantitis: An experimental study in dogs. J Clin Periodontol. 2011;38(1):58-64.

34.   Berglundh T, Zitzmann NU, Donati M. Are peri-implantitis lesions different from periodontitis lesions? J Clin Periodontol. 2011;38(s11):188-202.

35.   de Avila ED, van Oirschot BA, van den Beucken JJJP. Biomaterial-based possibilities for managing peri-implantitis. J Periodontal Res. 2020;55(2):165-73.

36.   Derks J, Tomasi C. Peri-implant health and disease. A systematic review of current epidemiology. J Clin Periodontol. 2015;42 Suppl 16:S158-71.

37.   Zitzmann NU, Berglundh T. Definition and prevalence of peri-implant diseases. J Clin Periodontol. 2008;35(8 Suppl):286-91.

38.   Renvert S, Hirooka H, Polyzois I, Kelekis-Cholakis A, Wang HL. Diagnosis and non-surgical treatment of peri-implant diseases and maintenance care of patients with dental implants - consensus report of working group 3. Int Dent J. 2019;69 Suppl 2:12-7.

39.   Heitz-Mayfield L, Lang N. Antimicrobial treatment of peri-implant diseases. Int J Oral Maxillofac Implants. 2004;19 Suppl:128-39.

40.   Roccuzzo A, De Ry S, Sculean A, Roccuzzo M, Salvi G. Current approaches for the non-surgical management of peri-implant diseases. Current Oral Health Reports. 2020;7:274-282.

41.   Mombelli A, Decaillet F. The characteristics of biofilms in peri-implant disease. J Clin Periodontol. 2011;38 Suppl 11:203-13.

42.   Heitz-Mayfield LJA, Salvi GE, Mombelli A, Loup PJ, Heitz F, Kruger E, et al. Supportive peri-implant therapy following anti-infective surgical peri-implantitis treatment: 5-year survival and success. Clin Oral Implants Res. 2018;29(1):1-6.

43.   Louropoulou A, Slot DE, Van der Weijden F. Influence of mechanical instruments on the biocompatibility of titanium dental implants surfaces: A systematic review. Clin Oral Implants Res. 2015;26(7):841-50.

44.   Ronay V, Merlini A, Attin T, Schmidlin PR, Sahrmann P. In vitro cleaning potential of three implant debridement methods. Simulation of the non-surgical approach. Clin Oral Implants Res. 2017;28(2):151-5.

45.   Persson GR, Samuelsson E, Lindahl C, Renvert S. Mechanical non-surgical treatment of peri-implantitis: A single-blinded randomized longitudinal clinical study. II. Microbiological results. J Clin Periodontol. 2010;37(6):563-73.

46.   Faggion Jr CM, Chambrone L, Listl S, Tu YK. Network meta-analysis for evaluating interventions in implant dentistry: The case of peri-implantitis treatment. Clin Implant Dent Relat Res. 2013;15(4):576-88.

47.   De Waal YCM, Vangsted TE, Van Winkelhoff AJ. Systemic antibiotic therapy as an adjunct to non-surgical peri-implantitis treatment: A single-blind RCT. J Clin Periodontol. 2021;48(7):996-1006.

48.   Berglundh T, Wennstrom JL, Lindhe J. Long-term outcome of surgical treatment of peri-implantitis. A 2-11-year retrospective study. Clin Oral Implants Res. 2018;29(4):404-10.

49.   Renvert S, Polyzois I. Treatment of pathologic peri-implant pockets. Periodontol 2000. 2018;76(1):180-90.

50.   Khoshkam V, Suarez-Lopez Del Amo F, Monje A, Lin GH, Chan HL, Wang HL. Long-term radiographic and clinical outcomes of regenerative approach for treating peri-implantitis: A systematic review and meta-analysis. Int J Oral Maxillofac Implants. 2016;31(6):1303-10.

51.   Tomasi C, Regidor E, Ortiz-Vigon A, Derks J. Efficacy of reconstructive surgical therapy at peri-implantitis-related bone defects. A systematic review and meta-analysis. J Clin Periodontol. 2019;46 Suppl 21:340-56.

190

52.   Mordini L, Sun N, Chang N, De Guzman JP, Generali L, Consolo U. Peri-implantitis regenerative therapy: A review. Biology. 2021;10(8):773.

53.   Haugen HJ, Lyngstadaas SP, Rossi F, Perale G. Bone grafts: Which is the ideal biomaterial? J Clin Periodontol. 2019;46 Suppl 21:92-102.

54.   Jepsen K, Jepsen S, Laine ML, Anssari Moin D, Pilloni A, Zeza B, et al. Reconstruction of peri-implant osseous defects: A multicenter randomized trial. J Dent Res. 2016;95(1):58-66.

55.   Block MS. The processing of xenografts will result in different clinical responses. J Oral Maxillofac Surg. 2019;77(4):690-7.

56.   Broggini N, McManus LM, Hermann JS, Medina R, Schenk RK, Buser D, et al. Peri-implant inflammation defined by the implant-abutment interface. J Dent Res. 2006;85(5):473-8.

57.   Roccuzzo M, Grasso G, Dalmasso P. Keratinized mucosa around implants in partially edentulous posterior mandible: 10-year results of a prospective comparative study. Clin Oral Implants Res. 2016;27(4):491-6.

58.   Roccuzzo M, Pittoni D, Roccuzzo A, Charrier L, Dalmasso P. Surgical treatment of peri-implantitis intrabony lesions by means of deproteinized bovine bone mineral with 10% collagen: 7-year-results. Clin Oral Implants Res. 2017;28(12):1577-83.

59.   Wen SC, Barootchi S, Huang WX, Wang HL. Surgical reconstructive treatment for infraosseous peri-implantitis defects with a submerged healing approach: A prospective controlled study. J Periodontol. 2022;93(2):197-209.

60.   Renvert S, Roos-Jansaker AM, Persson GR. Surgical treatment of peri-implantitis lesions with or without the use of a bone substitute-a randomized clinical trial. J Clin Periodontol. 2018;45(10):1266-74.

61.   Khoury F, Keeve PL, Ramanauskaite A, Schwarz F, Koo KT, Sculean A, et al. Surgical treatment of peri-implantitis - consensus report of working group 4. Int Dent J. 2019;69 Suppl 2:18-22.

62.   Zitzmann NU, Arnold D, Ball J, Brusco D, Triaca A, Verna C. Treatment strategies for infraoccluded dental implants. J Prosthet Dent. 2015;113(3):169-74.

63.   Jemt T, Ahlberg G, Henriksson K, Bondevik O. Tooth movements adjacent to single-implant restorations after more than 15 years of follow-up. Int J Prosthodont. 2007;20:626-32.

64.   Bernard JP, Schatz JP, Christou P, Belser U, Kiliaridis S. Long-term vertical changes of the anterior maxillary teeth adjacent to single implants in young and mature adults. J Clin Periodontol. 2004;31(11):1024-8.

65.   Duong H-Y, Roccuzzo A, Stähli A, Salvi GE, Lang NP, Sculean A. Oral health-related quality of life of patients rehabilitated with fixed and removable implant-supported dental prostheses. Periodontol 2000. 2022;88(1):201-37.

66.   Alsulaimani F, Batwa W. Incisors' proportions in smile esthetics. J Orthod Sci. 2013;2:109-12.

67.   Machado A. 10 commandments of smile esthetics. Dental Press J Orthod. 2014;19:136-57.

68.   Angkaew C, Serichetaphongse P, Krisdapong S, Dart MM, Pimkhaokham A. Oral health-related quality of life and esthetic outcome in single anterior maxillary implants. Clin Oral Implants Res. 2017;28(9):1089-96.

CHAPTER 8

Summary

8.1 Summary

The aims of this thesis were; (i) to assess the attitudes towards peri-implantitis of periodontists who are practicing in different parts of the world, (ii) to expand the knowledge on the microbial etiology of peri-implantitis, (iii) to explore the effectiveness of a non-surgical and a surgical therapeutic approach, and (iv) to evaluate the longitudinal changes in the position of single-implant prostheses adjacent to maxillary anterior teeth.

By sending anonymous surveys to periodontists in the U.S. and Europe, the considerations of risk factors, diagnostic criteria, and management of peri-implantitis between the U.S. and European periodontists were evaluated (chapter 2). Both groups rated poor oral hygiene, history of periodontitis, and smoking as very important, and, implant surface, occlusion and absence of keratinized tissue as relatively less important risk factors. However, European periodontists put a higher value on history of periodontitis and a lower value on implant surface, occlusion and presence of keratinized tissue than did U.S periodontists. Both groups based their diagnosis of peri-implantitis on clinical probing pocket depths, radiographic bone loss and presence of bleeding and suppuration on probing. For the management of peri-implantitis, both groups nearly always applied patient education, plaque control and mechanical debridement. U.S. periodontists were more likely to use antibiotics, lasers, allograft and regenerative approaches, but less likely to use resective surgery than European periodontists. All respondents acknowledged that peri-implantitis is an emerging, significant concern and that there is a need to educate general dentists better about identifying risk factors, diagnosing and referring peri-implantitis cases for treatment to specialists.

Using 16S rRNA gene pyrosequencing to analyze biofilm samples from peri-implant sites (chapter 3), differences were found in the composition of peri-implant microbiota based on probing depth, implant disease status, and dentition status. Well-recognized classical periodontal genera

such as *Fusobacterium*, *Prevotella* and *Treponema* were present in higher proportions in deep peri-implant pockets, in peri-implantitis pockets, and in peri-implant pockets of individuals still having natural teeth of their own. Bleeding on probing, implant system, time of functional loading, and sex did not seem to be associated with the composition of the peri-implant biofilms. Overall, these results add to the knowledge that the microbiome of peri-implant sites clearly shares common features with the periodontal microbiome. This understanding implies that therapeutic choices common in the treatment of periodontitis, can be applied in the treatment of peri-implantitis.

A randomized controlled clinical trial of peri-implantitis treatment, aimed to evaluate the clinical results of the combined use of systemic amoxicillin and metronidazole in conjunction with non-surgical treatment, in comparison to non-surgical treatment alone (chapter 4). A reduction in mean probing depth following non-surgical treatment plus administration of combination antibiotic therapy amoxicillin and metronidazole was observed after 12 weeks in both groups. Furthermore, neither of the tested treatment modalities achieved complete resolution of the disease with absence of bleeding on probing, i.e. inflammation was still present. Therefore, these results showed no significant additional clinical benefit from the adjunctive use of systemic antibiotics amoxicillin and metronidazole in the non-surgical treatment of peri-implantitis. It was concluded that although non-surgical treatment should always be the first step in peri-implantitis treatment, which provides some improvement in clinical parameters and allows for oral hygiene improvement and better patient compliance, sufficient probing depth reduction in severe peri-implantitis cases can only be accomplished after a surgical treatment phase.

In a randomized clinical pilot study, it was investigated whether xenograft Endobon® is non-inferior to xenograft BioOss when used in reconstructive surgery of peri-implant osseous defects (chapter 5). There were no differences in the treatment outcome between the two groups at 12 months post-treatment. The treatment with bovine-derived

xenografts resulted on average in radiographic defect reduction of approximately 3 mm and in probing depth reduction of approximately 4 mm in both groups. Furthermore, the proportion of implant sites with bleeding and suppuration on probing was reduced by approximately 50% and 80% respectively, in both groups. Nevertheless, this study showed limited success in the resolution of inflammation. Future studies on the treatment of peri-implantitis should include histologic analysis to evaluate the healing of the peri-implant intra-osseous defect and to prove true re-osseointegration of the diseased implant surface. Longer follow-up times are necessary to confirm the stability of the treatment outcomes.

Finally, a long-term retrospective study evaluated the effect of continuous tooth eruption on the outcomes of single-implant–supported restorations in the anterior maxilla of adult patients (chapter 6). Radiographs obtained at crown placement and at follow-up examinations from 1 to 15 years post-loading were analyzed with regard to vertical incisal plane changes of the implant-supported crown relative to adjacent teeth and it was found that infraocclusion increased over time by 0.08 ± 0.02 mm/year. Infraocclusion was more pronounced for delayed versus immediate implant placement and for younger versus older adults. It was therefore confirmed that adult patients with implant-supported restorations in the esthetic zone may display long-term changes which can jeopardize the implant aesthetics. However, the magnitude of infraocclusion was small, showing in most situations differences in incisor plane between teeth and implant restorations of less than 1 mm. Severe cases could be defined as the ones where infra occlusion exceeds 1 mm.

This thesis contributed in expanding the knowledge on the understanding and treatment of peri-implantitis, the most common pathological complication of implant dentistry, as well as in shedding light on long-term esthetic complications of implant dentistry especially in the upper front region, which are often overlooked.

8.2 Samenvatting

De doelstellingen van dit proefschrift waren; (i) het in kaart brengen van de professionele kennis en benadering van peri-implantitis door parodontologen die in verschillende delen van de wereld werkzaam zijn, (ii) het vergroten van de kennis over de microbiële etiologie van peri-implantitis, (iii) het onderzoeken van de effectiviteit van een niet-chirurgische en een chirurgische therapie van ernstige peri-implantitis, en (iv) om de longitudinale veranderingen in de positie van enkelvoudige kronen op implantaten in de bovenkaak naast natuurlijke elementen te evalueren.

Door anonieme enquêtes te sturen naar parodontologen in de VS en Europa, vergeleken werden de overwegingen van risicofactoren, diagnostische criteria en de behandeling van peri-implantitis vergeleken tussen de Amerikaanse en Europese parodontologen (hoofdstuk 2). Beide groepen beoordeelden slechte mondhygiëne, een voorgeschiedenis van parodontitis en roken als zeer belangrijk, en het implantaatoppervlak, de occlusie en de afwezigheid van gekeratiniseerde mucosa als relatief minder belangrijke risicofactoren. Europese parodontologen hechten echter meer waarde aan de voorgeschiedenis van parodontitis en achten het type implantaatoppervlak, occlusie en aanwezigheid van gekeratiniseerd weefsel minder van belang dan Amerikaanse parodontologen. Beide groepen baseerden hun diagnose van peri-implantitis op klinische pocketdieptes, röntgenologisch botverlies en de aanwezigheid van bloeding en pus na sonderen. Voor de behandeling van peri-implantitis gebruikten beide groepen bijna altijd patiëntenvoorlichting, plaquecontrole door middel van mondhygiëne instructies en mechanisch subgingivaal reinigen. Amerikaanse parodontologen gebruiken vaker antibiotica, lasers, allografts en andere regeneratieve benaderingen, maar zijn minder geneigd om resectieve chirurgie te gebruiken dan Europese parodontologen. Alle respondenten erkenden dat peri-implantitis een opkomend, belangrijk probleem is en dat er behoefte is om tandartsen beter voor te lichten over het identificeren van risicofactoren, het diagnosticeren en doorverwijzen van peri-implantitis gevallen voor behandeling naar specialistische tandartsen.

Met behulp van pyrosequencing van het 16S rRNA gen in de bacteriën in biofilm-monsters van peri-implantaire locaties (hoofdstuk 3), werden verschillen gevonden in de samenstelling van de peri-implantaire mi-

crobiota op basis van sondeerdiepte, status van het implantaat (gezond/peri-mucositis, peri-implantitis) en de (natuurlijke) gebitsstatus van de patiënt. De "klassieke" genera geassocieerd met parodontitis zoals Fusobacterium, Prevotella en Treponema, waren duidelijk aanwezig in hogere proporties in diepe peri-implantaire pockets, peri-implantitis en bij mensen die nog gedeeltelijk natuurlijke gebitselementen hadden. Bloeding na sonderen, implantaatsysteem, tijdstip van functionele belasting en geslacht leken niet geassocieerd te zijn met de samenstelling van de peri-implantaire biofilms. Over het algemeen dragen deze resultaten bij aan de kennis dat het microbioom van peri-implantaire pockets gemeenschappelijke kenmerken deelt met het parodontale microbioom. Dit begrip helpt clinici dat de therapeutische keuzes die gebruikelijk zijn bij de behandeling van parodontitis, kunnen worden toegepast bij de behandeling van peri-implantitis.

In een gerandomiseerde, gecontroleerde, klinische studie naar de niet-chirurgische behandeling van peri-implantitis, was het ten doel gesteld om de klinische resultaten te evalueren van het gecombineerde gebruik van systemisch amoxicilline en metronidazol in combinatie met de niet-chirurgische behandeling, in vergelijking met de niet-chirurgische behandeling alleen (hoofdstuk 4). Na 12 weken werd in beide groepen na de niet-chirurgische behandeling (met of zonder de antibiotica) een afname van de gemiddelde pocketdiepte waargenomen. Bovendien bereikte geen van de geteste behandelingsmodaliteiten een volledige genezing van peri-implantitis, nl. complete afwezigheid van ontsteking. Deze resultaten suggereren dat er geen significant klinisch voordeel is van het aanvullende gebruik van systemische antibiotica (amoxicilline en metronidazol) bij de niet-chirurgische behandeling van peri-implantitis. Er werd geconcludeerd dat niet-chirurgische behandeling altijd de eerste stap moet zijn in de behandeling van peri-implantitis; dit levert i.i.g. enige verbetering van klinische parameters op en een verbetering van de mondhygiëne en een betere therapietrouw van de patiënt. Maar voor de behandeling van ernstige peri-implantitis, om voldoende reductie van de sondeerdieptes te bewerkstelligen, blijkt een aanvullende chirurgische behandelingsfase noodzakelijk.

In een gerandomiseerde klinische pilotstudie werd onderzocht of het transplantaat materiaal van Endobon® niet inferieur is aan het transplantaatmateriaal van BioOss (beiden afkomstig van runder bot) bij het gebruik bij reconstructieve chirurgie van peri-implantaire botdefecten (hoofdstuk 5). Er waren geen verschillen in het behandelresultaat tussen de twee groepen 12 maanden na de behandeling. De behandel-

ing met de xenotransplantaten resulteerde in beide groepen in een reductie van gemiddeld ongeveer 3 mm van de röntgenologische defecten en in een reductie van de sondeerdieptes van ongeveer 4 mm. Bovendien waren plekken met bloeding en pus na sonderen in beide groepen substantieel verminderd (50% - 80% reductie). Desalniettemin toonde deze studie beperkt succes om een peri-implantitis defect geheel te "genezen", i.e. dat ook de ontsteking volledig is verdwenen. Toekomstige studies over de behandeling van peri-implantitis zouden ook histologische analyses moeten omvatten om de genezing van het peri-implantaire intra-ossale defect te evalueren en om daadwerkelijke re-osseo-integratie van het aangetaste implantaatoppervlak aan te tonen. Langere follow-up tijden zijn ook nodig om de stabiliteit van de behandelresultaten te bevestigen.

Ten slotte evalueerde een lange-termijn retrospectieve studie het effect van de continue tanderuptie op de uitkomsten van door een enkel implantaat ondersteunde restauraties in de anterieure bovenkaak bij volwassen patiënten (hoofdstuk 6). Röntgenfoto's verkregen bij het plaatsen van de kroon en bij vervolgonderzoeken van 1 tot 15 jaar na implantaat belasting werden geanalyseerd met betrekking tot veranderingen in het verticale incisale vlak van de door het implantaat ondersteunde kroon ten opzichte van aangrenzende tanden, derhalve of er zgn. infra-occlusie ontstaat in verloop van de tijd. De studie liet zien dat infra-occlusie in deze longitudinale studie met 0,08 ± 0,02 mm/jaar toenam. Infra-occlusie was minder uitgesproken bij implantaten die onmiddellijke geplaatst waren na tandextractie dan bij implantaten die geplaatste waren na eerst genezing van de extractie alveole. Ook was infra-occlusie wat groter bij jongere versus oudere volwassenen. Met deze studie werd aangetoond dat volwassen patiënten met kronen op implantaten in de esthetische zone na verloop van tijd een bepaalde mate van infra-occlusie kunnen vertonen dat de esthetiek van het implantaat in gevaar kan brengen. De omvang van de infra-occlusie ten opzichte van de natuurlijke buurelementen was echter beperkt en vertoonde in de meeste situaties verschillen in het incisale vlak van de tanden en implantaatrestauraties van minder dan 1 mm. Ernstige gevallen kunnen worden gedefinieerd als gevallen waarbij infra-occlusie groter is dan 1 mm.

Dit proefschrift heeft bijgedragen aan het vergroten van de kennis over peri-implantitis, de meest voorkomende pathologische complicatie van de tandheelkundige implantologie, en heeft ook licht geworpen op een esthetische complicatie, die vaak over het hoofd worden gezien.

CHAPTER 9

List of publications
Author contributions
Acknowledgements

9.1 List of publications

1. Polymeri A, van der Horst J, Anssari Moin D, Wismeijer D, Loos BG, Laine ML. "Non-surgical peri-implantitis treatment with or without systemic antibiotics; a randomized controlled clinical trial". Clin Oral Implants Res. 2022;33(5):548-557.

2. Polymeri A, Loos BG, Aronovich S, Steigmann L, Inglehart MR. "Risk Factors, Diagnosis and Treatment of Peri-implantitis: A Cross-cultural Comparison of U.S. and European Periodontists' Considerations". J Periodontol. 2022;93(4):481-492.

3. Polymeri A, van der Horst J, Buijs M.J, Zaura E, Wismeijer D, Crielaard W, Loos B.G, Laine M.L, Brandt B.W. "Submucosal microbiome of peri-implant sites - a descriptive cross-sectional study". J Clin Periodontol. 2021;48(9):1228-39.

4. Polymeri A, Anssari-Moin D, van der Horst J, Wismeijer D, Laine ML, Loos BG. "Surgical treatment of peri-implantitis defects with two different xenograft granules: A randomized clinical pilot study". Clin Oral Implants Res. 2020;31(11):1047-1060.

5. Polymeri A, Qing L, Laine ML, Loos BG, Wang HL. "Occlusal migration of teeth adjacent to implant prostheses in adults; A long-term study". Int J Oral Maxillofac Implants 2020;35(2):342-349.

6. Polymeri A, Giannobile WV, Kaigler D. "Bone marrow stromal stem cells in tissue engineering and regenerative medicine". Horm Metab Res. 2016;48(11):700-713.

7. Bajestan MN, Rajan A, Edwards S, Aronovich S, Cevidanes L, Polymeri A, Travan S, Kaigler D. "Stem cell therapy for reconstruction of alveolar cleft and trauma defects in adults". Clin Implant Dent Relat Res. 2017;19(5):793-801.

8. Fontana CR, Song X, Polymeri A, Goodson JM, Wang X, Soukos, NS. "The effect of blue light on periodontal biofilm growth in vitro". Lasers Med Sci. 2015;30(8):2077-86.

9. Polymeri A, Kodovazenitis G, Polymeris AD, Komboli M. "Bisphos-phonates: Clinical applications and adverse events in dentistry". Oral Health Prev Dent. 2015;13(4):289-299.

10. Nikitakis N, Polymeri AA, Polymeris AD, Karga H, Sklavounou A. "Metastatic papillary thyroid carcinoma to the maxilla: case report and literature review". Head Neck Pathol. 2012;6(2):216-223.

11. Polymeri A, Peponi H, Tsami A, Polymeris A, Vrotsos I, Antsaklis A. "Perception of gingival changes during pregnancy. Opinions of pregnant women". Perin Med Neonat. 2011;6(1):19-32.

12. Polymeri AA, Kontovazainitis GJ, Polymeris AD, Koboli MG. "Bisphosphonates: clinical applications and adverse events in dentistry". Hellenic Stomatological review. 2009;53(4):309-325.

13. Polymeri AA, Polymeris AD, Karga H. "Magnesium and periodontal disease". Iatriki. 2007;92(5):404-413.

14. Polymeris AD, Polymeri AA, Papapetrou PD. "Bisphosphonates and jaw osteonecrosis". Arch Hell Med. 2006;23(1):97-98.

9.2 Author contributions

Chapter 2 | Risk factors, diagnosis and treatment of peri-implantitis; a cross-cultural comparison of U.S. and European periodontists' considerations.

Angeliki Polymeri, Bruno G. Loos, Sharon Aronovich, Larissa Steigmann, Marita R. Inglehart

MRI and SA conceived and designed the study and the original survey and collected the data in the United States. MRI, AP, BGL and LS worked on the translations and pilot studies of the surveys into Dutch, German and Greek. AP and BGL collected the data in the Netherlands; AP collected the data in Greece; MRI and LS collected the data in Germany. MRI and AP analyzed the data and all authors worked on drafting the manuscript and gave final approval of the submitted version.

Chapter 3 | Submucosal microbiome of peri-implant sites: a cross-sectional study.

Angeliki Polymeri, Joyce van der Horst, Mark J. Buijs, Egija Zaura, Daniel Wismeijer, Wim Crielaard, Bruno G. Loos, Marja L. Laine, Bernd W. Brandt.

EZ, WC, DW, MLL and BGL conceived the idea, designed the study and achieved financial support. JH performed the clinical examination and collected the biofilm samples. MJB performed DNA extraction and sequencing. BWB, AP, MLL and EZ analyzed the data. AP drafted the manuscript and all authors critically revised the manuscript and gave final approval of the submitted version.

Chapter 4 | Non-surgical peri-implantitis treatment with or without systemic antibiotics; a randomized controlled clinical trial.

Angeliki Polymeri, Joyce van der Horst, David Anssari-Moin Daniel Wismeijer, Bruno G. Loos, Marja L. Laine.

D.W., B.G.L and M.L.L conceived the idea; J.H treated the patients; J.H. and D.A.M. collected the data; A.P., B.G.L and M.L.L analyzed the data; A.P. wrote the first draft of the manuscript and all authors critically

revised the manuscript. All authors read and approved the final manuscript.

Chapter 5 | Surgical treatment of peri-implantitis defects with two different xenograft granules: A randomized clinical pilot study.

Angeliki Polymeri, David Anssari-Moin, Joyce van der Horst, Daniel Wismeijer, Marja L. Laine, Bruno G. Loos.

M.L.L., B.G.L. , D.W. and D.A.M. conceived the idea; D.W. carried out the treatment; D.A.M., D.W., J.H. and A.P. collected the data; A.P. analyzed the data; A.P. wrote the manuscript; M.L.L., B.G.L., D.W., D.A.M and J.H. critically reviewed the manuscript. All authors read and approved the final manuscript.

Chapter 6 | Occlusal migration of teeth adjacent to implant prostheses in adults; A long-term study.

Angeliki Polymeri, Qing Li, Marja L. Laine, Bruno G. Loos, Hom-Lay Wang.

H.L.W conceived the idea; A.P, Q.L and H.L.W designed the study; A.P and Q.L collected and analyzed the data; A.P. wrote the first draft of manuscript; Q.L, H.L.W, M.L.L and B.G.L critically reviewed the manuscript. All authors read and approved the final manuscript.

shoulders and made this task easier for me. Last but not least, I would like to thank my good friend and artist Markella Kompitsa for designing the cover of this thesis.

A scientist apologizes
every day.
He does it in the name of truth.

A philosopher refuses
to apologize.
He does it in the name of freedom.

A humble man
neither apologizes
nor refuses to apologize.
Simply,
he is struggling to keep his name.

Nikos Soukos