Efficacy of GapSeal® in Preventing Microleakage at the Dental Implant Abutment Interface

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Keywords

GapSeal®

Implant Abutment Interface

Microleakage

Dynamic Loading

Chewing Simulator

Abstract

Background: Dental implants have proven to be a success in the past decades, however the inevitable presence of microgaps at the implant abutment interface leading to microleakage is still a distressing concern. Microbial leakage can lead to peri-implant disease and bone loss and reduces implants' success rates. Measures to decrease the effect of the microgap were introduced; amongst them is the application of silicone sealing gels, such as GapSeal®.

Aims: The aim was to test the ability of the sealing gel, GapSeal®, in preventing bacterial leakage at the implant abutment interface of dental implants, under dynamic loading in a chewing simulator**.**

Materials and Methods: In this *in-vitro* study**,** a total of 30 dental implants (SEVEN design, internal hexagon, standard platform, MIS) were divided into 2 groups: Group GS had GapSeal® applied to the internal aspect of the implants (case group) while Group GN (control group) had none applied. The implants were connected to their adjacent abutments at 30 Ncm, following the manufacturer's torque instructions. Microbiological analysis was done by immersing the implant assembly in *Streptococcus sanguinis* inoculated Brain Heart Infusion (BHI) suspension. The implant assemblies were mounted on a chewing simulator and subjected to dynamic forces of 80 N at 1 Hz for 200,000 cycles. Once the cycles were complete, the implants were dismantled from the chewing simulator and the abutments disconnected. Sterile paper points were used to obtain samples from the implants' interiors and immersed in sterile BHI. Serial dilution was performed, and the samples cultured on labelled agar plates and incubated for 24 hours at 37ºC and 5% CO^2, then colony forming units were counted and recorded.

Results: The results showed a significant difference (Levene's test of variances p=0.006) in the number of CFU/ml in GN (case) group in comparison to GS (control) group, with the mean CFU/ml of GS group (10.21) being less than that of GN group (87.79), which was statistically significant.

Conclusion: The application of GapSeal® to the implants interiors was effective in reducing microleakage of *S. sanguinis* at the implant abutment interface, under dynamic loading, to a negligible amount. However, it was not successful in completely preventing microbial leakage.

Declaration

I hereby declare, that the mini-thesis titled "*Efficacy of GapSeal® in preventing microleakage at the dental implant abutment interface*" is of my own work and has not been submitted previously for any examination or degree at any additional university, and that all the used or quoted sources have been fully indicated and acknowledged by complete references.

Hadeel Mohamed Badi Mohamed December 2020

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Dedication

To my sweet, sweet parents, I don't see how anyone can carry all this love you have given me, I appreciate you.

To my two brothers, it's because of you I try, you inspire me more than you can imagine.

And to my angel in heaven, thank you for always being the light shining down on my darkest days, I'm forever grateful.

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List of Abbreviations

IAI	Implant Abutment Interface
BHI	Brain Heart Infusion
S. sanguinis	*Streptococcus sanguinis*
PBS	Phosphate Buffered Saline
CFU	Colony Forming Units
GN	Group not containing GapSeal®
GS	Group containing GapSeal®

Chapter 1: Introduction

Dental implants are considered amongst the most convenient treatments in replacing missing teeth in our day and age. With success rates noted to be higher than 90%, dental implants are proving to be one of the best rehabilitation methods in modern dentistry (Kwon *et al.,* 2014). This success rate is attributed to multifactorial conditions, including accurate treatment planning, quantity and/or quality of bone and adequate oral hygiene, in addition to increase in the chemical, mechanical and physical properties of the implant and its components (Jimbo and Albrektsson, 2015). Nevertheless, the dental implant is still prone to failure. One of the causes for that is the microleakage that occurs at the implant abutment interface (IAI) (Nascimento *et al.,* 2012; Passos *et al*., 2013), leading to peri implant tissue infection which is linked to both early and late failure of implants (Sakka *et al*., 2012; Palma-Carrio *et al*., 2011).

The presence of a microgap at the IAI is inevitable, regardless of the implant abutment connection, and microbial leakage cannot be completely prevented even in contemporary implant systems (Do Nascimento *et al*., 2008). On this basis, there has been an increased interest in testing the use of sealing materials at the IAI to reduce or perhaps even prevent microbial leakage (Sousa CA *et al.,* 2019). One such material is GapSeal®, which was evaluated by Nayak et al. (2014) and showed promising results for its performance in reducing bacterial leakage, under static conditions.

However, studies investigating microbial leakage showed that implant systems were more susceptible to microleakage under dynamic loading, due to the so-called pumping effect (Steinebrunner *et al.,* 2005; Koutouzis *et al.,* 2014). This indicates that testing dental implants under dynamic loading is an essential part of the experimental design for evaluating bacterial leakage in dental implants and understanding IAI dynamics (Koutouzis *et al.,* 2014). Therefore, the aim of this *in-vitro* laboratory study was to investigate the sealing ability of GapSeal®, in preventing microleakage at the IAI under dynamic conditions. The hypothesis to be tested is that GapSeal® would form a seal and prevent microbial leakage at the IAI, even after dynamic loading.

Chapter 2: Literature Review

2.1 Implant Abutment Interface (Microgap)

Generally, dental implant systems are comprised of two main components: a fixture inserted surgically into the alveolar bone and an abutment connected transmucosally (McGlumphy *et al.*, 1998). Although this system permits a longer healing period and successful osteointegration (Watchel *et al*., 2016), it has a disadvantage of creating a microgap between the implant fixture and abutment, also known as the implant abutment interface (IAI) (Jansen *et al.*, 1997). The presence of microgaps at the IAI is an unavoidable outcome in two-piece dental implant systems (Texiera *et al.*, 2011; Canullo *et al.*, 2015), even after applying the recommended torque values provided by the manufacturer (Do Nascimento *et al.*, 2015).

The microgaps offer both biological and mechanical significance to the success of dental implants. The biological aspect is linked to the crestal bone remodelling, whereas the mechanical one is related to the possibility of screw loosening and abutment fracture (Scarano *et al.*, 2005). Microgaps at the IAI are generally ranging from 1 to 49 μm (Jansen *et al.*, 1997) however the diameter of a microbe is, on average, <2.0 μm (Cavalcanti *et al.*, 2016). It is also worth considering that the dimensions of the microgap are larger and more unpredictable than what is usually observed in studies carried out *in-vitro* (Scarano *et al.*, 2005).

2.2 Microleakage

The microgap allows the influx of fluids and bacteria into the implant from the oral cavity, due to the pumping action caused by chewing (Koutouzis *et al.*, 2014), stated to even occur in patients with optimum oral hygiene (Rimondini *et al*., 2001). It was also suggested that after loss of teeth, in completely edentulous patients, the species of microorganisms related to periodontal disease will still be present in the oral cavity, soft tissue, and the alveolar bone (Fernandes *et al.*, 2010). The interior wall of the implant becomes a reservoir for the bacterial colonies and the bacteria and micro-organisms found are capable of migrating in a bidirectional manner, that is, from the implant's interior to the outside environment as well as in the opposite direction (Jansen *et al*., 1997).

Microleakage can interfere with osseointegration in the healing phase of the surgical intervention (Arshad *et al.*, 2013). It can also result in an inflammatory reaction and a host response in the peri-implant soft tissues which can lead to bone loss and peri-implantitis (Adell *et al.*, 1981). Peri-implantitis is stated to be one of the major causes of implant failure (do Nascimento *et al*., 2012). In addition, studies have suggested that the changes in the crestal bone level relied on the location of the microgap from it (Adell *et al.*, 1981; Broggini *et al.*, 2006).

In addition, microleakage has been linked with mechanical problems, such as abutment screw loosening and fracture (Sahin and Ayyildiz 2014) which could be a consequence of the lubricous environment created by the microbial activities (Broggini *et al.*, 2006). Recent studies have determined that the use of two stage implant systems inevitably resulted in microgap formation and consequently microleakage (Canullo *et al.*, 2015). However, there are variations in the microbial leakage depending on precision of fit between the implant and abutment, the torque force used to attach them, the degree of micromovement between them (Steinebrunner *et al*., 2005; Harder *et al.*, 2010) and the position of the IAI with relation to the crestal bone (Broggini *et al.*, 2006). Additionally, attempts to reduce microleakage have been made by employing different types of implant-abutment connections (Goiato *et al.*, 2015;) as well as the use of a sealing material at the IAI (Nayak *et al*., 2014; Sousa *et al.*, 2019; Mohammadi *et al.*, 2019).

2.3 Reducing Microleakage

2.3.1 Implant-Abutment Connections

Implant systems are divided into two groups according to the connection geometry existing between the fixture and the abutment. The two types are the internal connection and the external hexagon connection. The internal connection is further subdivided into three designs: the internal hexagon, the internal conical design (Morse taper), and the internal octagon (Goiato *et al.*, 2015).

Studies conducted on external hexagon connection designs have shown presence of bacterial leakage irrespective of the technical variable used (do Nascimento *et al.*, 2011*)*. This is partly due to its short platform (Costa *et al.*, 2017) that leads to instability

and increases the microgap resulting in more bacterial leakage (Costa *et al.,* 2017). Studies indicated that the size of the microgap in external hexagon connection designs can be up to 86.6 μm (De Olivera *et al.*, 2014).

In comparison to the external hexagon design, the internal connection designs have shown superior sealing abilities at the IAI (Peñarrocha-Diago *et al.*, 2013). This can be credited to the smaller magnitude of the microgap in internal connection designs (Canullo *et al.*, 2015). For instance, the microgap in a conical internal connection design was found to be an average of 6.61± 3.17 μm (Ranieri *et al.*, 2015), up to 53.9 μm in an internal hexagon design (de Olivera *et al.*, 2014), and ranges from 7 to 10 μm in the internal octagon design (Rismanchian *et al.*, 2012).

Abutments are also manufactured with different materials such as titanium and zirconium. The magnitude of the microgap was found to be bigger in zirconium abutments when compared to titanium ones, and therefore presented with more microleakage (Sahin and Ayyildiz, 2014; Cavusoglu *et al.*, 2014).

A study in patients with implants inserted for five years was performed where microleakage of different types of implant abutment connections was compared, using polymerase chain reaction. The results concluded that the Morse taper connection showed the least colony forming units within the implants. However, none of the implant abutment connection designs was able to fully prevent microleakage (Canullo *et al.*, 2014).

Figure 2-1: Different dental implant connections (Lopez Navarro *et al*., 2017)

Figure 2-2: Internal octagon connection (Mode Implant)

Figure 2-3: Internal conical connection (Southern Implant)

2.3.2 Sealing the microgap

Sealing the microgap with different materials have been suggested as a mean of reducing microleakage. Some of the different materials attempted included chlorohexidine solution or gel (Koutouzis *et al.,* 2015; D'ercole *et al.,* 2009) gutta-percha (Proff *et al.,* 2006), Atridox (Mohammadi *et al.,* 2019) and GapSeal® (Nayak *et al.,* 2014).

Using Gutta percha was tested *in-vitro* and there was no significant success in prevention of microbial leakage (Proff *et al.*, 2006). A silicone gel sheet was tested in an in-vivo study and resulted in reduced microleakage after 90 days but did not completely prevent it (Castro Pimentel *et al.,* 2014). Atridox, a controlled-release doxycycline gel with antibacterial effects, applied at the IAI delayed the bacterial leakage significantly and limited endotoxin production. While the use of chlorohexidine did not prevent or provide any considerable delay in the microleakage, despite the bacteria being sensitive to it (Mohammadi *et al*., 2019). Koutouzis *et al.* (2015) also tested chlorohexidine solution in preventing endotoxin penetration and the results showed no significant outcome. Other studies attempted the application of 1% chlorohexidine gel to reduce microbial leakage, both *in-vitro* and *in-vivo*, and the results proved successful (D'ercole *et al.,* 2009; Ghannad *et al.,* 2015).

2.4 GapSeal®

GapSeal® (Hager & Werken, Duisburg, Germany) is a sealing gel added on highly viscous silicone matrix with 5% weight of thymol (Fritzemeier 2013). No allergic reactions were found due to Thymol which is also bactericidal (Basch *et al.*, 2004; Burt S. 2004). Its ability to seal the microgap in dental implants was observed, *in-vivo*. Split mouth study design was carried out, where the IAI of dental implants on one side had GapSeal® applied whereas Vaseline was added to the IAI of implants on the opposite side of the mouth. Results showed that after 6 months, microorganism growth was found on the implants with Vaseline but no bacterial growth was found in the ones with GapSeal® (Fritzemeier 2013). An *in-vitro* study using enterococci, compared the sealing abilities of GapSeal® and O-ring (a polysiloxane ring) in preventing microleakage. It was found that GapSeal® was more successful in reducing microbial

leakage. The better performance of GapSeal® proposed that it could flow easily through the IAI and provide an enhanced cover due to its viscosity.

Another study measured the microleakage and the microgap size in internal hexagon connection implants with and without GapSeal®. The results showed microgap size to be 0.99±0.39 µm with GapSeal® added, which was less in comparison to the 3.04±0.54 µm without it. No microleakage was detected in all 8 implant samples that contained GapSeal® (Nasser Mostofi *et al.*, 2019). Mohammadi *et al.* (2019) showed that GapSeal® could not stop microleakage, it did however lead to a considerable delay to its onset.

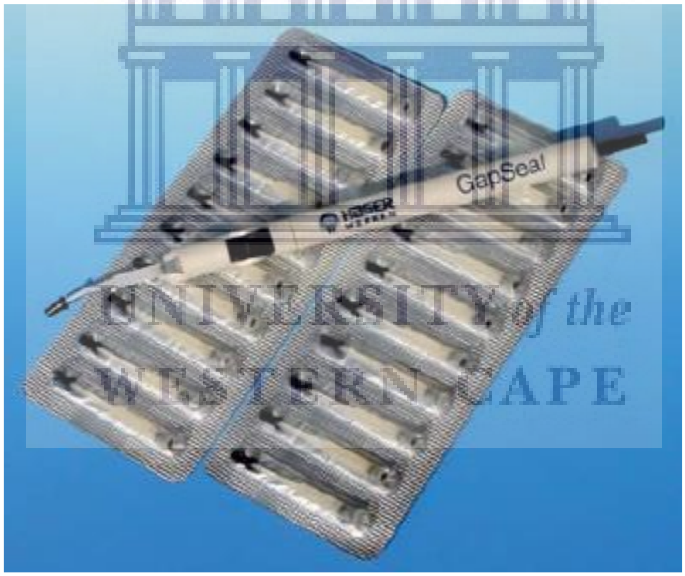

Figure 2-4: GapSeal® applicator with GapSeal® carpules (Fritzemeier, 2013).

2.5 Dynamic Loading

Investigating microleakage under loaded conditions is advised since it is more applicable to the clinical state. In addition, it was stated that the implant assemblies were more prone to microbial leakage following dynamic loading due to the pumping effect (Koutouzis *et al.,* 2014). In addition, deterioration of the IAI may occur due to altered functional adaptations of the dental implant-abutment connection (Al-Jadaa *et al.,* 2015). Studies testing different implant systems with different implant-abutment connections deduced that an increased potential for microleakage was present in the implant assemblies exposed to dynamic forces (Al-Jadaa *et al.,* 2015; Siadat *et al.,* 2016). Dynamic cycles ranging from 16,000 to 1,200,000 cycles were employed in testing the implants (Mishra *et al.,* 2017), in which 500,000 cycles was the equivalent average of 6 months *in-vivo* mastication (Cibirka *et al.,* 2001).

2.6 Microleakage testing methods

In this section the following will be discussed: studies evaluating bacterial microleakage, the bacterial species found in dental implants and the various testing methods used to assess microleakage. Several methodologies have been adopted to determine both the magnitude and the influence of the microgap on bacterial leakage (Harder *et al.,* 2010). Studies have investigated this microleakage either from the interior aspect of the implants to the outside environment (I/E) (Aloise *et al.,* 2010; Sousa *et al.,* 2019) or from the external environment to the internal aspect of the implant (E/I) (Ranieri *et al.,* 2015; Siadat *et al.,* 2016). The freehand inoculation of bacterial broth into the implant in the I/E method, in addition to the lack of determination of the implant's internal volume could generate false-positive results. Furthermore, the opening of the implant fixture is, in most cases, approximately 2 to 3 mm in diameter. Due to this, higher precision from the operator is required to avoid possible contact with the borders of the implant, enabling the passage of bacteria into the external environment (Da Silva-Neto *et al.,* 2012). When considering microleakage testing at the IAI with the E/I testing procedure, the total immersion of implant assemblies in the testing liquid could also lead to false-positive results owing to the potential penetration of the fluid through the abutment screw interface (Da Silva-Neto *et al.,* 2012).

Bacterial microleakage studies (both E/I and I/E) have made use of several bacteria, ranging from facultative to obligate anaerobes, differing in size from 1 to 10 μm. Their use was justified by their reduced size, their common residence around the peri-implant area and their permeability through the microgap (Koutouzis *et al*., 2014; Zipprich *et al.,* 2016). Nevertheless, elevated bacterial concentrations (>1.5 × 10^8 CFU/mL), limited volumes of inoculum, and the interior of the implant fixture having limited conditions of oxygenation and nutrition, provide extremely adverse circumstances for bacterial growth and survival. This could lead to false negatives resulting from the death of the bacteria within the implants. Therefore, extended monitoring periods (>7 days) must be avoided (Da Silva-Neto *et al.,* 2012).

Bacterial toxins (Koutouzis *et al.,* 2015), saliva (do Nascimento *et al*., 2011) and varying stains, such as methylene blue (Verdugo *et al*., 2014) were also used. The authors stated that the motivation of utilizing stains included the similar sizes of their particles to the size of bacterial toxins and ease of their use (Coelho *et al.,* 2008; Verdugo *et al.,* 2014). As for bacterial endotoxins, their smaller size and primary role in marginal bone loss proved advantageous (Da Silva-Neto *et al.,* 2012).

Numerous qualitative and/or quantitative microleakage testing methods were tried, including turbidity analysis (Silva-Neto 2012), checkerboard DNA-DNA hybridization (Do Nascimento *et al.,* 2015) radiotracer technique (Siadat *et al.,* 2016) and microbial counting (Nayak *et al.,* 2014). Do Nascimento *et al.* (2012) performed a study comparing the accuracy of the DNA checkerboard hybridization testing method and the conventional culture counting method. Their result showed that microbial leakage in the IAI was comparably noted in both methods. That suggested that either method would lead to similar conclusions (Refer to Table 2-1).

2.7 Rationale of the Study

After reviewing the literature, it is possible to conclude that the search for methods of eliminating microleakage at the IAI is still ongoing. The application of GapSeal® to the IAI showed promising potential in the prevention of microbial leakage (Nayak et al., 2014; Mohammadi et al., 2019). However, not enough studies were found testing its effectiveness under dynamic forces, using the E/I testing procedure with bacterial

species. Thus, the *in-vitro* study to test GapSeal®'s efficacy in preventing microleakage at the IAI, after exposing the implant assembly to dynamic loading, was conducted.

Table 2-1: *In-vitro* studies on microleakage at IAI using diverse testing techniques with various implant connection designs, different loading conditions and sealing agents.

Author	Type of implant connection/Number of implants (n)	Dynamic Loading	Bacteria/dye used	Method of evaluation	Result
Koutouzis *et al.,* 2014	Internal Morse taper/n=40	500,000 cycles/50 N	*E. coli*	CFU were counted	Morse-taper connection showed minimal leakage. Dynamic loading increased microleakage
Nayak *et al.,* 2014	n=45	No loading	*Enterococcus*	CFU were counted	Least growth was observed in the GapSeal® group followed by the O-ring group
Verdugo *et al.,* 2014	External connection; Internal Morse taper/n=42	No loading	0.2% methylene blue	Optical microscopy	Less microleakage in the Morse taper connection implants than external connection implants
Al-Jadaa *et al.,* 2015	Taper lock and internal hexagonal; Flat-to-flat interface and internal hexagonal mating surface; Flat-to-flat and a trilobe mating/n=30	Both static and dynamic loading/ 1,200,000 cycles/unknown N	Unknown bacteria	Gas Enhanced Permeation Test (GEPT)	Flat-to-flat interface and internal hexagonal mating surface showed least microleakage, under both static and dynamic conditions
Ranieri *et al.,* 2015	Internal Morse taper/n=4	No loading	*S. sanguinis*	Scanning electron microscopy (SEM)	Morse taper implant systems do not provide resistance to microleakage
Zipprich *et al.,* 2016	Conical implant abutment connection; Flat implant abutment connection/n=70	1,200,000 cycles/Max of 200N	*S. sanguinis, A. viscosus, S. mutans, V. parvula F. nucleatum,*	Florescence microscopy	Under dynamic loading conical implant abutment connection offers better seal
Sousa *et al.,* 2019	External hexagon; Internal Morse taper/n=216	No loading	*E. faecalis*	CFU counted and SEM	Presence of the sealing agent reduces or eliminates microleakage
Nasser Mostofi *et al.,* 2019	Internal hexagon/n=12	500,000 cycles/80 N	Methylene blue solution	SEM	Gapseal group showed no microleakage

Chapter 3: Aims and objectives

3.1 Aim:

The aim of this study was to test the ability of the sealing gel, GapSeal®, in preventing bacterial leakage through the implant abutment interface of internal hexagon dental implants, under dynamic loading in a chewing simulator.

3.2 Objectives:

1. To determine the microbial leakage in internal hexagon dental implants containing GapSeal®, after being exposed to dynamic loading in the chewing simulator.
2. To determine the microbial leakage in internal hexagon dental implants not containing GapSeal®, after being exposed to dynamic loading in the chewing simulator.
3. To compare the microbial leakage between the implants containing GapSeal® and the implants not containing GapSeal®.

Chapter 4: Materials and Methods

4.1 Study Design

This study conducted an *in-vitro* laboratorial analysis investigating the sealing ability of GapSeal® in reducing bacterial leakage at the implant-abutment interface, post being exposed to dynamic forces in a chewing simulator.

4.2 Study Site

This study was performed in the Oral and Dental Research Laboratory, Faculty of Dentistry, University of the Western Cape, Tygerberg Campus.

4.3 Sample Size

A total of 30 dental implants (SEVEN MIS design, internal hexagon, 4.2x10 mm, standard platform) (MIS Implants Technologies Ltd, Haifa, Israel) and their corresponding abutments were utilized in this study. This sample size was agreed upon after consultation with a statistician and considering the cost effectiveness. It also coincided with the literature, where the minimal number of samples used per group should be between 8 and 10 (Da Silva-Neto *et al*., 2012).

4.4 Method

The following methodological steps were implemented from previous studies (Koutouzis *et al*., 2011; Nayak *et al*., 2014).

4.4.1 Closing Torque Procedure

Each dental implant was stabilized and held upright with autopolymerising resin, in custom-made, polytetrafluoroethylene (PTFE) test chambers. This was done to create a compartment for the bacterial solution and to standardize the volume used for each implant assembly. In addition, the resin helped in mimicking intraoral environment in which some forces transported to the IAI would be absorbed by the bone (Steinnebrunner *et al*., 2005). For the preparation of the resin, an appropriate powder/liquid ratio was used in accordance to the manufacturer's instructions.

The dental implants embedded in the resin and their respective abutments were then put in sterilization pouches, assigned codes and sent for gas sterilization (Figure 4-1 and 4-2). Subsequently, in a disinfected laminar flow chamber, each implant was carefully connected to its corresponding abutment using a sterile torque wrench, at 30 Ncm according to the manufacturer's guidelines and protocols.

Figure 4-1: Dental implants embedded in resin placed in surgical pouches for sterilization

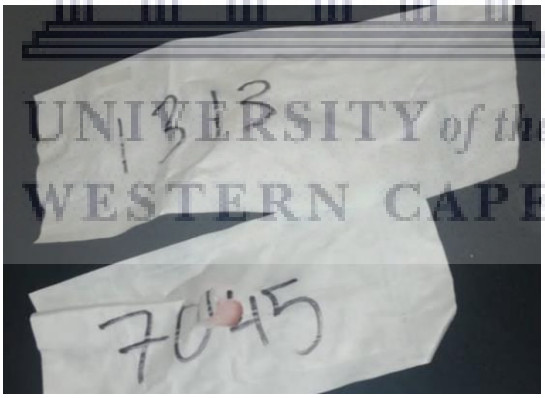

Figure 4-2: Surgical pouches with implant assembly codes

The dental assemblies were divided into two groups of 15 implants each: Group GS had GapSeal® applied to the internal aspect of the implant fixture prior to abutment connection while group GN implants had none applied. The GapSeal® was added to the maximum capacity of the internal aspect of the implant fixture according to the manufacturer's instructions to prevent air entrapment. GapSeal® was applied using an applicator provided by the manufacturer and sterilized before every use (Figure 4-3).

All dental assemblies were handled by one operator, in sterile conditions in the laminar flow chamber.

Figure 4-3: GapSeal® applicator and carpules

4.4.2 Bacterial Culture Preparation

The microorganism selected to test the bacterial sealing ability of GapSeal® was *Streptococcus sanguinis (ATCC10556)*. S. *sanguinis* is a gram positive, facultative anaerobic bacterium and is amongst the primary colonizers in the oral cavity. It attaches directly to oral surfaces and even more to titanium than other bacteria (Edgerton *et al.*, 1996). It has a relatively small size, 0.5-1.0µm (Bulleid 1938), and is capable of adhering to implant titanium surfaces, irreversibly (Rimondini *et al.*, 2001), as well as facilitating the adherence of secondary microbial colonizers (Ranieri *et al.*, 2015). *S. sanguinis* bacteria was obtained from the Oral and Dental Research Institute, University of the Western Cape, and cultured using the direct colony suspension method. Using a sterile loop, some colonies from the stock culture were transferred into sterile BHI and incubated for 24 hrs at 37ºC. Afterwards, it was inoculated in agar plates and incubated overnight. The bacterial inoculum was then extracted from the incubated culture and diluted in 2 ml of PBS with and adjusted to 0.5 McFarland standard (~1.5x 10^8 CFU/ml), using DensiCHEK. The 2 ml of inoculated PBS was then added to 2 ml of sterile BHI and mixed well using a vortex mixer for 5 seconds. This was the final inoculated BHI solution used for testing the microbial leakage.

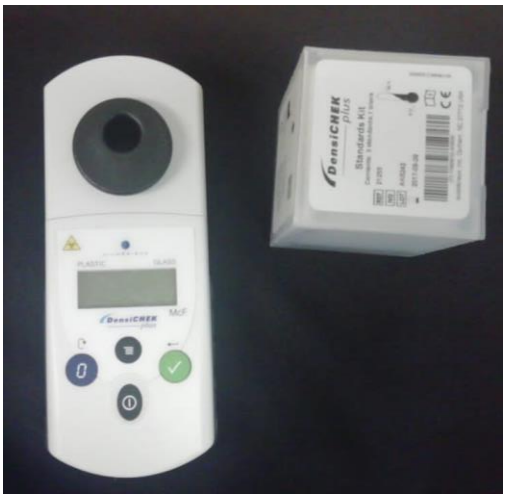

Figure 4-4: DensiChek device used to adjust the inoculum to 0.5 McFarland standard

4.4.3 Dynamic Loading

All implant assemblies from each group, placed in the custom-made PTFE chambers were then mounted in the chewing simulator. 2 ml of the inoculated BHI was transferred to the chamber of 28 of these assemblies, using a sterile pipette. This volume of solution was adequate to guarantee that the IAI was fully immersed but not the screw opening. This was to ensure that if leakage occurred, it would be due to leakage through the IAI and not from the screw opening. 1 implant assembly from Group GS and 1 from Group GN had 2 ml of sterilized BHI solution added to their test chambers instead of the inoculated BHI. This served as a negative control that ensured any microleakage into the implant was from the inoculum and not a result of external contamination.

The chewing simulator (CS-4, SD Mechatronik, Germany) housed two implants at the same time (Figure 4-5). A cyclic fatigue load of 80 N, considered in the physiologic range (Richter 1995; Morneburg *et al.*, 2002), was applied for a total of 200,000 cycles at 1 Hz to each implant assembly with a sterile round stainless-steel stylus. 200,000 cycles were completed in around the 24hour time frame which took into consideration the *S. sanguinis* livelihood. The chewing simulator operated via a computer program, therefore it calibrated automatically once the parameters mentioned above were input. The chewing simulator and its components were disinfected before and after every complete set of cycles.

Figure 4-5: Chewing Simulator (CS-4, SD Mechatronik)

4.4.5 Measuring Bacterial Colonies

After the completion of the chewing cycle, the assemblies were removed from the custom-made test chambers using sterile pliers, sprayed with 70% alcohol and positioned vertically for 10 minutes until the alcohol evaporated. The assemblies were carefully disconnected in a disinfected laminar flow chamber, using a sterile torque wrench. A sample for testing bacterial contamination from the interior aspect of each implant was taken using sterile paper points. The paper points were then immersed in 1000 µl of sterile BHI in sterilized eppendorf tubes, labelled with the implant code, and placed in an incubator at 37 °C for 20 minutes.

Serial dilution was performed for each sample. 200 µl was pipetted from the eppendorf tube and transferred to the wells in row A of the first three columns in the 96-well plate (Figure 4-6). Afterwards, 100 µl of PBS was added to the wells from of row B of the first column to row H of the third column, using a multichannel pipette. The solution was then diluted by two-folds by adding 100 µl from the wells in row A to the wells in row B and so forth up to the wells in row H.

Figure 4-6: 96 well plate used in serial dilution with the multichannel pipette

10 µl was then transferred from the wells E1, E2 and E3**,** using a single channel pipette, spread on 3 individual labelled agar plates using a sterile hockey stick and then incubated at 37°C for 24 hours. After the complete incubation period, the CFU in the plates were measured by means of a colony counter (Gerber, Switzerland) (Figure 4-7) and recorded. Individual colonies on the agar plates were tested for gram positive cocci to ensure *S. sanguinis* growth.

The usually accepted range of CFU per plate is 30 to 300, where any number of colonies above 300 is considered too numerous to count and any less than 30 too few to count. However, in this study any CFU less than 30 were recorded.

Figure 4-7: Automated colony counter

4.5 Data Collection and Analysis

Each sample was coded to permit blind analysis. The data was collected by the same investigator, recorded in Microsoft Excel© spreadsheets (Microsoft Corporation, USA) and processed using various statistical analysis techniques. IBM SPSS Statistics Version 20 for Windows (SPSS©, Inc. Chicago, IL, USA) and Microsoft Excel 2010 (Microsoft Corporation, USA) were used for all the statistical analysis. Descriptive analysis, Levene's test for equality of variances and an independent t test were performed for analysis and a value of $P < 0.05$ considered statistically significant.

4.6 Ethical Approval

Ethical approval from the Biomedical Research Ethics Committee of the University of the Western Cape was attained to conduct the study (Appendix C). This was a laboratorial investigation, with no usage of any human tissue. The study was performed in the Dental Research Laboratory at the Faculty of Dentistry, University of the Western Cape, Tygerberg. There was no conflict of interest found. The manufacturers of the materials used played no part in the research and had no access to the results during the testing period, as well as no influence on the ongoing experiment or upon completion of the study.

Chapter 5: Results

No bacterial growth was observed in the negative control of the GS group (Table 1, Appendix B) or in the negative control of the GN group (Table 2, Appendix B).

For the description of data, mean values and standard deviations were calculated. This is shown in Table 5-1.

Table 5-1: The descriptive analysis for *S. sanguinis* CFU/ml in GS and GN groups

	N	Mean	STD. Variation	Variance	Minimum	Maximum	Range
Overall mean	28	49.00	46.52	2163.84	0.00	171.67	171.67
GS	14	10.21	7.70	59.28	0.00	26.67	26.67
GN	14	87.79	34.57	1194.76	50.67	171.67	121.00

The data for both groups was subjected to statistical analysis using the Levene's test for equality of variances for comparison where a value of 5% ($P \leq 0.05$) was considered significant. The test resulted in a significant value of 0.006. Results of the test are shown in Table 5-2.

Table 5-2: Levene's test for equality of variances

	F	**Sig.**
Equal variances assumed	8.911	0.006
Equal variances not assumed		

The data obtained from both groups was compared by using an independent t-test. The results are shown in Table 5-3.

Table 5-3: Independent t-test

Independent Samples Test	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						Lower	Upper
Equal variances assumed	8.196	26	0.000	77.57	9.46	58.12	97.03
Equal variances not assumed	8.196	14.287	0.000	77.57	9.46	57.31	97.83

	Mean	Std. Deviation	Std. Error Mean
GN	87.79	34.57	9.24
GS	10.21	7.70	2.06

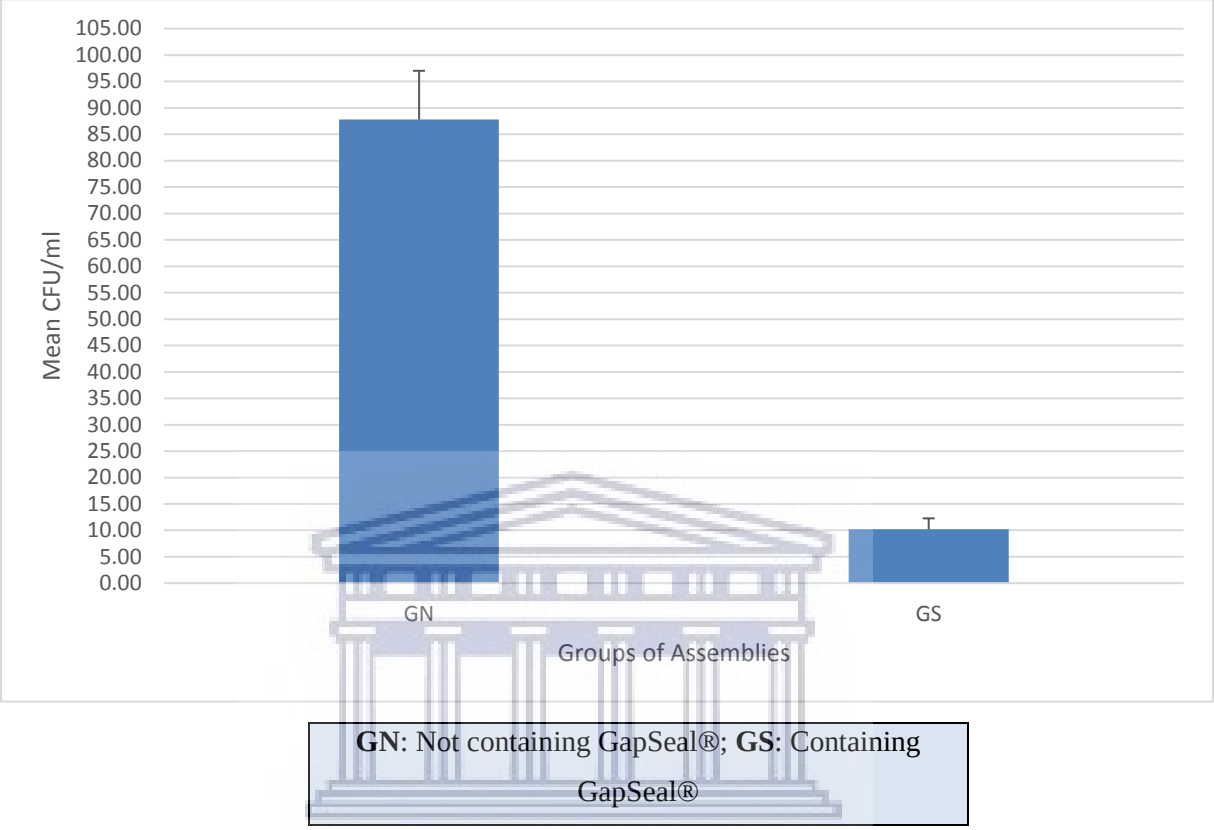

Figure 5-1: Graph illustrating the mean *S. sanguinis* CFU/ml in GS and GN groups.

Chapter 6: Discussion

This *in-vitro* study was conducted to investigate the effect of GapSeal® on microleakage at the IAI of internal a hexagonal connection dental implants after exposure to dynamic loading. According to the results, it was determined that GapSeal® was successful in reducing microleakage significantly. The results showed that the mean CFU detected in the case group **(GS) was 10.21**, whereas in the control group **(GN) the mean CFU was 87.79**. It was also interesting to note that the **maximum** CFU detected in the implants with GapSeal® was 26.67 which was **less** than the **minimum** CFU found in the control group which was 50.67 (Table 5-1). The above finding suggested that the addition of GapSeal® to the interior of the implant prior to abutment connection lead to less microleakage at the IAI than when none is applied.

Internal hexagon connection dental implants were used to test the microleakage in this study. They were connected at a torque of 30 Ncm in accordance with the manufacturer's guidelines. Results noted than in **implants of the control group (GN) colony growth was found**. Microleakage is stated to be dependent on multiple factors including the geometry of the implant abutment connection and the final closing torque (Ranieri *et al.*, 2015; Scarano *et al.*, 2016). Studies showed that internal hexagon designs, although causing less microleakage than external hexagon (Peñarrocha-Diago *et al.*, 2013), did not prevent microleakage and that Morse taper designs only reduced it but did not eliminate it (Aloise *et al.*, 2010; Koutouzis *et al.*, 2011). Rismanchian *et al.* (2012) studied microleakage at the IAI of four different implant abutment connections. Their results presented that different types of abutments had no significant effect on microbial leakage after the first 24 hours. Scrano *et al.* (2005) showed that bacteria were present in the interior walls of all implants removed from patients in their 16 years study. Therefore, microleakage seems to occur at the IAI irrespective of the connection type used (Passos *et al.*, 2013).

Another factor affecting microleakage is the micromovement caused by dynamic loading which leads to a pumping effect and increased flow of fluids and bacteria into the implant. Koutouzis *et al.* (2014) compared microleakage in internal hexagon implants in both static and loaded conditions. The implants were exposed to 500,000

cycles under 50 N in the chewing simulator. Their results showed significantly increased microleakage in the implants exposed to loading compared to the ones that were in static. Increased microleakage under dynamic loading is stated to also be due to deterioration in the implant abutment connection and alterations and deformations to the threaded portion which may aid in loosening of the screw (Al-Jadaa *et al.,* 2015; Siadat *et al.,* 2016). There is also a two-way relationship between microleakage at the IAI and screw loosening, in which microleakage can cause screw loosening which increases the microgap which in turn increases microbial leakage (Sahin *et al.,* 2014).

Various methods of reducing microleakage have been proposed, including the application of a sealing material (Nayak *et al.,* 2014; Mohammadi *et al.,* 2019) decontaminating the internal wall of the implant and using memory shape alloys (Yeo et al., 2014). Nayak *et al.* (2014) studied the effect of GapSeal® in microleakage and concluded that it's application significantly reduced microbial leakage. However, their study was performed in static conditions which could lead to an underestimation of microleakage. Ozdiler *et al.* (2018), studied microleakage in internal conical connections under dynamic loading. 50 N force was applied for 500,000 cycles and the results deduced that the use of sealants such as chlorohexidine gel and silicone material decreased bacterial leakage at the IAI significantly. In the present study, GapSeal® was tested under dynamic loading to mimic the chewing action and only **two** implants from the GS group showed **no** colony forming units (Table 1, Appendix B). This could mean that GapSeal® did not provide a complete seal against microleakage but did significantly reduce it and/or delay it. A study done by Mohammadi *et al.* (2019) showed results parallel to this by comparing the use of Artidox, GapSeal® and chlorhexidine in preventing microleakage. They concluded that GapSeal® did not prevent microleakage but significantly delayed it. However, their study tested the implants under static conditions and only recorded turbidity but did not count colonies which gave more of a qualitative result.

Taking the above findings into consideration, GapSeal® seemed to reduce microleakage significantly when compared to no GapSeal® present, however it was not successful in fully eliminating the leakage. This study tested GapSeal® in a 24hour period only, therefore additional studies for testing it in a longer time frame are needed

since it is a silicone gel that breaks down with time. This will add clinical significance to the mentioned findings.

Chapter 7: Limitations

Although the findings of this study agreed with previous studies (Nayak *et al.*, 2014; Jalalian *et al.*, 2019; Mohammadi *et al.*, 2019), certain constraints were encountered. First, this study performed an *in-vitro* experiment as opposed to an *in-vivo* one. Generally, dental implant *in-vitro* studies pose controlled conditions that do not equate to the exact oral cavity environment and surroundings. Therefore, *in-vivo* studies should be completed to assert the above findings.

Secondly, a larger sample size would have been preferred, since it was stated that using a smaller sample size usually leads to more standard deviation when compared to the mean value of a greater sample size, which yields more constant data (Nawafleh *et al.*, 2013). However, the proposed and tested sample size in this study was within the parameters of cost effectiveness.

Thirdly, the microgap size was not measured in this study, so no correlation between the microgap size and microleakage could be deduced. Using methods such as scanning electron microscopy to quantify the IAI microgap is encouraged in future studies to form a better understanding of GapSeal® sealing parameters. In addition, only a single microorganism species was tested as opposed to a biofilm.

Chapter 8: Conclusion

Considering the limitations found in the present study, the results showed that microbial leakage persistently occurred at the IAI in the internal hexagon implants after being exposed to dynamic loading. However, the application of the sealant material GapSeal® was successful in significantly reducing the microleakage of *S. sanguinis in-vitro* through the IAI, under dynamic loading.

Testing GapSeal®'s sealing longevity, in addition to whether the reduction in microleakage was due to GapSeal® reducing the size of the microgap or due to the antibacterial effect were not in the scope of this study and further research is required to investigate them.

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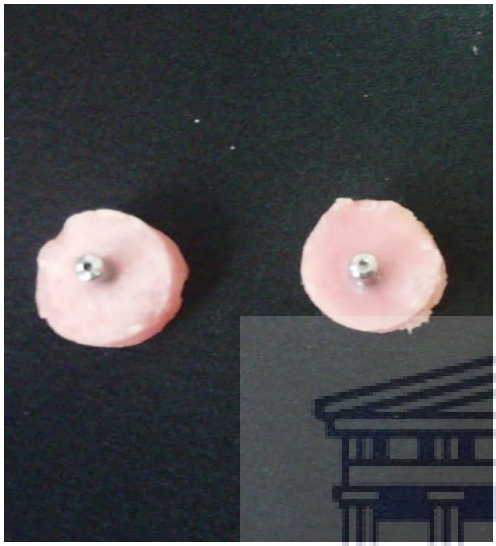
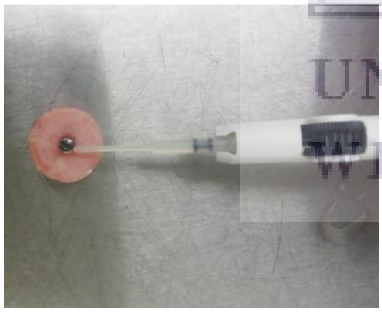
Appendices

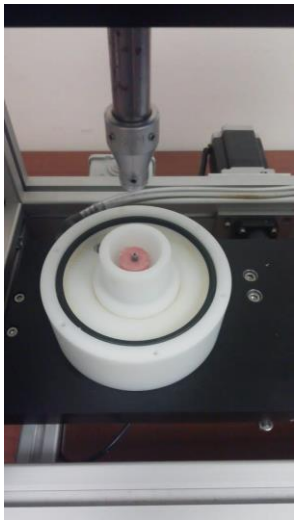
Appendix A
Methodology



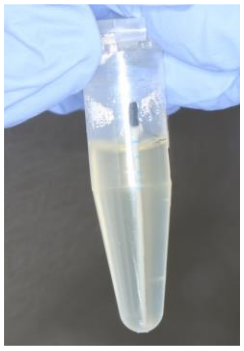
Embedding implants in autopolymerising resin.



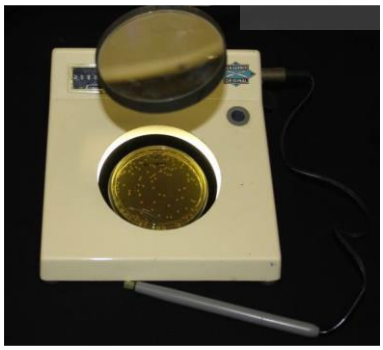
Addition of GapSeal® to interior of implant prior to abutment connection.



Placing of implant assembly in chewing simulator then adding BHI



Paper points used to take microbiological sample from interior of implant after completion of cycle. The paper point placed in sterile BHI.



Counting and recording colonies formed after incubation.

Appendix B

Table 1: Data collection sheet of GS group

Code	Sample	CFU/ml
2534	1	6
	2	3
	3	10
4747	1	8
	2	8
	3	4
6248	1	3
	2	9
	3	5
6128	1	23
	2	20
	3	25
3224	1	7
	2	8
	3	8
5643	1	0
	2	0
	3	0
1478	1	8
	2	15
	3	6
4307	1	0
	2	0
	3	0
7045	1	12
	2	18
	3	15
3312	1	4
	2	4
	3	9
8417	1	13
	2	16
	3	20
2536	1	8
	2	8
	3	15
9058	1	17
	2	28
	3	35
6608	1	9
	2	15
	3	7
1112 Control	1	0
	2	0
	3	0

Table 2: Data collection sheet of GN group

Code	Sample	CFU/ml
3874	1 2 3	65 50 60
3821	1 2 3	43 62 74
1313	1 2 3	57 81 88
2837	1 2 3	47 73 99
7148	1 2 3	60 85 62
1474	1 2 3	72 70 82
9827	1 2 3	44 53 55
5219	1 2 3	165 200 150
2730	1 2 3	140 166 135
5623	1 2 3	87 74 102
1824	1 2 3	99 78 86
2364	1 2 3	67 74 88
6252	1 2 3	122 105 125
5937	1 2 3	75 88 79
1984 Control	1 2 3	0 0 0

Appendix C

Ethical Approval

UNIVERSITY of the WESTERN CAPE

OFFICE OF THE DIRECTOR: RESEARCH
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6 November 2018

Dr H Badi Mohamed
Faculty of Dentistry

Ethics Reference Number: BM18/8/17

Project Title: Efficacy of GapSeal in preventing micro leakage at the dental abutment interface.

Approval Period: 07 November 2018 – 07 November 2019

I hereby certify that the Biomedical Science Research Ethics Committee of the University of the Western Cape approved the scientific methodology and ethics of the above mentioned research project.

Any amendments, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.

Please remember to submit a progress report in good time for annual renewal.

The Committee must be informed of any serious adverse event and/or termination of the study.

Ms Patricia Josias
Research Ethics Committee Officer
University of the Western Cape

BMREC REGISTRATION NUMBER -130416-050

FROM HOPE TO ACTION THROUGH KNOWLEDGE