Effects of Taper Angle and Sealant Agents on Bacterial Leakage Along the Implant-Abutment Interface: An In Vitro Study Under Loaded Conditions

Arda Ozdiler, DDS, PhD[1]/Nursen Bakir-Topcuoglu, DDS, PhD[2]/
Guven Kulekci, DDS, PhD[3]/Gulbahar Isik-Ozkol, DDS, PhD[4]

Purpose: *The aim of this study was to compare the bacterial leakage of conical internal connection implants with different taper angles (5.4, 12, 45, and 60 degrees) and examine the efficiency of a disinfectant agent and a silicone sealant agent in the prevention of bacterial leakage under loaded conditions.* ***Materials and Methods:*** *Twenty-one implant-abutment connections were studied from each implant system (Ankylos Implants, Dentsply; Bego Semados S Implants, Bego; Trias Implants, Servo-Dental; DTI Implants, DTI), for a total of 84 implants. Each system's implants were divided into three groups as follows: unsealed (control), 2% chlorhexidine gel-sealed, or silicone-sealed (n = 7 for each group). The insertion torque was applied to each abutment screw according to the manufacturers' recommendation. The specimens were partially immersed in an 8-mm* E faecalis *suspension. A cyclic load of 50 N was applied for a total of 500,000 cycles at 1 Hz to the specimens. Following disconnection of dental implants and abutments, microbial samples were taken from the inner threaded surface of the implants, plated, and counted under appropriate conditions.* ***Results:*** *There were no statistically significant differences in frequency of bacterial leakage and leaked bacterial counts among the four types of connections in all groups (P > .05). The statistically significant differences were found between sealant agents and control groups in four different connection types in terms of the amount of leaked bacteria (P < .05). There was no significant difference between the amount of leaked bacteria for four connection types when comparing the chlorhexidine and silicone sealant agents (P > .05).* ***Conclusion:*** *Differences in taper angles in the internal conical connections had no significant effect on leaked bacterial counts or the frequency of bacterial contamination under dynamic loading. The application of 2% chlorhexidine gel or a silicone sealant can reduce the leaked bacterial counts and reduce the frequency of bacterial leakage.* Int J Oral Maxillofac Implants 2018;33:1071–1077. doi: 10.11607/jomi.6257

Keywords: *bacterial leakage, dynamic loading, implant-abutment connection, sealant*

Colonization of bacteria along the implant-abutment interface microgap may establish a bacterial reservoir, which can result in soft tissue inflammation, thereby increasing the risk of peri-implantitis and marginal bone loss.[1,2] However, it is still not clear from clinical evidence whether bacterial leakage along the implant-abutment interface is one of the major contributing factors of peri-implantitis.

Microgaps along the implant-abutment interface can enlarge under loading conditions and create a "pump-like effect" around the peri-implant bone zone, leading to the introduction of large amounts of bacteria into the internal aspects of the implant.[3,4] This undesired result poses major challenges for the prevention of bacterial leakage through the implant-abutment interface microgaps.

Implant systems have been developed with different configurations between the transmucosal abutment and the dental implant. The main claim of these implants is a precise fit to minimize microgaps and avoid bacterial leakage along the implant-abutment interface.[5]

The geometry of the implant-abutment interface may affect the amount of bacterial leakage into the internal threads of dental implants.[4–10] Higher amounts

[1]Researcher, Department of Prosthodontics, Istanbul University, Istanbul, Turkey.
[2]Assistant Professor, Microbiology, Istanbul University, Faculty of Dentistry, Istanbul, Turkey.
[3]Associate Professor, Microbiology, Istanbul University, Faculty of Dentistry, Istanbul, Turkey.
[4]Associate Professor, Department of Prosthodontics, Istanbul University, Istanbul, Turkey.

Correspondence to: Dr Arda Ozdiler, Department of Prosthodontics, Istanbul University, Millet Street, Capa Faculty of Medicine Campus, 34097 Fatih/Istanbul, Turkey. Email: ardaozdiler@gmail.com

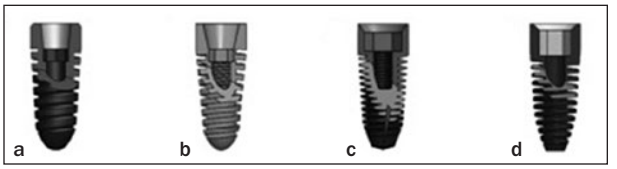

Fig 1 Connection geometries of the implant systems. *(a)* Ankylos Implant System, 5.4 degrees; *(b)* DTI Implant System, 12 degrees; *(c)* Bego Implant System, 45 degrees; *(d)* Trias Implant System, 60 degrees.

of bacterial leakage along the implant-abutment interface have been reported for external connections when compared with internal connections,[8] and reduced amounts of bacterial leakage have been reported for conical internal connections.[4,6,8] According to Harder et al, conical internal connections are not tight enough to prevent the leakage completely.[9] There are limited in vitro studies comparing bacterial leakage in conical internal connection systems with different taper angles.

Numerous attempts have been made to avoid bacterial leakage in internal connections by using disinfectants and sealants under unloaded conditions.[5,11,12] Some investigators examined silicone sealing materials, while others have focused on disinfectants such as chlorhexidine. Kern and Harder described the method of chlorhexidine application for filling implant cavities in order to avoid bacterial leakage.[13] Gel or varnish forms of chlorhexidine have been reported to reduce bacterial colonization in the internal threads of dental implants under unloaded conditions.[14,15] The silicone material used in the study by Duarte et al reduced the bacterial leakage but could not ensure a tight seal; however, it must be noted that the material used in their study was not designed to seal the implant-abutment interface.[16]

Thus, the present study aimed to compare the amount of bacterial leakage in four internal conical implant systems with different taper angles (5.4, 12, 45, and 60 degrees) using two different approaches to reduce bacterial leakage under loaded conditions.

MATERIALS AND METHODS

Implant Systems

Four internal conical implant systems with different taper angles at the implant-abutment connection were examined in the present study (Fig 1). Commercially packaged implants (Ankylos Implants, Dentsply; Bego Semados S Implants, Bego; Trias Implants, Servo-Dental; DTI Implants, DTI) and abutments (Ankylos Regular C/X, 2 mm; Bego Sub-TecPlus, 2 mm; Trias solo, 2 mm; DTI Standart Straight, 2 mm) for cement-retained prosthetic restorations were used from each system (Table 1). A total of 84 implant-abutment connections (21 for each implant system) were studied. To examine the effects of the sealing agents and the connection geometry on bacterial leakage, the implants in each system were divided into three groups (n = 7) as follows: unsealed (control); 2% chlorhexidine gel-sealed (Gluco-CHeX, Cerkamed PTT); and silicone-sealed (Kiero Seal, Kuss Dental).

Disinfecting and Sealing Agents

Gluco-CHeX Gel (disinfectant) and the silicone sealant Kiero Seal were used in this study. Gluco-CHeX Gel contains 2% chlorhexidine digluconate, which acts as the antimicrobial agent, and is used as a disinfectant in the oral cavity. It interacts with the lipophilic cell membranes of bacteria, causing osmotic imbalance and leading to cell death.[17]

Kiero Seal is a polyvinyl siloxane-based material that was specifically developed for sealing the implant-abutment interface. It has a low viscosity with a setting time of up to 3 minutes.

Preparation of the Samples

Twenty-one implants from each system were embedded in an auto-polymerizing resin, which had an elastic modulus equal to that of human bone (EpoFix, Struers). The 21 abutments from each system were restored with geometrically simplified molar crowns with a 30-degree cusp angle and an occlusal screw access hole to disconnect the dental implants and abutments for microbiologic detection. The crowns were fabricated by ProX 300 3D Laser Printer (3D Systems) with a CrCo alloy (ST2724G, SinT-Tech) and luted to the abutments using a dual-cure resin cement (RelyX U200, 3M ESPE).

Experimental Design

Prior to the experiment, all specimens were autoclaved for 15 minutes at 121°C; the sterility of the components was verified in pretests. The inner parts of each implant were filled with one of the two tested agents or left unfilled in the control group. Each abutment-crown was attached to the implant with an appropriate insertion torque according to the manufacturers' recommendations (15 N/cm for Ankylos Implants; 30 N/cm for Bego Semados, Trias, and DTI Implants) to ensure proper preload for all implant systems.

All 84 samples were attached to sterilized, custom-made test chambers. The specimens were partially immersed in an 8-mm bacterial suspension that was aligned with the crown (halfway up) to avoid bacterial penetration through the occlusal screw access hole (Fig 2). Then, the specimens were mounted in a dual-axis chewing simulator (Cs-4.2, Mechatronic) that

Table 1 Implant Systems Used in the Study

Implant	Manufacturer	Taper (degrees)	Abutment	Height (mm)	Diameter (mm)
Ankylos	Dentsply	5.4	Regular C/X 2 mm	9.5	3.5
Bego Semados	Bego	45	Sub-Tech plus 2 mm	10	3.75
Trias	Servo-Dental	60	Trias Solo 2 mm	10	3.8
DTI	DTI Implant Systems	12	Standart-Straight 2 mm	10	4.0

housed four specimens of each implant system at a time (Fig 3).

A cyclic load was applied to each crown with a round, stainless steel stylus, 2 mm away from the crown's occlusal center on the 30-degree tapered occlusal area (Fig 4). A force of 50 N was applied to the specimens for a total of 500,000 cycles at 1 Hz, which is within the physiologic range.[4,18–20]

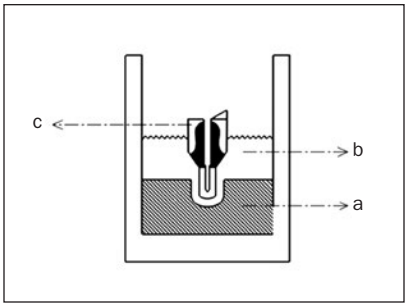

Fig 2 Experimental design. *(a)* Epoxy resin; *(b) E faecalis* suspension; *(c)* geometrically simplified crown.

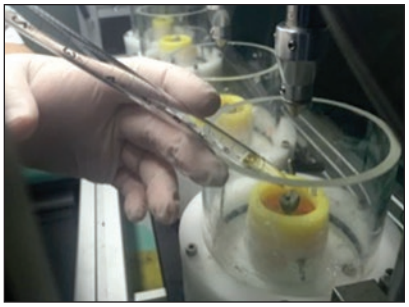

Fig 3 Mounted specimens in a chewing simulator.

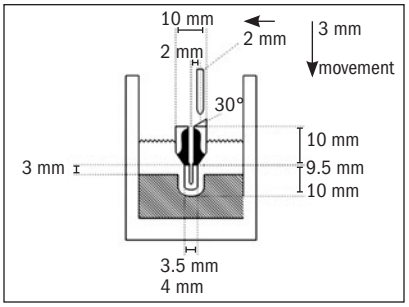

Fig 4 Experimental design under dynamic loading.

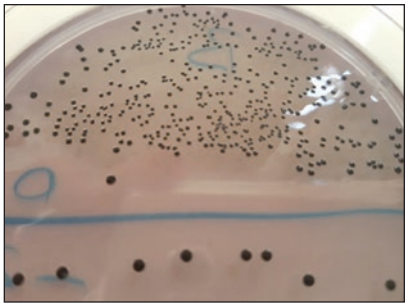

Fig 5 For each sample, colony forming units per mL (cfu/mL) were counted.

Microbiologic Sampling and Examination

Each specimen was immersed in a bacterial suspension (8 mL) prepared with cultures of *Enterococcus faecalis* that had been prepared for 24 hours (ATCC 29212) resuspended in fresh brain heart infusion (BHI, Difco Laboratories) broth (approximately 10^6 colony-forming units per mL [CFU/mL]). Dynamic loading was performed at room temperature for 4 days with the bacterial suspension, which was refilled every 48 hours with fresh BHI.

Following the disconnection of implant-abutments under sterile conditions, samples were taken from the inner threaded parts of the implant using two sterile paper points (Dentsply-Maillefer). To minimize the possibility of contamination during the sampling process, one investigator performed disconnection of the dental implants and abutments while another obtained samples from the inner part of the implants. Both investigators (A.O., N.B.T.) utilized aseptic techniques.

The samples were put into 1 mL of sterile saline solution and vortexed for 30 seconds; tenfold dilutions were prepared. Aliquots of 0.1-mL suspensions were inoculated onto Mitis Salivarius Agar (Difco Laboratories) plates and incubated at 37°C for 24 hours. For each sample, CFU/mL was counted (Fig 5).

Statistical Analyses

The G*Power Statistics 3.1.9.2 (Heinrich-Heine-Universitat) program was used to calculate the number of samples needed to detect a significant difference in the study. The IBM SPSS Statistics 22 program (IBM SPSS) was used for statistical analysis to evaluate the findings of the study. Compliance to normal distribution of parameters was evaluated using the Shapiro-Wilk test; it was determined that the parameters did not show normal distribution. Hence, the Kruskal-Wallis test was performed to compare the parameters between the groups for mean counts of leaked bacteria, and the Mann-Whitney *U* test was performed in the evaluation of post hoc analysis. The chi-square test was used to compare frequencies of bacterial leakage. A P value < .05 was considered statistically significant.

Table 2 The Frequencies of Bacterial Leakage

Sealant/Connection (taper [degrees])	Contamination: Yes n (%)	Contamination: No n (%)
Control		
Ankylos (5.4)	2 (28.6)	5 (71.4)
Bego (45)	6 (85.7)	1 (14.3)
Trias (60)	6 (85.7)	1 (14.3)
DTI (12)	5 (71.4)	2 (28.6)
P	.071	
Chlorhexidine		
Ankylos (5.4)	0 (0)	7 (100)
Bego (45)	4 (57.1)	3 (42.9)
Trias (60)	2 (28.6)	5 (71.4)
DTI (12)	1 (14.3)	6 (85.7)
P	.083	
Kiero Seal		
Ankylos (5.4)	0 (0)	7 (100)
Bego (45)	2 (28.6)	5 (71.4)
Trias (60)	4 (57.1)	3 (42.9)
DTI (12)	1 (14.3)	6 (85.7)
P	.083	

Table 3 Mean Counts of *E faecalis* Detected in the Internal Parts of the Implants

Sealant/Connection (taper [degrees])	Bacterial count Mean ± SD (median)
Control	
Ankylos (5.4)	45,314.29 ± 112,483.59 (0)
Bego (45)	282,014.29 ± 388,679.73 (60,000)
Trias (60)	462,868.57 ± 737,774.67 (100,000)
DTI (12)	87,131.43 ± 184,301.93 (7,900)
P	.196
Chlorhexidine	
Ankylos (5.4)	0 ± 0 (0)
Bego (45)	17.14 ± 41.12 (0)
Trias (60)	1, 437.14 ± 3,775.93 (0)
DTI (12)	1.43 ± 3.78 (0)
P	.084
Kiero Seal	
Ankylos (5.4)	0 ± 0 (0)
Bego (45)	4,578.57 ± 11,234.31 (10)
Trias (60)	6,147.14 ± 8,798.57 (30)
DTI (12)	142.86 ± 377.96 (0)
P	.066

Table 4 The Effects of Sealant Agents on the Number of Leaked Bacteria

Connection (taper [degrees])/Sealant	Bacterial count Mean ± SD (median)
Ankylos (5.4)	
Control	45,314.29 ± 112,483.59 (0)
Chlorhexidine	0 ± 0 (0)
Kiero Seal	0 ± 0 (0)
P	.037*
Bego (45)	
Control	282,014.29 ± 388,679.73 (60,000)
Chlorhexidine	17.14 ± 41.12 (0)
Kiero Seal	4,578.57 ± 11,234.31 (10)
P	.014*
Trias (60)	
Control	462,868.57 ± 737,774.67 (100,000)
Chlorhexidine	1,437.14 ± 3,775.93 (0)
Kiero Seal	6,147.14 ± 8,798.57 (30)
P	.046*
DTI (12)	
Control	87,131.43 ± 184,301.93 (7,900)
Chlorhexidine	1.43 ± 3.78 (0)
Kiero Seal	142.86 ± 377.96 (0)
P	.017*

*Statistically significant difference ($P < .05$).

RESULTS

The frequencies of bacterial leakage in three groups of each implant system (Ankylos, Bego, Trias, DTI) are shown in Table 2. Evidence of bacterial leakage was observed in all four types of connections in the control group. The incidence of leakage was 28.6% in Ankylos, 85.7% in Bego, 85.7% in Trias, and 71.4% in DTI implants. There were no statistically significant differences between the connection types in terms of incidence of leakage in the control group ($P = .071$). No statistically significant differences in the incidence of leakage ($P = .083$) were noted between the connection types following chlorhexidine gel application. Whereas no leakage was observed in the Ankylos implants, frequency of leakage was noted to be 57.1% in Bego, 28.6% in Trias, and 14.3% in DTI implants. Similarly, no statistically significant differences in incidence of leakage ($P = .083$) were observed between the connection types following silicone sealant use. Just as with the chlorhexidine gel, no leakage was observed in the Ankylos implants, whereas 57.1% of the Bego implants, 28.6% of Trias implants, and 14.3% of DTI implants presented with leakage.

The mean counts of *E faecalis* detected in the internal parts of the implants in each group are shown in Table 3. There were no statistically significant differences between the four types of connections in all groups (control, chlorhexidine-sealed, and silicone-sealed; $P > .05$).

Table 4 illustrates the effects of sealants on the amount of leaked bacteria in the different types of connection. In Ankylos, Bego, Trias, and DTI implants, statistically significant differences were observed in terms of leaked bacteria counts between the sealants and the control group ($P = .037$, .014, .046, and .017, respectively). No significant differences in leaked bacterial counts were noted between chlorhexidine and Kiero Seal in all groups ($P > .05$).

DISCUSSION

In this study, four internal conical implant systems with different taper angles at the implant-abutment connection were examined for potential bacterial leakage along the implant-abutment interface under dynamic loading. Additionally, the efficiency of 2% chlorhexidine gel and Kiero Seal in reducing bacterial leakage along the implant-abutment interface was evaluated. Since the main objective of this study was to examine the effects of taper angle differences on bacterial leakage, four implant systems with obviously distinct taper angles were chosen (5.4, 12, 45, and 60 degrees). Implants from each system were preferred to be closest to each other in terms of length and diameter.

The results of the present study showed that differences in taper angles in the conical connections had no significant effect on bacterial leakage under dynamic loading. A complete hermetic seal did not exist along the implant-abutment interface in the control group because bacterial contamination was detected in all implant systems. Application of 2% chlorhexidine and Kiero Seal could reduce bacterial leakage and reduce the amount of bacteria that invade the inner aspects of the implants in all tested implant systems. No significant difference in bacterial counts was found between the 2% chlorhexidine and Kiero Seal groups.

Bidirectional bacterial leakage along the implant-abutment interface under dynamic loading has been evaluated previously in several in vitro studies.[4,7,8,10,21] These studies had different methodologies and microorganisms. *E faecalis*, which was used for a recent similar study by Tripodi et al,[10] was chosen in the present study to evaluate bacterial leakage along the implant-abutment interface. *E faecalis* is a facultative anaerobic microbe with a size ranging from 1.0 to 1.5 µm, which is small enough for it to pass through the 2.3- to 100-µm microgaps of the implant-abutment interface.[22–24] It is a potential pathogen of the gastrointestinal tracts and oral cavity. Furthermore, it can be detected in individuals with peri-implantitis and can colonize in a dental implant after placement in the healed site or gingival sulcus.[25–27] In the present study, the specimens were partially immersed in an 8-mm *E faecalis* suspension that was aligned with the crown (halfway up) to avoid bacterial penetration through the occlusal screw access hole. However, leakage may also occur between the crown and abutment because the crowns were cemented to the abutments. The possibility of leakage between the crown and abutment can be marked as a limitation of this study.

According to Binon, the frequency of fluid and bacterial leakage along the implant-abutment interface is a multifactorial condition that is dependent on the precision of the implant-abutment union, degree of micromovement between the components, and the final torque value used to connect them.[28] Therefore, differences in the geometry of the implant-abutment connections and dynamic loading, which can increase the micromovement by creating a pump effect, have a direct and important effect on bacterial leakage.[7,21] Several in vitro studies have been performed to compare bacterial leakage in different connection geometries under dynamic loading. Do Nascimento et al compared the bacterial leakage of conical connections with internal and external connections under dynamic loading with 500,000 cycles at 120 N.[7] Conical connection implants showed the lowest bacterial counts, with significantly lower counts than internal and external connections. Aloise et al and Jansen et al reported similar results, confirming that contamination in conical connections was lower than that in other types of connections.[29,30] In a study by Steinebrunner et al, bacterial penetration occurred significantly later in the tube-in-tube connections of Camlog implants when compared with conical connections.[21] According to Steinebrunner et al, the Camlog implant system has a "positive locking tube-in-tube joint" connection that might minimize the micromovement and pumping effects, which may explain the low leakage observed in their study.[21]

In light of recent dynamic loading studies, the effect of taper angle differences in conical connections on bacterial leakage was evaluated in the present study. Researchers have examined bacterial leakage in different dynamic conditions that differ in magnitude (15 N to 160 N), direction (30 to 90 degrees), and cycles (200,000 to 1,200,000).[4,5,7,8,10,21] All the aforementioned magnitude, direction, and cycle values are within physiologic ranges.[4,19,20,31] In a dynamic loading study by Steinebrunner et al, one implant-abutment connection from the Frialit-2 group mechanically failed at 172,800 cycles (120 N).[21] Furthermore, in another mechanical resistance study by Ugurel et al, it was reported that three of four implant systems (Biohorizons, Xive, and Octo) had a maximum median failure at 539,719 cycles (120 N).[32] Based on these findings, in the present study, specimens were loaded at 500,000 cycles and 50 N to avoid any mechanical failures that might lead to false positive contamination results. The same dynamic loading conditions were also used in the study by Koutouzis et al.[4] However, alterations may be seen in the results under higher-level cycles and forces. Therefore, further in vitro dynamic loading studies under higher-level cycles and forces are needed to validate the results of the present study.

In terms of the scientific consistency of a study, it is very important to calculate the adequate number of samples for significant difference. In the recent in

vitro studies, different numbers of samples (n = 6 to 10) were used to evaluate bacterial leakage along the implant-abutment interface.[4,7,9,21] In the present study, sample size was determined by a power analysis, which was performed prior to the study period according to the similar work of Steinebrunner et al.[21] Based on the comparison of significantly different Camlog and Frialit-2 Systems groups in the Steinebrunner et al study, the number of samples that is adequate to detect a significant difference was calculated as n = 7 with an impact size of 1.8, one-way test, 80% power, and 5% Type I margin of error. However, according to the results of the present study, it was determined that the parameters did not show normal distribution, and variances were nonhomogeneous among the groups. For this reason, nonparametric tests with a lower level of evidence were preferred in the statistical examination, and post hoc analyses were performed to the results with significant differences. This is one of the most important limitations of the present study. It is quite possible that the main reason for this limitation may be the lack of sample size. Therefore, further in vitro or clinical studies with different sample sizes are needed to validate the findings of the present study.

According to the results of this study, there were no significant differences in leaked bacterial counts and frequencies of bacterial leakage along the implant-abutment interface between the four types of connections. However, when the degree of taper decreased, the leaked bacterial counts also decreased. Decreased taper angles in the conical implant-abutment connections form larger connective surfaces that lead to expansions on the interlocking surfaces. Expansions on interlocking surfaces can minimize micromovements and pump-like effects, which may be attributed to the reduced bacterial counts in the connections with lower taper degrees and larger connective surfaces.

The present study compared the effects of two different materials (2% chlorhexidine and Kiero Seal) in reducing bacterial leakage along the implant-abutment interface under dynamic conditions. Both materials succeeded in reducing leaked bacterial counts; significant differences between the control and sealant groups were noted ($P < .05$). The chlorhexidine group presented with lower bacterial counts when compared with the Kiero Seal group, statistical significance notwithstanding ($P > .05$). This finding is in accordance with those of previous studies, which used rinse[33] or gel[16,34,35] forms of chlorhexidine under unloaded conditions and succeeded in significantly decreasing the bacterial counts, especially in the chlorhexidine groups. In the present study, no bacterial contamination was noted following the use of 2% chlorhexidine in the Ankylos implant system. This may be attributed to the low viscosity as well as the antibacterial activity of chlorhexidine. Based on previous research,[36–38] the antibacterial activity of 2% chlorhexidine lasts from 3 days to 12 weeks, after which the gel or rinse forms of chlorhexidine dissolve into the fluids that penetrate along the implant-abutment interface. This inevitable outcome can cause further contamination in the implant-abutment interface. Therefore, use of chlorhexidine must be interpreted with great care.

The results of the present study appear to support those of numerous studies that used silicone materials to reduce bacterial leakage under unloaded conditions.[14,39,40] Polyvinyl siloxane-based silicone sealant can reduce bacterial counts. In the present study, no contamination was found in the Ankylos implant system following the use of Kiero Seal. On the other hand, silicone materials could not guarantee a tight seal along the implant-abutment interface because of their thermal contraction and expansion behaviors.[12] Moreover, silicone materials have some disadvantages concerning the application process: removal of excess material around the implant neck is a challenge; reapplication of silicone materials can be very difficult; and the removal of set material from the threaded parts of the implant and screw can be very challenging in the case of a necessary re-closure of the implant-abutment connection.

CONCLUSIONS

Within the limitations of this in vitro study, the following conclusions can be revealed. Differences in taper angles in the internal conical connections had no significant effect on leaked bacterial counts or the frequency of bacterial contamination under dynamic loading. Application of chlorhexidine and silicone sealants can reduce frequency of bacterial leakage and reduce the number of bacteria invading the inner threads of dental implants. However, there were no significant differences in frequency of leakage or amount of leaked bacteria between either material.

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