In Vitro Evaluation of Bacterial Leakage Along the Implant-Abutment Interface of Different Implant Systems

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Purpose: *Microbial leakage and colonization between implants and their abutments may cause inflammatory reactions in the peri-implant tissues. This study evaluated microbial leakage at the implant-abutment interface with a new in vitro model.* ***Materials and Methods:*** *Bacterial leakage was tested during dynamic loading in a 2-axis chewing simulator. The authors theorized that dynamic loading would decrease the stability of the implant-abutment connections and thereby lead to bacterial penetration along the gap. Five different implant systems with 8 standard implant-abutment combinations for single molar crowns were tested. The internal aspects of the implants were inoculated with a bacterial suspension and connected to the superstructure with the recommended torque. The specimens were immersed in a nutrient solution and loaded with 1,200,000 cycles of 120 N in the chewing simulator.* ***Results:*** *Statistically significant differences (P ≤ .05) between implant systems with respect to number of chewing cycles until bacterial penetration were found.* ***Discussion:*** *The degree of penetration in a specific implant system presumably is a multifactorial condition dependent on the precision of fit between the implant and the abutment, the degree of micromovement between the components, and the torque forces used to connect them.* ***Conclusion:*** *It was concluded that the newly developed test model is a sensitive tool for the detection of differences between current implant systems with respect to their ability to prevent bacterial penetration at the implant-abutment interface under dynamic loading conditions.* Int J Oral Maxillofac Implants 2005;20:875–881

Key words: *abutments, bacterial leakage, chewing simulation, dental implants*

Most dental implant systems consist of 2 components: the endosteal part (the implant), which is placed in a first surgical phase, and the transmucosal connection (the abutment), which is typically attached after successful implant osseointegration to support the prosthetic restoration. When the prosthetic abutment is placed on the subgingival implant, contact with the peri-implant soft tissue and bacterial dissemination into the implant body is nearly unavoidable. Penetration of oral microorganisms through gaps between these components may add to the risk of soft tissue inflammation or be responsible for the failure of peri-implantitis treatment.[1] The connective gap is located near the level of the alveolar bone crest for most implant systems; thus, microbial colonization of the gap may result in bone resorption. Location of the gap near the alveolar crest could also be responsible for the 1 mm of bone loss observed during the first year of functional loading of implants.[2]

Several in vitro studies have shown bidirectional fluid and bacterial leakage into and out of implant-abutment assemblies of common implant systems. Microbial penetration along the implant-abutment interface of the Brånemark System implant (Nobel Biocare, Göteborg, Sweden) has been reported; inward as well as outward leakage was demon-

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Table 1 Characteristics of the Implants Tested and Their Abutments

Product	Manufacturer	Implant	Abutment	Diameter (mm)	Design of the implant-abutment connection	Recommended screwing torque (N/cm)
Brånemark System	Nobel Biocare, Göteborg, Sweden	Mk II Wide Platform	CeraOne Wide Platform	5.0	External hex/cylindric	45
Frialit-2	Dentsply Friadent, Mannheim, Germany	Synchro	MH 6/A0, Hermetics	4.5	Guide rod with integrated hex and silicon washer/cylindric	24
Camlog	Altatec, Wurmberg, Germany	Promote	Standard	5.0	"Tube in tube" with cam-slot fixation/cylindric	20
Replace Select	Nobel Biocare, Göteborg, Sweden	Standard	Easy Abutment	5.0	"Tube in tube" with cam-slot fixation/cylindric	35
Screw-Vent	Zimmer Dental, Carlsbad, CA	Standard	Hexlock abutment 4/5	4.5	Internal hex with friction fit/tapered	30

strated for a solution of paranitrophenol and for microorganisms.[3] Similar results were obtained in another study showing bacterial colonization on internal aspects of Brånemark System implants.[4] Clinical studies have also demonstrated the presence of viable bacteria on the inside of implant assemblies. One study aimed to investigate the presence of microorganisms in the inner thread of Brånemark System implants that had been in place for 3 months.[5] The authors reported that all samples contained significant quantities of microorganisms, mainly coccoid cells and nonmotile rods. In another clinical study, a spectrum of microorganisms was found on the internal aspects of the intramobile element of IMZ implants (Dentsply Friadent, Mannheim, Germany) that corresponded more or less to the anaerobic flora of progressive periodontitis.[6]

Further investigations evaluated how the colonization of bacteria inside an implant system and the penetration of bacteria or their products via the microgap between the implant and the abutment influences the peri-implant tissues.[1,7] A study in the Labrador dog reported that 4 to 12 months after abutment connection, the apparently healthy peri-implant mucosa consistently harbored an infiltrate at the level of the implant-abutment connection.[7] This infiltrate was clearly separated from a marginal soft tissue lesion that had developed as a result of supragingival plaque formation. It was suggested that the infiltrate formed in response to bacterial contamination of the internal aspects of the implant system. Therefore, some manufacturers developed tighter connections with reduced microgaps.

An in vitro study evaluated whether newly developed implant-abutment interfaces can prevent microbial penetration better than commonly used older implant systems.[8] A promising solution for a fluid-tight connection was presented by a modification of the Frialit-2 interface (Dentsply Friadent) with a special silicone washer that clearly reduced bacterial penetration as compared to the standard Frialit-2 interface.[8] However, specimens were not subjected to functional cyclic loading.

The purpose of this study was to evaluate and compare bacterial leakage along the implant-abutment interfaces of common and newly developed implant systems with a new in vitro model. The null hypothesis of the study was that there is no difference between implant-abutment connection designs with respect to bacterial penetration during dynamic loading in a chewing simulator.

MATERIALS AND METHODS

Five implant systems with different components at the implant-abutment connection were evaluated: Brånemark (Nobel Biocare), Frialit-2/Hermetics (Dentsply Friadent), Replace Select (Nobel Biocare), Camlog (Altatec, Wurmberg, Germany), and Screw-Vent (Zimmer Dental, Carlsbad, CA). Commercially packaged implants and abutments were used. Eight identical implant-abutment combinations were studied for each implant system, for a total of 40. Abutments for cement-retained prosthetic restorations engaging the appropriate antirotational interface geometry were selected from each system (Table 1).

Preparation of the Implants

Eight implants of each system were embedded in an autopolymerizing resin (Luxatemp; DGM, Hamburg, Germany) with custom-made stainless steel ring forms (Fig 1). The implants were mounted in the resin to mimic intraoral conditions, where the bone may absorb some forces transmitted to the implant-abutment screw connection. Eight standard abutments of each system were restored with identical single molar crowns with an occlusal screw access opening to control possible abutment-screw loosening. The crowns were cast with a base-metal alloy (Wironit;

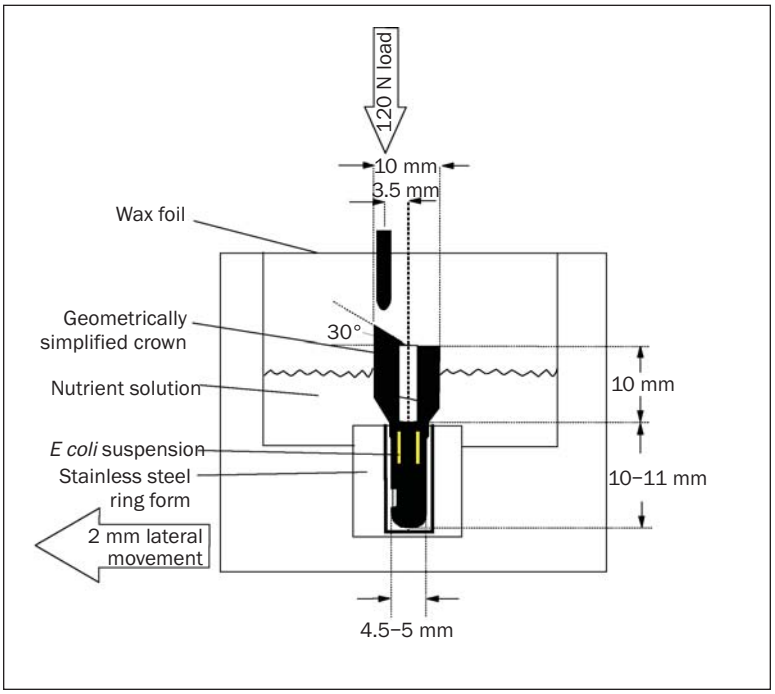

Fig 1 Test configuration in the test chamber.

Bego, Bremen, Germany) and luted to the abutments with a composite resin (Panavia 21 Ex; Kuraray, Osaka, Japan).

Microbiologic Examination

Test specimens were autoclaved for 15 minutes at 121°C. The internal aspect of each implant was inoculated with 5 µL of an *Escherichia coli* suspension (reference strain ATCC 11229; DSMZ, Braunschweig, Germany) with a micropipette (Eppendorf Reference; Eppendorf-Netheler-Hinz, Hamburg, Germany) under sterile conditions. *E coli* is a gram-negative, motile bacteria measuring 1.1 to 1.5 µm in diameter and 2 to 6 µm in length. To produce the solution, bacteria were selectively cultivated aerobically for 24 hours at 37°C in a tryptic soy broth (Merck, Darmstadt, Germany), resulting in a concentration of approximately 1.5×10^9 CFU/mL. After the implant was inoculated, the abutment-crown combination was assembled with sterile gloves to the implant with an abutment screw according to the manufacturers' protocols. An electronic implant torque controller (Intrasurg; Kavo, Biberach, Germany) was used to ensure proper seating torque for all implants.

Subsequently, the assembled specimens were tested for inadvertent *E coli* contamination of the outer surface using an *E coli*–indicating growth medium (ENDO-agar; Merck). Each specimen was rolled along a prepared agar plate. In addition, each specimen was extrusion-coated with warmed, liquid agar at the height of the implant-abutment interface. After the agar coating had solidified, each specimen was placed on the corresponding plate. The plates were incubated at 37°C for 24 hours. Growth of *coli* on the agar turned the color of the medium from light red to fuchsin red within 6 to 12 hours. This color change was also an indicator of possible contamination with other bacteria. The test also enabled the detection of assemblies that were contaminated with *E coli* on the external surface. Assemblies found to be contaminated were excluded from further evaluation.

Experimental Model

All 8 specimens of each implant system were fixed in custom-made autoclaved test chambers of polyoxymethylen immediately after the inoculation procedure. The specimens were partially immersed in a tryptic soy broth solution that came halfway up the crown (Fig 1). This ensured bacterial penetration along the implant-abutment interface but not along the occlusal screw access hole. The chambers were then mounted in a dual-axis chewing simulator (Willytec, Munich, Germany) that housed 8 specimens at a time, and each chamber was covered with wax foil. A cyclic fatigue load was applied to each crown with a round stainless steel stylus through a hole in the wax foil, 3.5 mm away from the crown's occlusal center on the tapered occlusal area. The chewing simulation contained an additional horizontal sliding motion of 2 mm (Fig 1). A force of 120 N, which is within the physiologic clinical range,[9] was applied for a total of 1,200,000 cycles at 1 Hz. The sur-

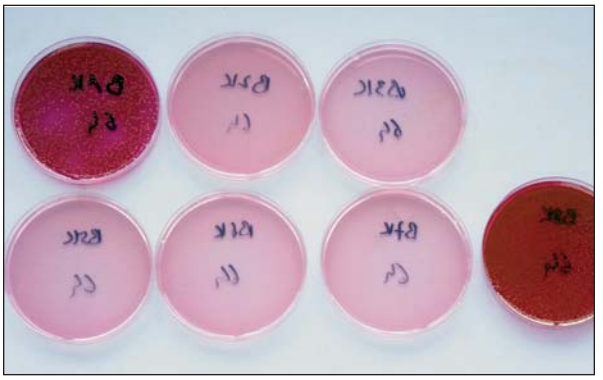

Fig 2 *E coli* colonization indicated on ENDO-agar plates (Brånemark 21,600 cycles).

rounding solution was probed for *E coli* during the cyclic loading at increasing intervals.

The testing was performed by drawing 0.5 mL of the nutrient solution from the corresponding test chamber with sterile 1-time-use syringes. The solution was dripped on additional prepared ENDO-agar plates and incubated aerobically for 24 hours at 37°C. Whenever *E coli* colonization of the samples was indicated on the agar plate (Fig 2), the number of completed chewing cycles was recorded, and the corresponding specimen was excluded from further investigation of bacterial leakage.

Since the data were not normally distributed (Kolmogorov-Smirnov test), statistical analyses were performed with the Kruskal-Wallis test, followed by the Mann-Whitney test modified by Bonferroni-Holm for multiple testing. $P \leq .05$ was considered statistically significant.

RESULTS

The numbers of chewing cycles until *E coli* contamination was detected are shown in Table 2. All specimens showed bacterial leakage. The median chewing cycles until *E coli* was detected in the respective surrounding solution were 172,800 for the Brånemark System, 43,200 for the Frialit-2/Hermetics System, 64,800 for the Replace-Select System, 345,600 for the Camlog System, and 24,300 for the Screw-Vent System (Fig 3). Statistically significant differences were found between the Camlog and the Frialit-2 Systems, as well as between the Camlog and the Screw-Vent Systems. The Camlog System showed bacterial leakage at significantly higher numbers of chewing cycles than the Frialit-2 ($P = .004$) or the Screw-Vent System ($P = .005$).

DISCUSSION

This test model showed bacterial leakage along the interfaces of the tested implants and their abutments under functional loading in an artificial chewing simulator. Inward penetration was not tested because it would have required disconnection of the implant-abutment assemblies. Given the present experimental setup, the implant-abutment assemblies could be disconnected only once and after a specific period of time to test bacterial colonization at the internal implant aspect. This procedure would not have allowed evaluation of bacterial penetration and possible changes over time. Multiple assemblies of the same parts as well as nonsterile conditions could have caused false-positive results because of bacterial migration from external portions of the components to the internal aspects. On the other hand, disinfection of the implant-abutment assemblies before disconnection for testing may have caused false-negative results in the event that disinfecting agents reach the internal aspects. Therefore, a reverse testing technique was applied in this study; it was supposed that bacteria leaking outward from the internal aspect of the implant also migrate in the opposite direction.

E coli was chosen for this study because it is a widely used test microorganism for in vitro studies, especially for sterilization, disinfection, and contamination purposes. It is not fastidious and it is easy to handle in the laboratory, with a short generation time of 20 minutes. Furthermore, it can be found in the oral cavity of healthy individuals.[10]

Bidirectional fluid leakage and bacterial penetration via the interface of implant-abutment assemblies without mechanical loading has been previously shown in several in vitro studies.[3,5,8,11] Jansen and associates[8] demonstrated that even implant systems with a high degree of precision fit between components could not completely prevent bacterial penetration and colonization. Therefore, the need for modification of the interfaces of common implant systems to better seal the implant-abutment contact area has been postulated. A silicone washer for the Frialit-2 abutment significantly reduced bacterial penetration compared to the standard Frialit-2 abutment and seemed to be a promising alternative. However, results previously generated with this system are not in agreement with the findings of the present study, in which bacterial leakage at the implant-abutment interface occurred very early during functional loading in all Frialit-2 specimens. The Brånemark, Replace-Select, and Screw-Vent systems also showed bacterial leakage relatively early. The degree of penetration in a specific implant system

Table 2 No. of Chewing Cycles Before Bacterial Leakage Was Detected

	Chewing cycles									
Implant/specimen	**0***	**5,400**	**10,800**	**21,600**	**43,200**	**86,400**	**172,800**	**345,600**	**691,200**	**1,200,000**
Brånemark										
4	+									
8	+									
1	–	–	–	+						
3	–	–	–	–	+					
6	–	–	–	–	–	–	+			
7	–	–	–	–	–	–	+			
2	–	–	–	–	–	–	–	–	+	
5	–	–	–	–	–	–	–	–	–	+
Frialit-2										
1	+									
3	–	–	+							
5	–	–	+							
4	–	–	–	+						
2	–	–	–	–	+					
7	–	–	–	–	–	+				
6	–	–	–	–	–	–	(+)†			
8	–	–	–	–	–	–	+			
Replace Select										
3	+									
4	+									
1	–	+								
2	–	–	+							
5	–	–	–	–	+					
6	–	–	–	–	–	+				
8	–	–	–	–	–	+				
7	–	–	–	–	–	–	–	–	+	
Camlog										
1	+									
4	–	–	–	–	–	+				
2	–	–	–	–	–	–	+			
3	–	–	–	–	–	–	–	+		
5	–	–	–	–	–	–	–	+		
7	–	–	–	–	–	–	–	+		
6	–	–	–	–	–	–	–	–	–	+
8	–	–	–	–	–	–	–	–	–	+
Screw-Vent										
1	+									
4	+									
2	–	+								
5	–	+								
8	–	+								
6	–	–	–	–	+					
3	–	–	–	–	–	+				
7	–	–	–	–	–	–	+			

*Specimen was excluded from testing.
†Abutment connection failed before the end of the test series.

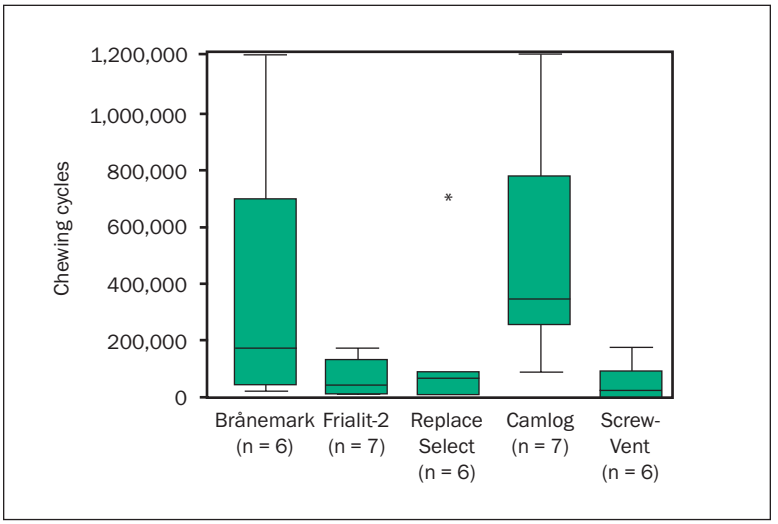

Fig 3 Box-plot diagram of recorded chewing cycles until bacterial penetration occurred. Asterisk depicts an extreme value for Replace select specimen no. 7. The boxes represent the first and third quartiles of the recorded chewing cycles; the line, the medians.

presumably is a multifactorial condition depending on the precision of fit between the implant and abutment,[12] the degree of micromovement between the components,[12, 13] and the torque forces used to connect them.[14] Some studies have shown a high precision of fit at the outer interface of implant-abutment assemblies,[8,15] but evaluations of connective or antirotational elements at different interfaces are still needed. Transverse occlusal forces on the prosthetic restoration during function may induce bending of or micromovement within the implant system, thereby increasing the gap at the components' interface[16] and inducing a "pump effect" between the inside of the implant and the surrounding peri-implant tissue.

This study showed that, under dynamic loading, bacterial penetration occurred significantly later in the specimens of the Camlog System as compared to the Frialit-2 and the Screw-Vent System; thus, the tested hypothesis was disproved. It should be noted that the latter 2 implant systems had smaller diameters than the Camlog; this could be a possible explanation for their relatively poor performance. However, the diameters of the Brånemark and Replace-Select components were identical to the Camlog components, and bacterial penetration also occurred earlier with these systems than with Camlog. Accordingly, the length of the implant-abutment joint could be a reason for differences in the bacterial penetration. The manufacturer of the Camlog System claims a so-called "positive locking tube-in-tube joint," which (in mechanical engineering terms) means that the ratio of the tube diameter to length of the joint is larger than 1.4. The tube diameter/joint-length relation was 1.68 for the tested Camlog implants but only 1.3 for the Replace-Select System, 1.13 for the Frialit-2 System, 0.43 for the Screw-Vent System, and 0.16 for the Brånemark System. This may be an explanation for higher structural strength of the Camlog connection compared to the other systems; this structure may minimize micromovement and pumping effects between the inside of the implant and the surrounding peri-implant sulcus. Further investigation is necessary to verify this hypothesis.

CONCLUSIONS

1. A new in vitro model was able to show bacterial leakage along the implant-abutment interface during dynamic loading.
2. Bacterial leakage along the interface was shown for all tested implant systems.
3. The number of load cycles until bacterial penetration occurred differed significantly between implant systems and their specific connection designs.

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