An *in vitro* assessment of the bacterial sealing capacity of narrow diameter implants with Morse-taper abutment connections.

A mini-thesis submitted as partial fulfillment for the requirements of the MChD. in Oral Medicine and Periodontics at the Faculty of Dentistry University of the Western Cape.

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KEYWORDS

Dental implants

Narrow implants

Narrow diameter implants

Bacterial sealing

Bacterial penetration

Morse taper abutment

Conical abutment

Implant-abutment interface

Microleakage

Tapered implant-connections

ABSTRACT

Background: Lack of appropriate bone thickness is a common clinical limitation for tooth replacement, often requiring narrow implants, which have shown better results when combined with Morse taper connections. Little is known about the sealing of the abutment-implant interface of narrow implants with Morse taper connections against oral bacteria.

Aims: To investigate the *in vitro* ability of four commercially available narrow diameter implant (< 3.5 mm) with Morse-taper type implant abutment connections to impede bacterial penetration of their implant abutment interface (IAI).

Material and Methods: Four commercially available narrow implant systems with Morse taper connections were subjected to *Streptococcus sanguinis* cultures *in vitro* and evaluated for contamination and microgaps through Scanning Electron Microscopy (SEM).

Results: Bacterial penetration of the IAI was observed in all systems (n=12), ranging from 65 to >300 CFU. There were no statistically significant differences in the average log CFU between the four implant groups ($\chi 2 = 5.244$, $P=0.155$). Microgaps ranging from 5-10 µm were observed in all assemblies when analyzed under SEM, with no statistically significant differences between the different systems ($P>0.05$).

Conclusions: Despite the advantages of Morse taper systems, the evaluated narrow diameter implants using this type of abutment geometry failed to provide bacterial sealing. The observed microgaps can form reservoirs and potentially lead to inflammation in the peri-implant tissues and micromovements.

DECLARATION

I hereby declare that “An in vitro assessment of the bacterial sealing capacity of narrow diameter implants with Morse-taper abutment connections” is my own work and that it has never been submitted before for any examination or degree at any University. All the sources that I have used and quoted have been acknowledged by complete references.

Dr. Mubarak Alriyahi

Signature: 

Date: 23.9.2020

ACKNOWLEDGEMENT

I would like to show my sincere gratitude to the following colleagues for their invaluable contribution and assistance during this research project:

Dr. Peck – thanks for the open communication, constructive discussions, guidance and patience through every part of this project.

DEDICATION

I would like to dedicate this thesis to my wife and my family, as it would not have been possible to conclude it without their complete help, support, encouragement and understanding.

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LIST OF ABBREVIATIONS

NDI: Narrow Diameter Implants

IAI: Implant-Abutment Interface

SEM: Scanning Electron Microscopy

CFU: Colony-Forming Unit

S. sanguinis: *Streptococcus sanguinis*

µm: micrometer

CHAPTER I: INTRODUCTION

Titanium implants, initially used by Dr. P. Branemark (Branemark et al. 1977), have caused a revolution in the oral rehabilitation of partially and totally edentulous patients (Brånemark et al., 1977). Despite the high success rate of dental implants, complications can result in implant failure. Peri-implantitis is undoubtedly one of the most prevalent biological complications seen in clinical practice, it has the potential to cause irreversible bone loss and result in loss of the implant (Berglundh et al., 2018). Although the etiology of peri-implantitis is not completely understood, several studies have suggested that bacterial colonization of the implant interface is one of the most important risk factors in the development of this condition (Broggini et al., 2003; Heydenrijk et al., 2002; Mombelli & Décaillet, 2011).

In vitro studies indicate that microgaps and misfits in the implant abutment interface can facilitate bacterial penetration and potentially contribute to the development and progression of peri-implantitis; the design of the interface have been found to influence its bacterial sealing capacity (Broggini et al., 2006; do Nascimento et al., 2011).

The Morse taper connection has been shown to present superior results in relation to other designs and therefore, it has been considered as a promising option. In many clinical situations, due to lack of appropriate bone thickness associated with post-extraction alveolar bone resorption, the use of narrow implants can be beneficial for the patient, as it potentially decreases treatment length, cost and morbidity by avoiding bone augmentation procedures (Schiegnitz & Al-Nawas, 2018). In the literature, narrow diameter implants (NDI) have been presented promising results when combined with a Morse taper abutment (Ricomini Filho, et al. 2010).

To our knowledge, there are no studies evaluating the sealing capacity of NDI implants with Morse taper connections. The aim of the present study is to compare the *in vitro* ability of four commercially available narrow implants (< 3.5 mm) with Morse-taper abutment connections to facilitate bacterial penetration of the implant abutment interface.

CHAPTER II: LITERATURE REVIEW

Implant-abutment connections

The implant-abutment connection is an important area that plays a crucial role in the stability and strength of an implant-supported prosthesis. There are two main types of implant-abutment connections, internal and external connections, presenting different geometric characteristics (Goiato et al., 2015). External hex connections were widely used in the past, usually presenting an external hexagon. The presence of microgaps in the implant-abutment interface (IAI), low stability and high stress over the screw were the main problems associated with this type of connection, resulting in increased risk for crestal bone loss (Almeida et al., 2013).

Over the last decade, external connections have been replaced by internal connections, which can be divided into three main categories: internal hexagon, internal octagon and morse taper (conical abutment). Internal hexagon and octagon connections were developed to improve contact between implant and abutment and better dissipate forces, however, none of them present frictional locking, ultimately relying on the screw preload (Maeda, Satoh, & Sogo, 2006).

Morse taper connections

In 1993, Sutter et al. presented a tapered implant-abutment connection (8-degree), which was shown to increase contact between implant and abutment, thus improving resistance and stability (Norton, 2000; Sutter et al., 1993). There are Morse taper connections with and without screws, however, it does not rely on the screw itself, but on frictional resistance. For implant connections that depend exclusively on screws for stability, incidence of screw

loosening have been reported to be as high as 40%, while the incidence of screw fracturing for tapered connections has been reported to be as low as 3.6-5.3% (Merz et al., 2000).

Thus, Morse taper connections have been associated with superior mechanical properties, improved stability, better stress distribution, and increased resistance to rotational and bending forces, which results in decreased risk for abutment loosening and fracture, being indicated for anterior and posterior single tooth restorations, partial fixed prostheses and overdentures (Bozkaya & Müftü, 2005; Goiato et al., 2015; Merz et al., 2000; Norton, 2000).

Narrow diameter implants

The constant challenge of lack of bone volume at implant sites has led to the investigation of narrow-diameter implants (NDI), considering that bone augmentation is not possible for every single patient that lacks alveolar bone. The concept of NDI varies, with some considering implants from 3 to 3.75mm, and others considering a diameter of 3 to 3.5mm (Arsan, et al. 2010; Assaf et al., 2015). Guided bone regeneration is frequently used for bone augmentation, with well described histological and clinical results. Despite the evidence behind this technique, the results depend on a variety of factors, such as grafting material, membrane and stabilization of the wound (Botticelli, Berglundh, & Lindhe, 2004; Donos, Mardas, & Chadha, 2008). Other complex surgical options are available to increase horizontal bone width, including ridge expansion, autogenous bone graft and osteogenic distraction, however they increase treatment time and morbidity, and have the potential for result in complications (Sierra-Sánchez et al. , 2014).

Narrow implants offer a simple, straightforward solution for situations where optimal horizontal bone augmentation cannot be achieved, due to patient or site-related factors

(Badran et al., 2017). They were initially developed in the nineties to replace teeth with small crowns, such as maxillary and mandibular lateral incisors, or for areas with limited space. Their use in posterior areas with thin alveolar ridges has also been evaluated. One trial evaluated 316 NDI with mean follow up time of 9.1 years. The observed average marginal bone loss was 1.3 mm after 10 years, the overall success rate was 91.4% and the cumulative survival rate was 92.3%, which is comparable to standard diameter implants. In this study, smoking and molar areas increased the risk for complications. None of the implants included in the study fractured (Arsan et al., 2010).

One longitudinal study compared 122 NDI to 208 standard implants after 1 to 7 years of follow-up. The results showed comparable success rates, cumulative survival rates and marginal bone loss, suggesting that NDI can be result in successful treatment outcome when replacing lost teeth in partially edentulous patients(Romeo et al., 2006).

A total of 510 narrow implants were evaluated for several clinical and radiographic parameters, and presented high survival rates (99.4%, only 3 implants were lost). The results showed slightly higher marginal bone loss for NDI subjected to immediate loading, as compared to delayed loading (Degidi, Piattelli, & Carinci, 2008).

A recent review of the literature presented positive results of NDI when used with overdentures (Marcello-Machado et al., 2018). The average survival rate was 98%, average success rate was 96% and average marginal bone loss of 0.3mm after 3 years of follow-up.

Another review article evaluated long-term results of narrow, standard and wide-diameter implants when placed in the posterior areas of the maxilla. The authors concluded that implant diameter did not influence success rates when the three variations were compared.

The factors that influenced long-term survival were primary stability at surgery, and adherence to maintenance programs (Javed & Romanos, 2015).

Altogether, several in vivo studies indicate that narrow diameter implants seem to be a reliable solution, decreasing morbidity and treatment length, with similar results in terms of survival and success rates, as well as marginal bone loss as compared to standard diameter implants (Arsan et al., 2010; Enkling et al., 2017; Galindo-Moreno et al., 2012; Mangano et al., 2017; Papadimitriou et al., 2015; Zinsli et al., 2004). The use of NDI 3.3 to 3.5 mm is well documented for use in posterior mandibular and maxillary areas (Klein, Schiegnitz, & Al-Nawas, 2014). Based on survival rates, marginal peri-implant bone loss and incidence of complications, narrow implants present high predictability irrespective of the area and the prosthetic restoration (Sierra-Sánchez et al., 2014).

NDI with Morse taper connections

Studies on standard implants have shown that the implant-abutment connection can affect survival rates. Survival rates are lowest for external hexagon and internal hexagon and highest with Morse taper connections (Almeida et al., 2013).

Few studies have evaluated those variables on narrow implants. One *in vitro* trial compared different types of connections in narrow and standard diameter implants. Irrespective of implant diameter, Morse taper connection groups presented highest resistance to failure under loading and probability of survival than internal hexagon connections (Freitas et al., 2016).

Different types of abutment-implant connection were compared in relation to reliability of NDI through fatigue failure tests. Internal and external hexagon connections resulted in the

lowest reliability when subjected to loading, as compared to internal conical connections. Failure mode varied, with external hexagon presenting mostly screw-related problems, internal hexagon presenting implant and screw fracture and conical connections presenting failure related to the prosthesis (Bordin et al., 2016).

More studies on NDI and Morse taper connections are needed to further elucidate their performance in relation to standard-diameter implants, different commercial brands and connection design.

Bacterial sealing of the implant-abutment interface

Comparison of Morse taper to other designs

The design of the implant-abutment interface has been shown to play a crucial role in bacterial penetration (Mishra, Chowdhary, & Kumari, 2017). Studies have shown that wide microgaps are present when using external hexagon abutments, allowing bacterial penetration in the IAI. The search for better seal, less bacterial penetration and potentially decreased risk for peri-implantitis brought attention to different abutment designs (Assenza et al., 2012; Dibart et al., 2005; do Nascimento et al., 2011).

Morse taper connections have been shown to present more stability, less micromovement and less bone loss as compared to other abutment designs (Binon, 2000; Bozkaya & Müftü, 2005; Dibart et al., 2005; Mangano et al., 2009; Sannino & Barlattani, 2013). Several studies have investigated the sealing capacity of Morse taper abutment systems.

When comparing Morse taper connections to internal hex abutments for *in vitro* bacterial infiltration, lower amounts of bacteria were present in the Morse taper group, although not statistically significant (D'Ercole et al., 2014). One trial compared the sealing capacity of

screwless Morse taper implants to tapered screw-retained implants with and without loading. The best seal was reported for Morse-tapered implants under static conditions (Alves et al., 2016).

One in vivo study evaluated the sealing capacity of internal hexagon compared to Morse taper implants through a volatile organic compounds (VOCs) emission test. Hexagon connections presented higher VOCs than Morse taper connections, indicating a better resistance to bacterial penetration for the latter (Scarano, Lorusso, Di Giulio, & Mazzatenta, 2016). When Morse taper abutment was compared to external hexagon, although both systems showed bacterial penetration, the Morse taper presented better seal (Jaworski et al., 2012).

Different types of Morse taper connections

Deconto and coworkers compared two commercially available Morse taper systems for bacterial infiltration and showed no difference between them (Deconto, Salvoni, & Wassall, 2010b). Similarly, another *in vitro* study evaluated two types of wide implants with Morse taper connections when subjected to bacteria; the results were similar with both presenting an appropriate seal (Dibart et al., 2005). In contradiction to the previous studies, one trial evaluated four commercially available Morse taper implant systems for bacterial penetration and reported that all of them presented micro gaps large enough to allow penetration of oral bacteria, ranging from 4.5 to 9.9 µm (Ranieri et al., 2015). In implants with internal connections, the microgap has been reported to range between 1-49 µm, according to the abutment type, while the most common oral bacteria have sizes ranging from 1-10 µm (Nassar & Abdalla 2015).

A study compared indexed to non-indexed Morse taper connections and found that, although both systems presented bacterial infiltration, indexed abutments presented superior sealing (Peruzetto et al., 2016). In another trial, screw-tightened Morse taper connections presented greater bacterial contamination when compared to mini friction abutments. Interestingly, increasing insertion torque from 20 to 30Ncm decreased contamination (Alves et al. 2014). When comparing two different conometric Morse taper systems, gold coping and Peek coping presented similar low bacterial counts (Bressan et al., 2017).

Thus, the literature suggests that most IAI present micro gaps and bacterial infiltration and microleakage increases under loading. Morse taper abutments present better sealing capacity and less microleakage as compared to other connections (Mishra et al., 2017). To our knowledge, currently there are no studies evaluating bacterial penetration in narrow implants with morse taper connections. Narrow implants have gained attention in the latest years as a potential solution for inadequate alveolar bone thickness at edentulous sites.

Bacterial species used in in vitro studies

Different species of bacteria have been used in *in vitro* studies on bacterial infiltration and colonization of the implant-abutment interface. *Streptococcus sanguinis* have been used in a few studies studies (Rogerio Ranieri et al., 2015; Ricomini Filho et al., 2010). It has been described as an early colonizer in the formation of dental plaque; it has been demonstrated to adhere to implant surfaces (Rodríguez-Hernández et al., 2011; Tamura et al., 2013). Other species of bacteria used in studies on the seal of Morse taper implants include *Escherichia coli* (Alves, Carvalho, & Martinez, 2014), *Pseudomonas aeruginosa* and *Aggregatibacter actinomycetemcomitans* (D'Ercole et al., 2014) and one study used a mixture

of *Aggregatibacter actinomycetemcomitans*, *Streptoccus oralis* and *Fusobacterium nucleatum* (Dibart et al., 2005).

To date, there are no studies comparing the different species of bacteria for *in vitro* studies of the implant abutment interface. It should be highlighted that the use of different species of bacteria might partly explain different results. *Escherichia coli* for instance is much larger the *S. sanguinis,* thus it might not be able to penetrate smaller microgaps, which could in turn become colonized by smaller species present in dental plaque.

In conclusion to this chapter, Morse taper implants have been shown to present superior results in relation to other abutment designs and few in vitro studies have evaluated the sealing capacity of Morse connections; some have reported a good sealing while others have not. None of these studies have focused on narrow-diameter implants, a treatment option that has become very relevant as many patients refuse to undergo regeneration procedures. Thus, this topic has clinical importance and requires further elucidation. In the current study, *S. sanguinis* will be used because it constitutes a small cocci present in oral biofilms, which is able to penetrate even the most reduced microgaps in the implant abutment interface.

CHAPTER III: CONTRIBUTIONS OF THE STUDY

It is crucial to understand the bacterial invasion of the implant abutment interface in order to control inflammation, bone loss and ultimately implant failure, especially with narrow implants. This study will shed light into the sealing capacity of Morse taper connections in narrow-diameter implants and compare four commercially available Morse taper implant systems. The results from the present study might ultimately help improve the long-term success of narrow-diameter implants in clinical practice through an investigation of the seal in the most critical area, the implant abutment interface.

CHAPTER IV: AIMS AND OBJECTIVES

Aims: The aim of the present study is to investigate the *in vitro* ability of four commercially available narrow diameter implant (< 3.5 mm) with morse-taper type implant abutment connections to impede bacterial penetration of their implant abutment interface.

Objectives

- To analyze the capacity of Streptococcus Sanguinis to invade the implant abutment interface of the following narrow implants:
    - MiNi, Megagen Implant Co, Korea
    - Toureg Closefit UNP, Adin Dental Implant Systems, Israel.
    - Bone Level Implant, Straumman, Switzerland.
    - V3, MIS, Israel

CHAPTER V: METHODOLOGY

Study design

The current study is based on in vitro laboratorial analysis to test the sealing ability of different Morse Taper narrow implant systems. All analysis were carried out at the Department of Oral Medicine and Periodontology and at the laboratory of Oral and Dental Research, Faculty of Dentistry, University of the Western Cape, Tygerberg Campus.

Implant assemblies

Four commercially available tapered narrow implants with Morse taper connections were tested in this *in vitro* study: MiNi (Megagen Implant Co, Korea); Toureg Closefit UNP (Adin Dental Implant Systems, Israel), Bone Level Implant (Straumman, Switzerland) and V3 (MIS, South Africa). In total, 12 implants were used in the present study (n=3 for each system). The specifications for the implants included in the study are presented in Table 1.

Table 1. Implant systems included in the study and their general characteristics.

Implant system	Size	Surface	Connection	Material
Touareg Closefit UNP (Adin)	3 x 10 mm	Calcium phosphate blasted (Osseofix)	Conical	Titanium alloy
Mini (Megagen)	3 x 10 mm	SLA - sandblasted with large grit, and acid etched (xSPEED)	11º Conical	Titanium alloy
Bone Level (Straumann)	2,9 x 10 mm	SLA - sandblasted with large grit, and acid etched (SLActive)	8º Conical	Roxolid
V3 (Mis)	3,3 x 10 mm	Sand blasted acid etched	Conical	Titanium alloy

Bacterial Culture

The gram-positive oral bacteria *Streptococcus sanguinis* (*S. sanguinis ATCC10556*) was used for evaluation of the sealing capacity of Morse taper narrow implants in the current study. In the oral biofilm, *S. sanguinis* constitutes a facultative anaerobe of small size (0.5 to 1µm). This early colonizer is capable of adhering directly to titanium implants and is often detected in peri-implant plaque (Rodriguez-Hernandez et al. 2011, Silva Neto et al. 2012). *S. sanguinis* was cultured in a brain-heart infusion (BHI) broth, incubated at 37^{o}C and 5% CO^{2}. In total, 200ml of the BHI broth was divided equally into two sterile glass containers.

Bacteria were freeze-dried for 24 hours, after which they were cultured in the BHI broth and transferred to labelled agar plates. Those were incubated at 37^{o}C for 18-24 hours. After 18 hours in the culture medium, the bacterial inoculum was extracted and diluted in PBS (phosphate-buffered saline). The turbidity of the bacterial suspensions was adjusted according to the McFarland standard number 0.5 for approximate cell density of 10^{8} CFU/ml (Ranieri et al. 2015).

Bacterial Challenge

The protocol used in this study was based on the protocol from the study of Ranieri et al. (2015). All implants and abutments included in the study were sterilized 24 hours before bacterial inoculation and removed from their packaging using sterile pliers. Then, under sterile conditions, 0.05 ml of BHI broth was injected into the internal well of each implant in order to simulate penetration of oral fluids during implant placement and facilitate bacterial growth. The implants were connected to their respective conical abutments following the manufacturer's instructions, and immediately transferred to plastic tubes containing 4ml of sterile BHI broth. The implant assemblies were submerged to a level above the IAI, just below

the screw opening, and incubated for 48h at 37°. Before bacterial inoculation, the tubes containing the implant assemblies in BHI were autoclaved for 15 minutes at 121°C.

The BHI broth in each tubes was inoculated with 0.01ml of the *S.sanguinis* solution and incubated at 37°C with 5% CO^2 (Nassar & Abdulla 2015).

Figure 1. Implant assemblies used in the study.

After 48 hours, the implant assemblies were carefully removed from the tubes using sterile pliers, sprayed with 70% alcohol and let to dry in a vertical position in a stand for 10 minutes. Using sterile torque wrenches, the implants were carefully disconnected from their conical abutments. Sterile paper points were used to collect bacterial samples from the inner surface of each implant. The paper points were labelled and placed into test tubes containing 10ml of BHI for 20 minutes. In order to exclude contamination of the BHI broth, sterile paper points were incubated in the broth (negative controls). All tubes containing BHI and paper points were cultured on labelled agar plates (TSA with LTHTh-ICR contact plates GRN:ST16/2018) and incubated for 24 hours at 37°C. The number of viable bacteria was estimated through

manual counting of colony-forming unit (CFU). All experiments were run in triplicate and data were collected in Excel spreadsheets.

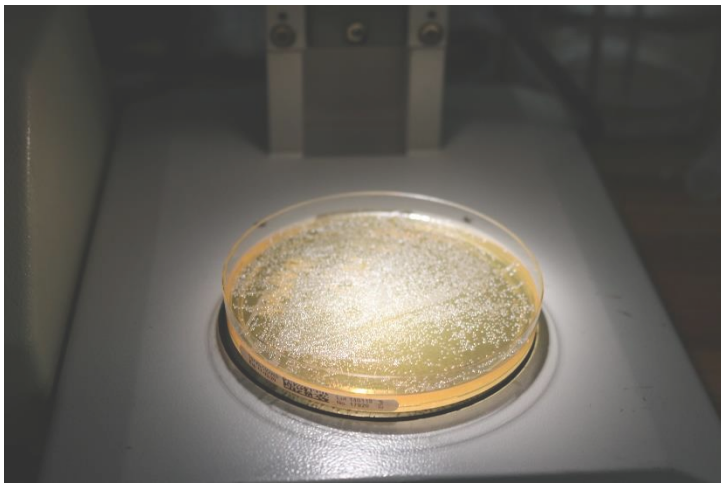


Figure 2. Agar plate used for bacterial growth

Figure 2. Agar plate subjected to CFU count

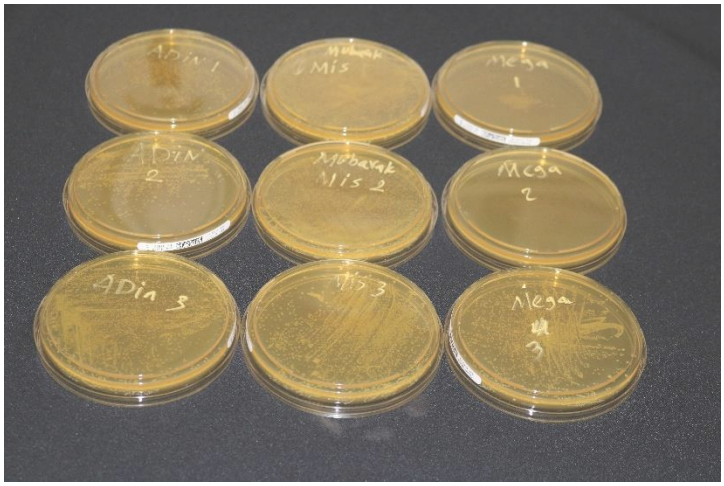
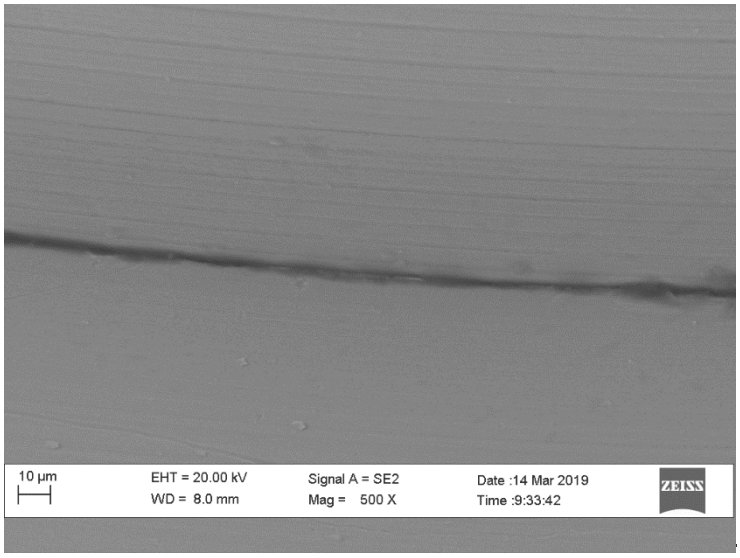


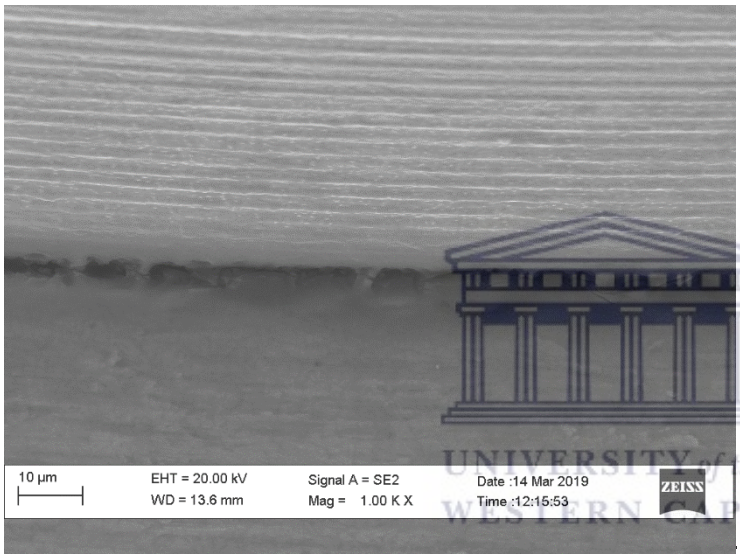
Figure 3. Agar plates after CFU count.

Scanning Electron Microscopy (SEM)

SEM was employed to determine the size of the microgaps. After the 48h incubation period, the bacterial broth was removed from the culture glasses, the assemblies were rinsed twice in phosphate-buffered saline (PBS) and subsequently fixed in modified Karnovsky solution (2.5% glutaraldehyde plus 2.5% formaldehyde in cacodylate buffer) for 24h. The assemblies were dehydrated in increasing alcohol concentrations (25%, 50%, 75%, and 90%, 30 minutes each and 100% for 1h) and placed in an incubator for the next 24h.

The units were analysed as a whole for the investigation of bacterial penetration in the implant abutment interface. To evaluate the extent of bacterial penetration, abutments and implants were disconnected. For the last analysis, abutments were put back in the implants and cut with diamond disks 1 mm above the implant crest module.





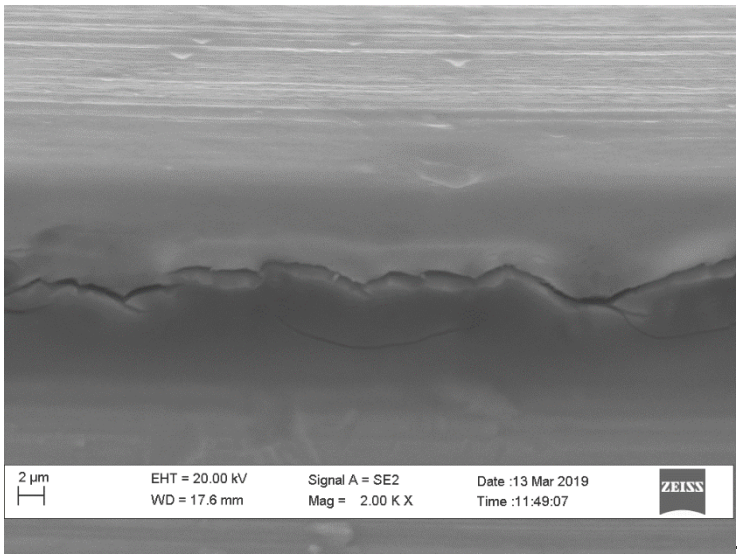


Figure 3. Scanning electron microscopy of three different assemblies. A: Straumman assembly, B: Megagen assembly, C: Adin assembly.

Statistical Analyses

Statistical analysis was performed using STATA software with a 5% level of significance. Due to the small sample size and the skewness of the data, CFU absolute numbers were log transformed (log CFU) and analysed through the Kruskal-Wallis test. Data for log CFU was presented as average and standard deviation.

CHAPTER VI: RESULTS

Bacterial penetration

In the present study, bacterial penetration of the IAI was observed in all evaluated implant systems (n=12), ranging from 65 to >300 CFU (Table 1).

All Megagen (n=3) and all Adin implants systems (n=3) presented bacterial amounts above 300 CFU/ml. For the MIS implants (n=3), all presented bacterial amounts ≥150 CFU. For the Straumman implants (n=3), one presented less than 150 CFU, while the others had values above 300 CFU.

Implant systems	Implant number	CFU
Megagen	1	>300
	2	>300
	3	>300
Mis	1	65
	2	130
	3	150
Adin	1	>300
	2	>300
	3	>300
Straumann	1	80
	2	>300
	3	>300

Table 2. Recovery of bacteria in absolute numbers, determined through CFU counting (CFU=colony-forming units) for all evaluated implant systems.

The log transformation for bacterial counting through CFU is presented in Table 2.

Implant systems	N	Mean LOG CFU	SD
Megagen	3	5,9	0,0
Mis	3	4,7	0,4
Adin	3	5,9	0,0
Straumann	3	5,4	0,6

Table 3. Log transformation for bacterial counting through CFU for each type of implant.

Megagen and Adin implant systems presented average log CFU of 5,9 ± 0. The Straumann assemblies had a mean log CFU of 5,4 ± 0.6, while the assemblies from Mis Implants presented mean log CFU of 4,7 ± 0.4.

There were no statistically significant differences in the average log CFU between the four implant groups ($\chi 2 = 5.244$, $p = 0.155$).

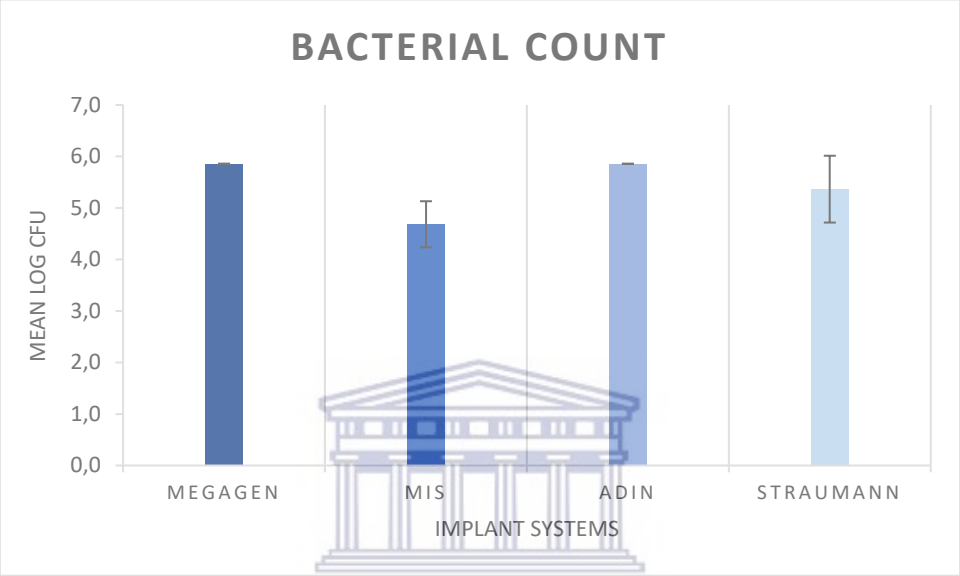


Figure 4. Bacterial counting for the different implant systems, presented as average log CFU (CFU=colony-forming units) ± SD (standard deviation).

SEM

All implant assemblies presented microgaps ranging from 5-10 micrometers when analyzed under SEM, with no statistically significant differences between the different systems ($P>0.05$).

CHAPTER VII: DISCUSSION

Similarly to natural teeth, the supporting tissues around dental implants are susceptible to inflammatory changes, described as peri-implant mucositis in the initial stages and peri-implantitis when it affects the alveolar bone, leading to bone loss and potential implant failure (Berglundh et al., 2018). Observational studies report that deficient plaque control and lack of regular maintenance can predispose to peri-implantitis, which constitutes a risk factor for late implant loss (Schwarz, Derks, Monje, & Wang, 2018). Thus, microbiological factors play a crucial role in the aetiology of peri-implantitis (Sakka, Baroudi, & Nassani, 2012). Therefore, in order to increase success rates, it is important for implant systems to provide an optimal seal that decreases the risk of bacterial penetration of the IAI (Broggini, et al., 2006; Dibart, et al., 2005).

Findings from the current study show that all tested narrow-diameter Morse taper implant systems presented bacterial penetration and colonization ranging from 65 to >300 CFU. This is in accordance with the results presented by Ranieri et al. (2015), where four regular diameter Morse taper implant systems were positive for penetration of *S. sanguinis*. The authors discussed that the small size of this microorganism (0.8 µm) could explain its penetration in all observed microgaps (Ranieri et al., 2015). The same is valid in our study, as the SEM analysis reported microgaps ranging from 5-10 µm.

Most studies on hexagon implant systems have reported microgaps in the IAI (Ricomini Filho, et al., 2010; Rismanchian, et al., 2012; Silva-Neto et al., 2012). In order to overcome limitations of hexagon systems, Morse taper abutments were later introduced, based on the concept developed in 1864 by Stephen Morse, which had been used successfully in Orthopaedics (Hernigou, Queinnec, & Flouzat Lachaniette, 2013). Morse taper systems were suggested to provide a better seal against bacterial penetration due to its inherent design

(cone inside a cone) and higher torque (Dibart et al., 2005). However, only few Morse taper systems have been reported to fully avoid bacterial penetration (Deconto, Salvoni, & Wassall, 2010; Khorshidi, et al. , 2016, Deconto et al., 2010; Dibart et al., 2005). Most in vitro studies corroborate our findings on the lack of optimal bacterial sealing in Morse taper implants (Teixeira et al., 2011; Aloise et al., 2010; D'Ercole et al., 2014; Dibart et al., 2005; Ranieri et al., 2015; Ricomini Filho et al., 2010).

Ranieri et al. (2015) suggested that torque intensity and the angle of the Morse connection can influence its sealing ability, with smaller angles potentially providing higher attrition between abutment and implant body (Ranieri et al., 2015). The current study might have been underpowered to detect differences among the systems due to the small sample sizes and one limitation is that duplicate and triplicate analysis should have been performed.

According to Steinebrunner et al. (2005), bacterial penetration of the IAI is defined by a multitude of factors, including torque, precision of the fit and the degree of micro movement of the IAI components once the implant is subjected to occlusal loading (Steinebrunner et al., 2005). In our study, the presence of microgaps ranging from 5 to 10 µm in all assemblies suggest less than ideal fit of the components in the IAI in the commercial brands evaluated. Microgaps are unfavourable due to their potential for harbouring bacteria and promoting micromovements, thus increasing the risk for bone loss and implant failure (Hermann, et al., 2001). The results from the current study are comparable to those from the study from Ranieri et al. (2015), who used similar methodology to evaluate Morse taper implants with regular diameter, reported microgaps ranging from 4.9 to 9.9 µm. (Ranieri et al. 2015) In terms of design, narrow diameter implants present a narrower cross-section than regular diameter implants, which mean that microgaps in narrow implants are in closer proximity to the alveolar bone and surrounding tissues. (Klein et al. 2014)

Another variation factor in studies on bacterial sealing of dental implants is the use of different species or combinations of bacteria. While some studies used *S. sanguinis* (Ranieri et al., 2015; Ricomini Filho et al., 2010), others tested *Streptococcus mutans*, *Streptococcus aureus*, *Escherichia coli* (Alves, et al., 2014), *Pseudomonas aeruginosa* and *Aggregatibacter actinomycetemcomitans* (D'Ercole et al., 2014), and a combination of *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Pophyromonas gingivalis* (Dibart et al., 2005). These microorganisms vary not only in size and shape, but also in biological characteristics and clinical significance for disease, making comparison of studies using different species a challenging task.

According to a recent meta-analysis, there is a need for studies on potential risk factors for biological complications associated with NDI (Schiegnitz & Al-Nawas, 2018). To our knowledge, this is the first study evaluating microleakage in narrow implants with Morse taper abutments, hence our results cannot be directly compared to any other studies. Considering the lack of appropriate bacterial sealing and the presence of microgaps in all implant systems in the current study, investigation of procedures to reduce bacterial colonization in the IAI and implant lumen can be clinically relevant to decrease the risk for peri-implant diseases (Podhorsky et al., 2016).

The limitations of the present study include the small sample size and the in-vitro nature of the study, which can differ from the clinical environment in the oral cavity. Different commercial implants systems need to be tested, using sample sizes that are big enough to detect potential differences.

CHAPTER VIII: CONCLUSION

Results from the present study indicate that Morse taper connections in narrow diameter implants might not present an ideal sealing of the IAI, despite its mechanical advantages regarding design and mechanical characteristics over non-tapered connections. The sealing capacity of narrow diameter implants with Morse taper connections was comparable to regular dimeter implants with Morse taper connections. Ideally, implant systems should provide optimal marginal fit to prevent bacterial penetration and provide better biologic and biomechanical results. Within the limitations of the study, it can be concluded that narrow diameter implant systems using Morse taper connections failed to provide bacterial sealing and presented microgaps. The observed microgaps can form reservoirs and potentially lead to inflammation in the peri-implant tissues and micromovements. Further studies with higher sample sizes are required to further elucidate if the angle of the Morse taper can influence the bacterial seal and if the use of disinfectant products can decrease bacterial load.

REFERENCES

Almeida, E. O., Freitas Jr, A. C., Bonfante, E. A., Marotta, L., Silva, N. R. F. A., & Coelho, P. G. (2013). Mechanical Testing of Implant-Supported Anterior Crowns with Different Implant/Abutment Connections. *The International Journal of Oral & Maxillofacial Implants*, *28*(1), 103–108. https://doi.org/10.11607/jomi.2443

Aloise, J. P., Curcio, R., Laporta, M. Z., Rossi, L., da Silva, A. M. Á., & Rapoport, A. (2010). Microbial leakage through the implant-abutment interface of morse taper implants *in vitro*. *Clinical Oral Implants Research*, *21*(3), 328–335. https://doi.org/10.1111/j.1600-0501.2009.01837.x

Alves, D. C. C., Carvalho, P. S. P. de, & Martinez, E. F. (2014). In vitro microbiological analysis of bacterial seal at the implant-abutment interface using two morse taper implant models. *Brazilian Dental Journal*, *25*(1), 48–53. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24789292

Alves, D. C. C., de Carvalho, P. S. P., Elias, C. N., Vedovatto, E., & Martinez, E. F. (2016). In vitro analysis of the microbiological sealing of tapered implants after mechanical cycling. *Clinical Oral Investigations*, *20*(9), 2437–2445. https://doi.org/10.1007/s00784-016-1744-0

Arsan, V., Bölükbaş, N., Ersanli, S., & Özdemir, T. (2010). Evaluation of 316 narrow diameter implants followed for 5-10 years: A clinical and radiographic retrospective study. *Clinical Oral Implants Research*, *21*(3), 296–307. https://doi.org/10.1111/j.1600-0501.2009.01840.x

Assaf, A., Saad, M., Daas, M., Abdallah, J., & Abdallah, R. (2015). Use of Narrow-Diameter Implants in the Posterior Jaw. *Implant Dentistry*, *24*(3), 1. https://doi.org/10.1097/ID.0000000000000238

Assenza, B., Tripodi, D., Scarano, A., Perrotti, V., Piattelli, A., Iezzi, G., & D'Ercole, S. (2012). Bacterial Leakage in Implants With Different Implant–Abutment Connections: An In Vitro Study. *Journal of Periodontology*, *83*(4), 491–497. https://doi.org/10.1902/jop.2011.110320

Badran, Z., Struillou, X., Strube, N., Bourdin, D., Dard, M., Soueidan, A., & Hoornaert, A. (2017). Clinical

Performance of Narrow-Diameter Titanium-Zirconium Implants. *Implant Dentistry*, *26*(2), 316–323. https://doi.org/10.1097/ID.0000000000000557

Berglundh, T., Armitage, G., Araujo, M. G., Avila-Ortiz, G., Blanco, J., Camargo, P. M., … Zitzmann, N. (2018). 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. *Journal of Clinical Periodontology*, *45*, S286–S291. https://doi.org/10.1111/jcpe.12957

Binon, P. P. (2000). Implants and components: entering the new millennium. *The International Journal of Oral & Maxillofacial Implants*, *15*(1), 76–94. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10697942

Bordin, D., Witek, L., Fardin, V. P., Bonfante, E. A., & Coelho, P. G. (2016). Fatigue Failure of Narrow Implants with Different Implant-Abutment Connection Designs. *Journal of Prosthodontics*. https://doi.org/10.1111/jopr.12540

Botticelli, D., Berglundh, T., & Lindhe, J. (2004). Hard-tissue alterations following immediate implant placement in extraction sites. *Journal of Clinical Periodontology*, *31*(10), 820–828. https://doi.org/10.1111/j.1600-051X.2004.00565.x

Bozkaya, D., & Müftü, S. (2005). Mechanics of the taper integrated screwed-in (TIS) abutments used in dental implants. *Journal of Biomechanics*, *38*(1), 87–97. https://doi.org/10.1016/j.jbiomech.2004.03.006

Brånemark, P. I., Hansson, B. O., Adell, R., Breine, U., Lindström, J., Hallén, O., & Ohman, A. (1977). Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. *Scandinavian Journal of Plastic and Reconstructive Surgery. Supplementum*, *16*, 1–132. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/356184

Bressan, E., Stocchero, M., Jimbo, R., Rosati, C., Fanti, E., Tomasi, C., & Lops, D. (2017). Microbial Leakage at Morse Taper Conometric Prosthetic Connection. *Implant Dentistry*, *26*(5), 756–761.

https://doi.org/10.1097/ID.0000000000000657

Broggini, N., McManus, L. M., Hermann, J. S., Medina, R., Schenk, R. K., Buser, D., & Cochran, D. L. (2006). Peri-implant Inflammation Defined by the Implant-Abutment Interface. *Journal of Dental Research*, *85*(5), 473–478. https://doi.org/10.1177/154405910608500515

Broggini, N., McManus, L. M., Hermann, J. S., Medina, R. U., Oates, T. W., Schenk, R. K., … Cochran, D. L. (2003). Persistent Acute Inflammation at the Implant-Abutment Interface. *Journal of Dental Research*, *82*(3), 232–237. https://doi.org/10.1177/154405910308200316

D'Ercole, S., Scarano, A., Perrotti, V., Mulatinho, J., Piattelli, A., Iezzi, G., & Tripodi, D. (2014). Implants With Internal Hexagon and Conical Implant-Abutment Connections: An In Vitro Study of the Bacterial Contamination. *Journal of Oral Implantology*, *40*(1), 30–34. https://doi.org/10.1563/AAID-JOI-D-11-00121

de Freitas, R. M., Spin-Neto, R., Junior, E. M., Pereira, L. A. V. D., Wikesjö, U. M., & Susin, C. (2015). Alveolar Ridge and Maxillary Sinus Augmentation Using rhBMP-2: A Systematic Review. *Clinical Implant Dentistry and Related Research*, *17*, e192–e201. https://doi.org/10.1111/cid.12156

Deconto, M. A., Salvoni, A. D., & Wassall, T. (2010a). In vitro microbiological bacterial seal analysis of the implant/abutment connection in morse taper implants: a comparative study between 2 abutments. *Implant Dentistry*, *19*(2), 158–166. https://doi.org/10.1097/ID.0b013e3181cc48b1

Deconto, M. A., Salvoni, A. Da., & Wassall, T. (2010b). In Vitro Microbiological Bacterial Seal Analysis of the Implant/Abutment Connection in Morse Taper Implants: A Comparative Study Between 2 Abutments. *Implant Dentistry*, *19*(2), 158–166. https://doi.org/10.1097/ID.0b013e3181cc48b1

Degidi, M., Piattelli, A., & Carinci, F. (2008). Clinical Outcome of Narrow Diameter Implants: A Retrospective Study of 510 Implants. *Journal of Periodontology*, *79*(1), 49–54. https://doi.org/10.1902/jop.2008.070248

Dias, E. C. L. de C. e M., Bisognin, E. D. C., Harari, N. D., Machado, S. J., da Silva, C. P., Soares, G. D. de

A., & Vidigal, G. M. (2012). Evaluation of implant-abutment microgap and bacterial leakage in five external-hex implant systems: an in vitro study. *The International Journal of Oral & Maxillofacial Implants*, *27*(2), 346–351. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22442774

Dibart, S., Warbington, M., Su, M. F., & Skobe, Z. (2005). In vitro evaluation of the implant-abutment bacterial seal: the locking taper system. *The International Journal of Oral & Maxillofacial Implants*, *20*(5), 732–737. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16274147

do Nascimento, C., Miani, P. K., Watanabe, E., Pedrazzi, V., & de Albuqerque, R. F. (2011). In vitro evaluation of bacterial leakage along the implant-abutment interface of an external-hex implant after saliva incubation. *The International Journal of Oral & Maxillofacial Implants*, *26*(4), 782–787. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21841988

Donos, N., Mardas, N., & Chadha, V. (2008). Clinical outcomes of implants following lateral bone augmentation: systematic assessment of available options (barrier membranes, bone grafts, split osteotomy). *Journal of Clinical Periodontology*, *35*(8 Suppl), 173–202. https://doi.org/10.1111/j.1600-051X.2008.01269.x

Enkling, N., Saftig, M., Worni, A., Mericske-Stern, R., & Schimmel, M. (2017). Chewing efficiency, bite force and oral health-related quality of life with narrow diameter implants - a prospective clinical study: results after one year. *Clinical Oral Implants Research*, *28*(4), 476–482. https://doi.org/10.1111/clr.12822

Freitas, G. P., Hirata, R., Bonfante, E. A., Tovar, N., & Coelho, P. G. (2016). Survival Probability of Narrow and Standard-Diameter Implants with Different Implant-Abutment Connection Designs. *The International Journal of Prosthodontics*, *29*(2), 179–185. https://doi.org/10.11607/ijp.4232

Galindo-Moreno, P., Nilsson, P., King, P., Becktor, J., Speroni, S., Schramm, A., & Maiorana, C. (2012). Clinical and radiographic evaluation of early loaded narrow diameter implants - 1-year follow-up.

Clinical Oral Implants Research, *23*(5), 609–616. https://doi.org/10.1111/j.1600-0501.2011.02254.x

Goiato, M. C., Pellizzer, E. P., da Silva, E. V. F., Bonatto, L. da R., & dos Santos, D. M. (2015). Is the internal connection more efficient than external connection in mechanical, biological, and esthetical point of views? A systematic review. *Oral and Maxillofacial Surgery*, *19*(3), 229–242. https://doi.org/10.1007/s10006-015-0494-5

Hermann, J. S., Schoolfield, J. D., Schenk, R. K., Buser, D., & Cochran, D. L. (2001). Influence of the Size of the Microgap on Crestal Bone Changes Around Titanium Implants. A Histometric Evaluation of Unloaded Non-Submerged Implants in the Canine Mandible. *Journal of Periodontology*, *72*(10), 1372–1383. https://doi.org/10.1902/jop.2001.72.10.1372

Hernigou, P., Queinnec, S., & Flouzat Lachaniette, C. H. (2013). One hundred and fifty years of history of the Morse taper: from Stephen A. Morse in 1864 to complications related to modularity in hip arthroplasty. *International Orthopaedics*, *37*(10), 2081–2088. https://doi.org/10.1007/s00264-013-1927-0

Heydenrijk, K., Meijer, H. J. A., van der Reijden, W. A., Raghoebar, G. M., Vissink, A., & Stegenga, B. (2002). Microbiota around root-form endosseous implants: a review of the literature. *The International Journal of Oral & Maxillofacial Implants*, *17*(6), 829–838. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12507243

Javed, F., & Romanos, G. E. (2015). Role of implant diameter on long-term survival of dental implants placed in posterior maxilla: a systematic review. *Clinical Oral Investigations*, *19*(1), 1–10. https://doi.org/10.1007/s00784-014-1333-z

Jaworski, M. E., Melo, A. C. M., Picheth, C. M. T., & Sartori, I. A. de M. (2012). Analysis of the bacterial seal at the implant-abutment interface in external-hexagon and Morse taper-connection implants: an in vitro study using a new methodology. *The International Journal of Oral &*

Maxillofacial Implants, *27*(5), 1091–1095. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23057021

Khorshidi, H., Raoofi, S., Moattari, A., Bagheri, A., & Kalantari, M. H. (2016). *In Vitro* Evaluation of Bacterial Leakage at Implant-Abutment Connection: An 11-Degree Morse Taper Compared to a Butt Joint Connection. *International Journal of Biomaterials*, *2016*, 1–5. https://doi.org/10.1155/2016/8527849

Klein, M. O., Schiegnitz, E., & Al-Nawas, B. (2014). Systematic review on success of narrow-diameter dental implants. *The International Journal of Oral & Maxillofacial Implants*, *29 Suppl*, 43–54. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24660189

Maeda, Y., Satoh, T., & Sogo, M. (2006). In vitro differences of stress concentrations for internal and external hex implant-abutment connections: a short communication. *Journal of Oral Rehabilitation*, *33*(1), 75–78. https://doi.org/10.1111/j.1365-2842.2006.01545.x

Mangano, C., Mangano, F., Piattelli, A., Iezzi, G., Mangano, A., & La Colla, L. (2009). Prospective clinical evaluation of 1920 Morse taper connection implants: results after 4 years of functional loading. *Clinical Oral Implants Research*, *20*(3), 254–261. https://doi.org/10.1111/j.1600-0501.2008.01649.x

Mangano, F. G., Mastrangelo, P., Luongo, F., Blay, A., Tunchel, S., & Mangano, C. (2017). Aesthetic outcome of immediately restored single implants placed in extraction sockets and healed sites of the anterior maxilla: a retrospective study on 103 patients with 3 years of follow-up. *Clinical Oral Implants Research*, *28*(3), 272–282. https://doi.org/10.1111/clr.12795

Marcello-Machado, R. M., Faot, F., Schuster, A. J., Nascimento, G. G., & Del Bel Cury, A. A. (2018). Mini-implants and narrow diameter implants as mandibular overdenture retainers: A systematic review and meta-analysis of clinical and radiographic outcomes. *Journal of Oral Rehabilitation*, *45*(2), 161–183. https://doi.org/10.1111/joor.12585

Merz, B. R., Hunenbart, S., & Belser, U. C. (2000). Mechanics of the implant-abutment connection: an 8-degree taper compared to a butt joint connection. *The International Journal of Oral & Maxillofacial Implants*, *15*(4), 519–526. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10960985

Mishra, S. K., Chowdhary, R., & Kumari, S. (2017). Microleakage at the Different Implant Abutment Interface: A Systematic Review. *JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH*, *11*(6), ZE10–ZE15. https://doi.org/10.7860/JCDR/2017/28951.10054

Mombelli, A., & Décaillet, F. (2011). The characteristics of biofilms in peri-implant disease. *Journal of Clinical Periodontology*, *38*, 203–213. https://doi.org/10.1111/j.1600-051X.2010.01666.x

Nassar, H.I. & Abdalla, M.F (2015). Bacterial leakage of different internal implant/abutment connection. *Future Dental Journal*, 1(1), 1-5.

Norton, M. R. (2000). An in vitro evaluation of the strength of a 1-piece and 2-piece conical abutment joint in implant design. *Clinical Oral Implants Research*, *11*(5), 458–464. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11168238

Papadimitriou, D. E. V., Friedland, B., Gannam, C., Salari, S., & Gallucci, G. O. (2015). Narrow-Diameter versus Standard-Diameter Implants and Their Effect on the Need for Guided Bone Regeneration: A Virtual Three-Dimensional Study. *Clinical Implant Dentistry and Related Research*, *17*(6), 1127–1133. https://doi.org/10.1111/cid.12224

Peruzetto, W. M., Martinez, E. F., Peruzzo, D. C., Joly, J. C., Napimoga, M. H., Peruzetto, W. M., … Napimoga, M. H. (2016). Microbiological Seal of Two Types of Tapered Implant Connections. *Brazilian Dental Journal*, *27*(3), 273–277. https://doi.org/10.1590/0103-6440201600604

Podhorsky, A., Biscoping, S., Rehmann, P., Streckbein, P., Domann, E., & Wöstmann, B. (2016). Transfer of Bacteria into the Internal Cavity of Dental Implants After Application of Disinfectant or Sealant Agents In Vitro. *The International Journal of Oral & Maxillofacial Implants*, *31*(3), 563–570.

https://doi.org/10.11607/jomi.4408

Ranieri, R., Ferreira, A., Souza, E., Arcoverde, J., Dametto, F., Gade-Neto, C., … Sarmento, C. (2015). The Bacterial Sealing Capacity of Morse Taper Implant–Abutment Systems in Vitro. *Journal of Periodontology*, *86*(5), 696–702. https://doi.org/10.1902/jop.2015.140623

Ricomini Filho, A. P., Fernandes, F. S. de F., Straioto, F. G., da Silva, W. J., & Del Bel Cury, A. A. (2010). Preload loss and bacterial penetration on different implant-abutment connection systems. *Brazilian Dental Journal*, *21*(2), 123–129. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20640358

Rismanchian, M., Hatami, M., Badrian, H., Khalighinejad, N., & Goroohi, H. (2012). Evaluation of Microgap Size and Microbial Leakage in the Connection Area of 4 Abutments With Straumann (ITI) Implant. *Journal of Oral Implantology*, *38*(6), 677–685. https://doi.org/10.1563/AAID-JOI-D-11-00167

Rodríguez-Hernández, A., Juárez, A., E. E.-J. of M. (2011). Streptococcus sanguinis adhesion on titanium rough surfaces: effect of shot-blasting particles. *Springer*. Retrieved from https://link.springer.com/article/10.1007/s10856-011-4366-8

Romeo, E., Lops, D., Amorfini, L., Chiapasco, M., Ghisolfi, M., & Vogel, G. (2006). Clinical and radiographic evaluation of small-diameter (3.3-mm) implants followed for 1-7 years: a longitudinal study. *Clinical Oral Implants Research*, *17*(2), 139–148. https://doi.org/10.1111/j.1600-0501.2005.01191.x

Sakka, S., Baroudi, K., & Nassani, M. Z. (2012). Factors associated with early and late failure of dental implants. *Journal of Investigative and Clinical Dentistry*, *3*(4), 258–261. https://doi.org/10.1111/j.2041-1626.2012.00162.x

Sannino, G., & Barlattani, A. (2013). Mechanical Evaluation of an Implant-Abutment Self-Locking Taper Connection: Finite Element Analysis and Experimental Tests. *The International Journal of Oral &*

Maxillofacial Implants, *28*(1), e17–e26. https://doi.org/10.11607/jomi.2058

Scarano, A., Lorusso, C., Di Giulio, C., & Mazzatenta, A. (2016). Evaluation of the Sealing Capability of the Implant Healing Screw by Using Real Time Volatile Organic Compounds Analysis: Internal Hexagon Versus Cone Morse. *Journal of Periodontology*, *87*(12), 1492–1498. https://doi.org/10.1902/jop.2016.160076

Schiegnitz, E., & Al-Nawas, B. (2018). Narrow-diameter implants: A systematic review and meta-analysis. *Clinical Oral Implants Research*, *29*, 21–40. https://doi.org/10.1111/clr.13272

Schwarz, F., Derks, J., Monje, A., & Wang, H.-L. (2018). Peri-implantitis. *Journal of Clinical Periodontology*, *45*, S246–S266. https://doi.org/10.1111/jcpe.12954

Sierra-Sánchez, J.-L., Martínez-González, A., García-Sala Bonmatí, F., Mañes-Ferrer, J.-F., & Brotons-Oliver, A. (2014). Narrow-diameter implants: are they a predictable treatment option? A literature review. *Medicina Oral, Patologia Oral y Cirugia Bucal*, *19*(1), e74-81. https://doi.org/10.4317/MEDORAL.19306

Silva-Neto, J. P. da, Prudente, M. S., Carneiro, T. de A. P. N., Nóbilo, M. A. de A., Penatti, M. P. A., & Neves, F. D. das. (2012). Micro-leakage at the implant-abutment interface with different tightening torques in vitro. *Journal of Applied Oral Science : Revista FOB*, *20*(5), 581–587. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23138747

Steinebrunner, L., Wolfart, S., Bössmann, K., & Kern, M. (2005). In vitro evaluation of bacterial leakage along the implant-abutment interface of different implant systems. *The International Journal of Oral & Maxillofacial Implants*, *20*(6), 875–881. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16392344

Sutter, F., Weber, H., Sorensen & Belser R. (1993). The new restorative concept of the ITI dental implant system: design and engineering. *The International Journal of Periodontics and Restorative Dentistry*, *13*(6), 409-431.

Tamura, N., Ochi, M., Myakaw,a H. & Nakazawa, F. (2013). Analysis of bacterial flora associated with peri-implantitis using obligate anaerobic culture technique and 16S rDNA gene sequence. *The International Journal of Oral & Maxillofacial Implants*, *28*(6), 1521–9. . Retrieved from http://europepmc.org/abstract/med/24278920

Teixeira, W., Ribeiro, R. F., Sato, S., & Pedrazzi, V. (2011). Microleakage into and from two-stage implants: an in vitro comparative study. *The International Journal of Oral & Maxillofacial Implants*, *26*(1), 56–62. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21365038

Warrer, K., Buser, D., Lang, N. P., & Karring, T. (1995). Plaque-induced peri-implantitis in the presence or absence of keratinized mucosa. An experimental study in monkeys. *Clinical Oral Implants Research*, *6*(3), 131–138. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7578788

Zinsli, B., Sägesser, T., Mericske, E., & Mericske-Stern, R. (2004). Clinical evaluation of small-diameter ITI implants: a prospective study. *The International Journal of Oral & Maxillofacial Implants*, *19*(1), 92–99. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14982361