

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

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ABSTRACT

Introduction

Dental implants have proven to be a success, however the microleakage at the implant-abutment interface is still a major concern. Suggested measures to decrease microleakage include the application of silicone sealing gels, such as GapSeal®.

Aims and objectives

To test the ability of GapSeal, in preventing microleakage at the implant-abutment interface of internal hexagon design dental implants, under dynamic loading.

Design

In-vitro experimental study.

Methods

30 dental implants were equally divided into 2 groups: one group had GapSeal (GS) added and the other group had none (GN). The implants were connected to their adjacent abutments and microbiological analysis was done by immersing the implant assembly in *Streptococcus sanguinis* inoculated BHI suspension. The implants were subjected to dynamic forces of 80 N at 1 Hz for 200,000 cycles in a chewing simulator. They were then dismantled and samples from the implants' interiors were obtained and cultured on agar plates incubated for 24 hours. Finally, colony forming units were recorded.

Results

Significant difference (Levene's test of variances $p=0.006$) in the number of CFU/ml in GS group compared to GN group, with the mean CFU/ml of GS group (10.21) being less than the GN group (87.79).

Conclusions

Application of GapSeal to the implants interiors was effective in reducing microleakage at the implant abutment interface, under dynamic loading. However, it did not completely prevent it.

Keywords

GapSeal, Implant Abutment Interface, Microleakage, Dynamic Loading, Chewing Simulator

Abbreviations

IAI: Implant Abutment Interface

BHI: Brain Heart Infusion

CFU: Colony Forming Units

INTRODUCTION

With success rates higher than 90%, dental implants are one of the best rehabilitation methods in modern dentistry.¹ Nevertheless, microleakage that occurs at the microgap in the implant abutment interface (IAI) is still a concerning factor for implants' success.² Dental implant systems generally consist of two main components: a fixture inserted surgically into the alveolar bone and a transmucosal abutment,³ which inevitably creates a microgap between the implant fixture and abutment.^{4,5} Chewing causes a pumping action that allows the influx of fluids and bacteria into the microgap,⁶ even in clinically healthy conditions.⁷ The interior well of the implant becomes a reservoir for microorganisms and their metabolic products which affect the periodontal tissues directly and are capable of migrating in a bidirectional manner.⁵ Tissues adjacent to the IAI can subsequently present with a marked infiltration of inflammatory substances regardless of the accumulation of plaque present.⁸

Microleakage can interfere with osseointegration in the healing phase of the surgical intervention, and it can result in an inflammatory reaction and a host response in the peri-implant soft tissues which can lead to bone loss and peri-implantitis.^{5,8,9} Microleakage can also cause abutment screw loosening as a consequence of the lubricous environment created by the microbial activities.¹⁰

The type of implant abutment connection used plays a crucial part in microleakage.⁸ Internal hexagon connections showed less microleakage in comparison with external connections,⁸ however microleakage still occurred regardless of the implant abutment connection used.¹¹ Numerous studies showed that microbial leakage cannot be completely prevented even in contemporary implant system designs.^{12,13}

Studies deduced that masticatory loads can cause an increase in microgap size due to micromovements in the prosthetic components,¹⁴ and cause a so-called pumping

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effect,^{6,14} which increases the susceptibility of the implant systems to microleakage.^{15,16} Dynamic loading also causes deterioration of the IAI over time.¹⁵ Therefore, it is proposed that testing microleakage under dynamic loading be a standard step in all future in-vitro studies conducted.¹⁶

There has been an increased interest in testing the use of sealing materials at the IAI to reduce or perhaps even prevent microbial leakage at the IAI. Studies tested the use of different sealing materials including chlorhexidine gel,^{18,19} Atridox¹⁹ and GapSeal.¹⁹⁻²³ GapSeal® (Hager & Werken, Duisburg, Germany) is a sealing gel added on a highly viscous silicone matrix with 5% weight of thymol,²⁴ and showed promising results in reducing microleakage and decreasing microgap size.¹⁹⁻²³ However, not enough studies were conducted testing the effectiveness of GapSeal under dynamic forces, in internal hexagon connections, thus this study was conducted.

MATERIALS AND METHODS

Closing Torque Procedure

A total of 30 dental implants (SEVEN MIS design, internal hexagon, standard platform) (MIS Implants Technologies Ltd, Haifa, Israel) and their corresponding abutments were utilized in this study, after consultation with the statistician and considering the cost effectiveness. Each dental implant was stabilized and held upright with autopolymerizing resin, prepared according to the manufacturer's instructions, 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. The resin also helped in mimicking the intraoral environment in which some forces transported to the IAI would be absorbed by the bone.²⁵ The dental implants and their respective abutments were then put in sterilization pouches, assigned codes and sent for gas sterilization.

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. Subsequently, each implant was carefully connected to its corresponding abutment using a sterile torque wrench, at 30 Ncm according to the manufacturer's guidelines. All dental assemblies were handled by one operator, in sterile conditions in a laminar flow chamber.

Bacterial Culture Preparation

Streptococcus sanguinis, a gram positive, facultative anaerobic bacterium is amongst the primary colonizers in the oral cavity. It attaches directly to oral surfaces and even more to titanium than other bacteria.²⁶ It has a relatively small size, 0.5-1.0µm,²⁷ and is capable of adhering to implant titanium surfaces, irreversibly,²⁸ as well as facilitating the adherence of secondary microbial colonizers.²⁹ *S. sanguinis* was cultured using the direct colony suspension method. The bacterial inoculum was then extracted from the incubated culture and diluted in 2 ml of PBS and adjusted to 0.5 McFarland standard (~1.5x 10⁸ 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.

Dynamic Loading

All implant assemblies from each group were then placed in the custom-made PTFE chambers and 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 was applied for a total of 200,000 cycles at 1 Hz to each implant assembly with a sterile round stainless-steel stylus in the axial direction. 200,000 cycles were completed in around the 24 hour 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.

Measuring Bacterial Colonies

After the completion of the chewing cycle, the assemblies were removed from the 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 inside 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. Afterwards, 100 µl of PBS was added to the wells from 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.

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) 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.

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.

RESULTS

Evidence of bacterial leakage was observed in most GS group samples, with the exception of 2 samples (Table I), and in all connections of group GN (Table II).

No bacterial growth was observed in the negative control of the GS group (Table I) or in the negative control of the GN group (Table II).

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 I: GS group CFU/ml

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

Table II: GN group CFU/ml

For the description of data, mean values and standard deviations were calculated, shown in Table III.

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 IV.

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

A comparison of the mean counts of *S. sanguinis* detected in the internal well of the implants in both groups is shown in Figure 4.

	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

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

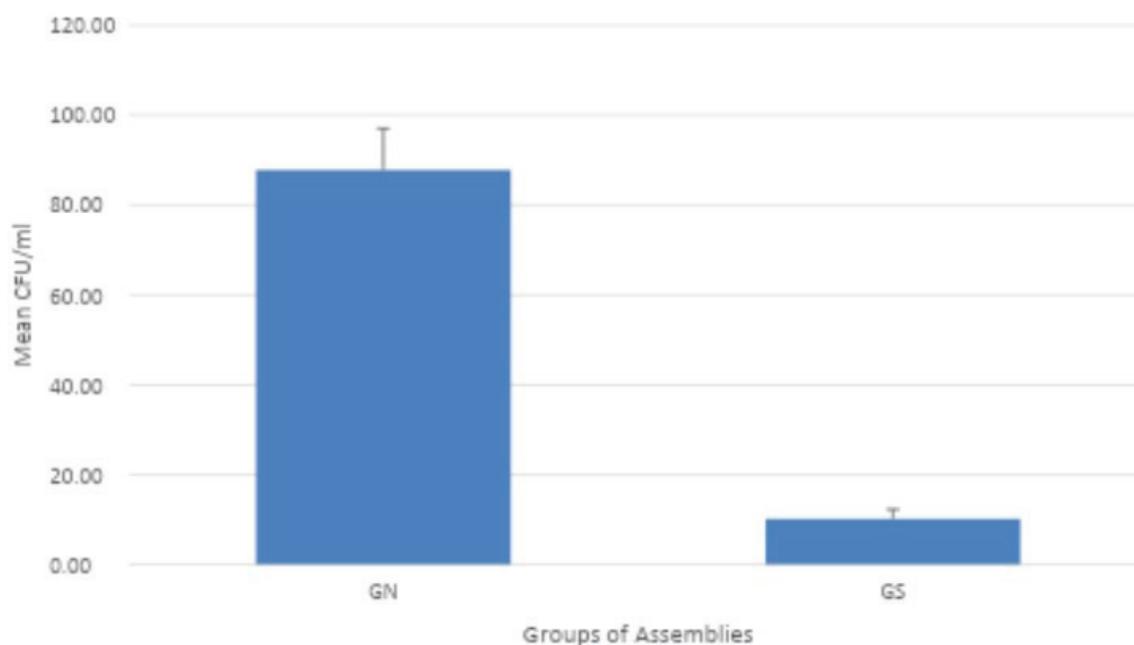
	F	Sig.
Equal variances assumed	8.911	0.006
Equal variances not assumed		

Table IV: Levene's test for equality of variances.

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

Table V: Independent t-test.

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



GN: Not containing GapSeal;
 GS: Containing Gapseal

Figure 4: Graph illustrating the mean *S. sanguinis* CFU/ml in GS and GN groups.

DISCUSSION

The present in-vitro experimental study was conducted to investigate the efficacy of GapSeal® in preventing microleakage at the implant abutment interface of internal 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, which was significantly less ($p=0.006$), than in the control group (GN) with a mean CFU of 87.79.

Microleakage is stated to be dependent on multiple factors, including the geometry of the implant abutment connection and the final closing torque.²⁹ Studies showed that in comparison to the external hexagon design, the internal connection designs produced superior sealing abilities at the IAI, with the Morse taper connection showing the least CFU within the implants. Nevertheless, none of the implant abutment connection designs was able to fully prevent microleakage.^{8,11,13} Internal hexagon connection dental implants were used to test the microleakage in the current study. They were connected at a torque of 30 Ncm in accordance with the manufacturer's guidelines, since studies showed that to acquire the best seal in the IAI, the manufacturer's torque recommendations should be followed strictly.^{8,30}

Studies showed that 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.^{6,14} Increased microleakage under dynamic loading is stated to also be due to deterioration in the IAI and deformations to the threaded portion which may aid in loosening of the screw.¹⁵ Sahin and Ayyildiz (2014) established 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.¹⁰ Mao et al. (2023) upon conducting a systematic review and meta analysis on in-vitro studies testing microleakage, concluded that testing bacterial leakage under dynamic loading should be a set standard in future studies.¹⁶ Steinebrunner et al. (2005) showed an implant-abutment connection mechanically failed at 172,800 cycles (120 N) of dynamic loading.²⁵ The present study provided dynamic loading in a chewing simulator for 200,000 cycles with a magnitude of 80 N, considered within the physiologic ranges and in line with previous studies that employed dynamic conditions with magnitudes ranging from 15 N to 160 N and cycles between 200,000 to 1,200,000.³¹

Different studies investigated microleakage with several methodologies.^{8,16} Studies investigated leakage either from the external environment to the internal part of the implant (inward method),^{21-23,31} or from the inner parts of the implants to the outside environment (outward method).³²⁻³⁴ The freehand inoculation of bacterial broth into the implant in the outward method, in addition to the lack of determination of the implant's internal volume could generate false-positive results.^{12,35} Moreover, 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.³⁵ When considering microleakage testing at the IAI with the inward testing procedure, the total immersion of implant assemblies in the testing liquid could lead to false-positive results owing to the potential penetration of the fluid through the abutment screw interface.³⁵ In addition, assembling and disassembling the implant abutment

complex in a sterile environment, and extracting a sample from inside the implant without cross contamination from the exterior aspect is considered a methodological challenge.¹² This study tested microleakage using the inward method, and assembled and disassembled the implant systems in a laminar flow chamber for sterile conditions. Also, the exact volume of bacterial solution added was measured and was adequate to cover the IAI without submerging the abutment screw interface to avoid false positives. 1 implant from each group (GS and GN) was tested with sterile BHI to check for external contamination issues, and the results showed no bacterial growth in either implant after dynamic loading.

Numerous qualitative and/or quantitative microleakage testing methods were tried, including turbidity analysis, checkerboard DNA-DNA hybridization, radiotracer technique, and microbial counting.⁸ This study used CFU counting since Do Nascimeto et al. (2012) concluded that the accuracy of conventional culture counting methods gave comparable results as that of DNA checkerboard hybridization testing methods.³⁶

Various methods of reducing microleakage were proposed, including the application of sealing materials,^{19,20} decontaminating the internal wall of the implant and using memory shape alloys.³⁷ Gutta percha showed no success in prevention of microleakage,³⁸ and silicone gel sheets were tested in-vivo and resulted in reduced microleakage after 90 days.³⁹ Chlorhexidine gel was studied in-vitro¹⁸ and in-vivo⁴⁰ and contradicting results were found. Ozdiler et al. (2018) performed a study on 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 chlorhexidine gel and silicone material decreased bacterial leakage at the IAI significantly, with no significant difference between the 2 materials.³¹ Yu et al. (2020) tested a silicone sealing gel in 3 different implant systems under dynamic loading of 20 N to 200 N, using the outward method with toluidine blue dye. They concluded that the gel enhanced the immediate fastening and anti-loosening performances of implant connections, and reduced the IAI microleakage and abutment screw thread abrasion.⁴¹

Nayak et al. (2014) compared the effect of GapSeal® and O-Ring in microleakage using the inward method with Enterococcus bacteria, and concluded that GapSeal® application significantly reduced microbial leakage.²⁰ However, their study was performed in static conditions which could lead to an underestimation of microleakage. Mohammadi et al. (2019) compared the use of Atridox, chlorhexidine and GapSeal® in preventing microleakage using the outward method with *A. actinomycetemcomitans*. They concluded that GapSeal® did not have an antimicrobial effect and was not successful in preventing microleakage, rather delaying it.¹⁹ Their study was performed under static conditions and only recorded turbidity but did not count colonies which gave more of a qualitative result. Smojver et al. (2022) compared GapSeal, Flow.sil, and Oxysafe gel in preventing microleakage in conical and straight internal abutment connections. They tested the implants in a suspension of *Candida albicans* and *Staphylococcus aureus* using the inward method and colony counting. They found that only GapSeal® was successful in significantly reducing microleakage, however their study was also conducted under static conditions.²²

Mostofi et al. (2019) studied the effect of GapSeal® on microleakage in internal hexagon connection implants,²¹ and

Zarbakhsh et al. (2018) in external hexagon implants,³¹ with similar methodologies. They both tested microleakage under dynamic loading using the inward method with methylene blue dye, as well as measuring the microgap size using scanning electron microscopy. Both studies concluded that GapSeal® does not provide a complete seal but significantly decreases microleakage and number of gaps.^{21,23} However, studies that utilized bacteria to measure microleakage showed more consistent results with reality compared to methylene blue dyes.²¹ In addition, methylene blue dye leakage was reported qualitatively.^{21,23}

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 I). This means that GapSeal® did not provide a complete seal against microleakage. It did nonetheless significantly reduce microleakage through the IAI, which is in line with previous studies. One of the limitations of this study was not measuring the microgap size, so no correlation between the microgap size and microleakage could be deduced. Also, this study tested GapSeal® in a 24 hour 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. The GapSeal manufacturer recommends the material be replaced every 5 years,²⁴ so it is crucial to conduct in-vivo studies regarding its biological effects and longevity.

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, even with the application of GapSeal. However, the application of 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 decreasing 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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