

The Effect of Dynamic Loading on Bacterial Colonization of the Dental Implant Fixture–Abutment Interface: An In Vitro Study

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Bacterial colonization of the fixture-abutment interface (FAI) microgap may contribute to increased marginal bone loss. The contribution of loading on bacterial colonization has not been thoroughly evaluated with in vitro experiments. The aim of this study was to evaluate the effect of dynamic loading on the colonization of oral microorganisms in the FAI microgap of dental implants with internal Morse-taper connection. Forty implants were divided into two groups (n = 20/group) based on subjection to dynamic loading conditions. Both Group 1 and 2 were comprised of fixtures that connected to standard abutments and allowed to incubate in a bacterial solution of *Escherichia coli*. The specimens of Group 2 were loaded with 500 000 cycles of 50 N using a chewing simulator. Following disconnection of fixtures and abutments, microbial samples were taken from the threaded portion of the abutment, plated and cultured under appropriate conditions. One of the 20 implants of Group 1 and 4 of the 20 implants of Group 2 had FAI microgaps colonized by *E coli*. With the limits of this study, it indicates that implants with internal Morse-taper connection exhibited minimal bacterial penetration down to the threaded part of the FAI and that dynamic loading increases the potential for such bacterial penetration.

Key Words: dental implants, bacterial leakage, in vitro, bacterial counts, titanium

INTRODUCTION

The importance of the position, size, and geometry of the fixture-abutment interface (FAI) microgap on marginal bone levels has been a subject of various studies^{1–4} demonstrating that bacterial colonization of the FAI microgap may contribute to the observed increased marginal bone loss. Microorganisms may grow into this FAI microgap^{5–7} and establish a bacterial reservoir, resulting in an area of inflamed soft tissue facing the fixture-abutment junction.⁸ In addition, the close proximity FAI

microgap to bone may have a role in the development of peri-implant inflammation and bone loss.^{1–3,9} Therefore, prevention of microbial leakage at the FAI is a major challenge for the construction of two-piece implant systems to minimize inflammatory reactions and to maximize peri-implant bone stability.

Several in vitro studies have evaluated the potential microbial leakage at the FAI, utilizing either non-loading^{10–15} or loading conditions.^{16,17} Despite the fact that these types of in vitro studies poorly mimic the biologic reality, they can be useful for understanding the dynamics of the FAI and thus contribute to the improvement of the FAI design. For instance, in vitro studies have demonstrated that the design of the FAI can impact the amount of microbial penetration into the internal part of the

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implant, where implants with an external hex design have failed to prevent microbial penetration at the FAI both under loading¹⁶ and non-loading conditions.^{10,11} This may in part explain the histological findings from in vivo studies evaluating implants with external hex connection design, which demonstrate an area of inflamed connective tissue facing the fixture-abutment junction.^{8,18}

Even though complete prevention of microbial penetration into the internal part of the implants has not been demonstrated in vitro, the most favorable results have been reported when implants with an internal Morse-taper connection have been utilized.^{12,13,15,17} Specifically, in our recent study,¹² we reported that under non-loading conditions, 3 out of 10 implants with internal Morse-taper connection demonstrated microbial leakage into the FAI. Interestingly, our follow-up study¹⁷ demonstrated that under loading conditions, only 1 out of 14 implants with similar internal Morse-taper connections demonstrated microbial leakage into the FAI. Importantly, in vivo implants are also exposed to dynamic loading conditions, as those associated with mastication. Therefore, the aim of this study was to evaluate the effect of dynamic loading on the colonization of oral microorganisms into the FAI microgap of dental implants with a Morse-taper internal connection between the fixture and abutment.

MATERIALS AND METHODS

Experimental design

The colonization of fixtures with an internal Morse-taper connection (Implant One Fixtures, 4.0 × 12 mm, Custom Dental Implants, Norwalk, Wis), connected to standard straight abutments (Standard Abutment 5.0 mm, Custom Dental Implants) (Figure 1) was evaluated following non-loading (Group 1) or dynamic loading conditions (Group 2). Twenty implants were tested in each experimental group. Group 1: The abutments were connected to the fixtures with a torque of 25 Ncm according to manufacturer's protocol. Group 2: Abutments were connected to the fixtures as described for Group 1, after which implants were subjected to dynamic loading as described as follows. All fixtures and abutments were connected in a sterile environment and placed in a bacterial solution, which covered the FAI interface (Figure 2).

In vitro dynamic loading

Dynamic loading was applied using a chewing simulator (CS-4.2 chewing simulator, Mechatronic, Feldkirchen-Westerham, Germany). Specifically, implants were placed in custom-made test chambers and secured with autopolymerizing resin (Luxa-temp, DGM, Hamburg, Germany). The test chambers with implants were mounted in a dual-axis chewing simulator and partially immersed in a bacterial solution that covered the FAI (Figure 2). A cyclic fatigue load was applied to each abutment with a round stainless steel stylus at an angle of 30°. A force of 50 N was applied for a total of 500 000 cycles at 1 Hz.

Bacterial culture conditions

Escherichia coli DH5 α was cultured in luria broth, shaking at 37°C until it reached mid-logarithmic growth phase, after which the bacterial solution was diluted 1:100 in additional luria broth, and implants were submerged above the FAI microgap. Implants were incubated in the bacterial culture at room temperature for five days, with bacterial suspensions replaced every 18 hours with fresh 1:100 dilution of mid-logarithmic phase *E coli*.

Microbial sampling and detection

Following disconnection of fixtures and abutments under sterile conditions, microbial samples were taken from the threaded portion of the abutment using sterile cotton swabs. To minimize the possibility of contamination during the sampling process, one investigator (RM) performed the disconnection of the fixtures from the abutments, and another investigator (TK) obtained the samples from the threaded portion of the abutments. Both investigators utilized sterile technique. Before and after the immersion of the implants to the bacterial solution, the outer surface of the abutment area was sampled and served as a negative and a positive control, respectively. Samples were immediately plated onto luria broth agar plates and incubated at 37°C for 24 hours, after which individual colony forming units (CFU) were counted and recorded.

Statistical analysis

For description of data, mean values and standard deviations (SD) were calculated. In addition, the

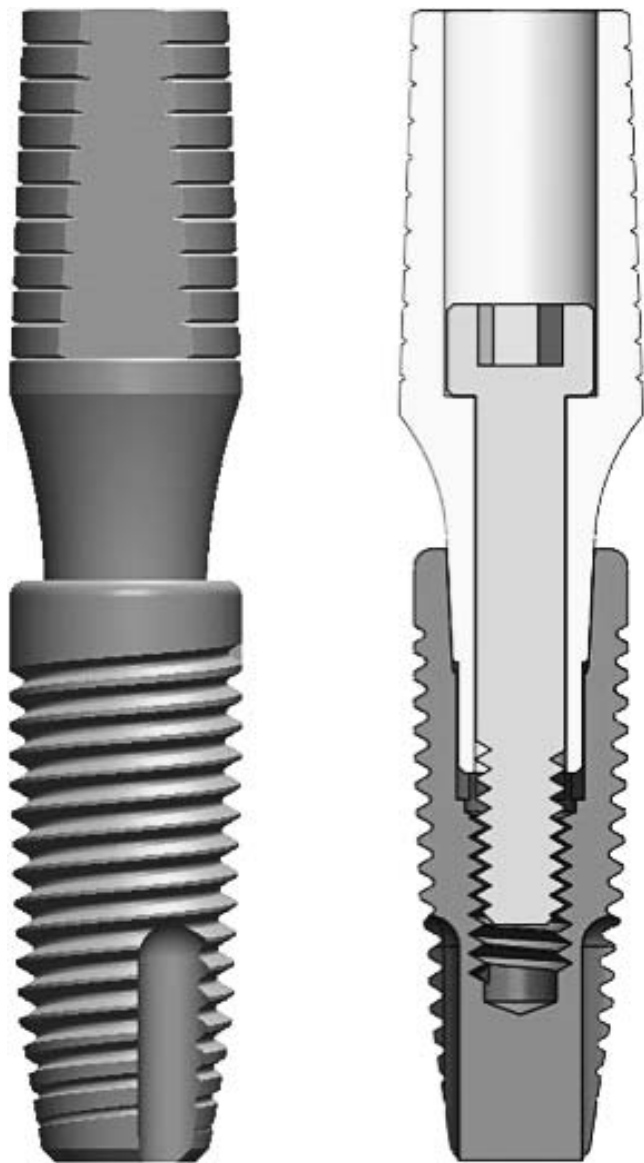


FIGURE 1. Schematic illustration of the experimental implant.

total number of implants per group exhibiting bacterial colonization of the FAI microgap was calculated. The Mann-Whitney *U* test was applied to evaluate differences between the two groups regarding number of CFU for *E. coli*. The Fisher exact test was used to evaluate differences in the number of implants exhibiting bacterial colonization of the FAI microgap between the two groups. A *P*-value of $<.05$ was considered significant.

RESULTS

To validate the colonization and detection techniques, microbial samples were taken from the outer surface of the abutment area from all specimens before and after the immersion in the bacterial solution, which served as negative and positive controls, respectively. No CFUs of *E. coli* were detected from samples taken prior to immersion of the implants to the bacterial solution (negative controls) for both groups. All positive controls in Group 1 and Group 2 developed multiple CFU (Group 1: mean 224, SD 173.3, Group 2: mean 213, SD 116.1).

For the samples taken from the threaded portion of the abutment, Group 1 exhibited significantly lower numbers of CFU for *E. coli* (mean 3, SD 13.4, minimum value 0, maximum value 60) compared to Group 2 (mean 19.2, SD 55.5, minimum value 0, maximum value 200), ($P < .05$).

One of the 20 implants of Group 1 and 4 of the 20 implants of Group 2 had FAI microgaps colonized by *E. coli* (one-sided Fisher exact test $P > .05$).

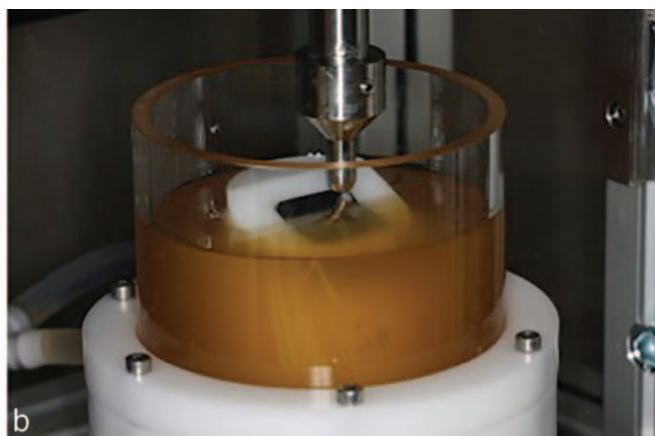


FIGURE 2. (a) Implant and abutment of Group 1 in a plastic container with the bacterial solution. (b) Implant and abutment of Group 2 in a bacterial solution subjected to dynamic loading.

DISCUSSION

In this study, implants with a Morse-taper connection were evaluated for potential invasion of oral microorganisms into the FAI microgap under non-loading and loading conditions. The results showed that dynamic loading of dental implants has an effect on bacterial penetration down to the threaded portion of the FAI. Specifically, non-loaded implant exhibited a lower number of CFU for *E coli* compared to loaded implants. In addition 1/20 of the non-loaded implants and 4/20 of the loaded implants became colonized by *E coli*.

In the present study, we tested for microbial colonization of FAI microgap by *E coli* because the experiment had to be executed in aerobic conditions, and this microorganism has been used frequently with in vitro experiments of similar design.^{10,11,16,17} The utilization of enterics such *E coli* seems relevant for in vitro studies because these microorganisms have been found frequently in peri-implantitis lesions.^{19,20}

The results of the present study are in line with several studies evaluating the potential of microbial leakage for implants with an internal Morse-taper connection under non-loaded conditions.^{12,13,15} Indeed our group¹² evaluated the potential risk for colonization of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* at the fixture-abutment interface of implants with Morse-taper internal connection under non-loaded conditions and found low colonization by these microorganisms. Similar results have been reported from Aloise et al,¹³ where internal Morse-taper connection implants were inoculated internally with *Streptococcus sanguinis*. Following assembly and total immersion of implants in a sterile solution for 14 days, only 2 implants showed evidence of bacterial leakage. In addition, a recent study¹⁵ utilized a similar experimental method but different bacterial species (*Pseudomonas aeruginosa* and *A actinomycetemcomitans*) and evaluated three different connection designs. It was determined that 1 out of 10 implants with Morse-taper connection exhibited microbial contamination through the FAI microgap. In spite of these data, there are studies reporting less favorable results for colonization of implants with Morse-taper connection.¹⁴ Under non-loading conditions, the degree of microbial penetration to the internal parts of the implant is mainly influenced by the precision fit between the implant and the abutment²¹ and

the torque forces used to connect them.²² Thus, the conflicting results among studies utilizing implants with internal Morse-taper connections may reflect differences in the precision fit between the implant components.

Unfortunately, there is limited information from in vitro studies evaluating microbial contamination of the FAI microgap under loading conditions. Steinebrunner et al¹⁶ used a 2-axis chewing simulator to apply a 120 N force for a total of 1 200 000 cycles, where statistically significant differences among five implant systems with respect to number of chewing cycles and bacterial leakage were reported. However, in this study, implants with internal Morse-taper connections were not evaluated. In a recent study from our group,¹⁷ an ACTA wearing simulator was used to apply load to implants with two different types of internal conical connections. Here, we reported that 1 of the 14 implants with an internal Morse-taper connection and 12 of the 14 implants with a four-groove internal conical connection exhibited multiple colony forming units for *E coli* following loading with a force of 15 N for 500 000 cycles. In this study Teflon material was used as an antagonist that had to be replaced every 2 hours due to wear. In the current study, we reported higher frequency of implants exhibiting CFU under loading conditions compared to the previously mentioned study. These differences may be attributed to differences in the experimental loading conditions (50 N vs 15 N), the agonist material (steel vs Teflon), as well as to differences in the internal connection characteristics of the studied implants.

In general, the reliability and the stability of an implant-abutment connection mechanism is an essential prerequisite for long-term success of dental implants.²³ The tightening and loosening torques are main factors in determining the stability of the abutments. Bozkaya and Müftü²⁴ evaluated the effect of parameters such as friction, geometric properties of the screw, the taper angle and the elastic properties of the materials on the mechanics of the tapered implant-abutment interface with a screw integrated at the bottom of the abutment. They reported that the tapered section of the abutment carries most of the tightening load and that the efficiency of the system—defined as the ratio of loosening torque to the tightening torque—depended on the values of taper angle and the

friction coefficient. In addition to these findings, Norton²⁵ reported that the interfacial surface area seemed to have a profound effect on the efficiency of the connection. This might explain some of the different findings among studies evaluating the contamination of the FAI microgap for implants with internal Morse-taper connections but with different degree of taper.^{12,14} In the present study, we have utilized implants with a 3° taper and an abutment with nonintegrated screw. The findings for the non-loaded part of this study are similar with studies that evaluated implants with internal Morse-taper connection, a taper of 5.5° and a screw integrated at the bottom of the abutment.^{12,13}

The mechanics of the Morse-taper implant connection under loading conditions have been studied by Merz et al²⁶ using a finite element model and an implant with an 8° taper with a screw integrated at the bottom of the abutment. They reported that tightening of the abutment only resulted in a symmetric stress distribution in the connection area of the implant and abutment. When the implant was loaded with a 30° off-axis, the threads of the abutment experienced stress that reached higher than the yield point, and the area of stress was limited to the thread root on the tension side. The results of this study indicate that dynamic loading may have an effect on the efficiency of the connection. In fact, Zipprich et al²⁷ evaluated the dynamic behavior of dental implants with different designs of the fixture–abutment connection designs. The authors reported micromovement of the fixture–abutment complex of implants loaded at an angle of 30° when a force of up to 200 N was applied. When implants with Morse-taper internal connection, 8° taper and a screw integrated at the bottom of the abutment evaluated a microgap of 0.1–4µm was observed. This finding supports the results of the present study that dynamic loading can have an effect on contamination of the FAI microgap for implants with internal Morse-taper connections. Thus, micromovement of the fixture–abutment complex can introduce a pumping effect between the fixture and the abutment with detrimental effects on the marginal bone stability.¹

In conclusion, this study indicates that implants with internal Morse-taper connection, a 3° taper, and an abutment with nonintegrated screw exhibited minimal bacterial colonization of the FAI under in vitro conditions. These findings may have

positive clinical implications for stable peri-implant bone levels for implants with such internal connection characteristics, but further clinical studies are needed to confirm the in vitro findings. Additionally dynamic loading affects the potential for invasion of oral microorganisms into the FAI. This indicates that testing dental implants under dynamic loading is an important part of the experimental design for evaluating bacterial colonization of dental implants and understanding FAI dynamics. Further studies utilizing loading conditions that resemble clinical loading conditions in magnitude and direction and evaluating penetration of bacterial products such as endotoxins to the FAI would be useful in the evaluating the dynamics of the FAI. However, findings from in vitro studies should always be confirmed with clinical studies for evaluation of dental implant characteristics.

ABBREVIATIONS

CFU: colony forming units
FAI: fixture–abutment interface
SD: standard deviation

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