In Vitro Comparison of Microbial Leakage of the Implant-Healing Abutment Interface in Four Connection Systems

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This study sought to assess microbial leakage through the implant-healing abutment interface in 4 dental implant connection systems. Ten implants of each of the 3i (double hexagon + flat to flat; group 1), IDI (internal hexagon + Morse taper; group 2), Swiss Plus (external bevel + internal octagon; group 3), and Tapered Screw-Vent (internal bevel + internal hexagon; group 4) systems were used in this in vitro, experimental study. Healing abutments were screwed to the implants with 10 Ncm torque. Implants were immersed in *Escherichia coli* suspension for 24 hours. Samples were taken of the internal surface of implants and cultured. The number of grown colonies was counted after 24 hours of culture and after 7 and 14 days of immersion in microbial suspension. The same was repeated with healing abutments torqued to 10 and 20 Ncm. With 10 Ncm torque, all specimens in all groups showed microleakage at one day with the highest microleakage in one sample in group 3. At 7 days, the highest microleakage was noted in one specimen in group 2. With 20 Ncm torque, group 3 showed significantly higher microleakage than other groups at 1 and 7 days ($P < .05$). Increasing the torque decreased microleakage in all groups except for group 3. Microbial leakage occurred in almost all implant systems in our study. In one-stage implant placement, healing abutments should be preferably torqued to 20 Ncm to minimize microleakage. Optimal torque for healing abutment insertion should be analyzed individually for each system.

Key Words: dental implants, implant-abutment connection, microleakage, microbiology, implant-abutment interface

INTRODUCTION

Dental implants are increasingly used to replace lost teeth.[1–3] This modality has a high success rate and yields favorable and long-term stable results.[4–10]

Considering the increasing demand and popularity of one-stage implant placement and consequently longer retention of healing abutments in the oral cavity,[11,12] microleakage through the implant-healing abutment interface is an interesting research topic. Microgap at the implant-abutment interface can serve as a suitable area for accumulation and proliferation of bacteria,[2,4] and can result in halitosis and peri-implant diseases such as peri-implant mucositis and peri-implantitis.[4,13,14] Such inflammatory conditions may impair bone healing and may eventually result in bone loss.[4,15,16] Also, positioning of implant-abutment interface at the level of alveolar bone crest in submerged implants is one reason for peri-implant bone loss during the first year after loading of implants.[13]

Dental implant manufacturers have designed several connection systems to minimize microgap at the implant-healing abutment interface.[16–19] However, the efficacy of these systems needs to be further scrutinized.[16]

Colonization of bacteria in the internal implant space and leakage of bacteria and their products through the implant-abutment interface have been the topic of many studies and the results have mainly indicated inflammatory infiltrates in peri-implant soft tissue.[4,13,14,20–23] This infiltration appears to be due to bacterial contamination of internal surfaces of implants.[24]

Quirynen et al[17] evaluated the presence of viable microorganisms in the internal surface of Branemark dental implants and found that all specimens harbored considerably high levels of microorganisms, mainly cocci and immobile rods. In a clinical study, Wahl et al[25] isolated a wide range of microorganisms from the internal surface of IMZ implants, which were relatively similar to the anaerobic flora in patients with periodontitis. An in vitro study on this particular type of connection showed that it had higher capability to prevent microbial leakage than the older types.[17] The IDI (internal hexagon + Morse taper), 3i (double hexagon + flat to flat), Tapered Screw-Vent (internal bevel + internal hexagon), and Swiss Plus (external bevel + internal octagon) are among the commonly used dental implants with different connection systems.

Escherichia coli is a gram-negative, facultative anaerobe

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https://doi.org/10.1563/aaid-joi-D-18-00311

with 1.5 μm diameter and 2–6 μm length.[23,26] It is found in the oral microbial flora of healthy individuals.[27] It is classified as a medium-sized oral bacterium.[28] Due to its easy culture and fast proliferation rate, it is commonly used in many in vitro studies on microleakage.[27]

To the best of the authors' knowledge, no previous study has compared microleakage at the implant-healing abutment interface among different connection systems available in the market. Thus, this study aimed to compare microleakage at the implant-healing abutment interface in four implant systems with different connection types.

Materials and Methods

This in vitro, experimental study was conducted on 10 implants from each of the following 4 systems:

- double hexagon + flat to flat; group 1 (3I, Implant Innovations, Palm Beach, Fla)
- internal hexagon + Morse taper; group 2 (IDI, Montreuil, France)
- external bevel + internal octagon; group 3 (Tapered Swiss Plus, Zimmer Dental Inc, Carlsbad, Calif)
- internal bevel + internal hexagon; group 4 (Tapered Screw-Vent, Zimmer Dental)

Sample size was calculated to be 10 in each group according to previous study by Jansen et al.[27] The implants along with their respective healing abutments were directly purchased from the manufacturers.

Sterile healing abutments were screwed to the implants using a motor and handpiece (Surgical Motor, W&H, Austria) with 10 Ncm torque. Implants were held by a locking plier to torque the healing abutment. To ensure that implant fixtures were sterile in the first place, they were placed in sterile brain heart infusion broth (Merck, Darmstadt, Germany) and incubated at 37°C for 24 hours. Next, samples were obtained of the broth and the internal surface of implants and cultured on blood agar medium (suitable for growth of almost all bacteria). Sterile implants (no turbidity in the plate) were used for the next step.

E. coli was cultured in MacConkey medium and *E. coli* suspension with 0.5 McFarland standard concentration was prepared. Each 1 cc of this bacterial suspension contained 1.5×10^8 bacteria. Bacterial suspension was transferred to 20 test tubes (2 mL per each tube). Test tubes for each system were coded from 1 to 10. To ensure equal bacterial concentration in all test tubes, a spectrophotometer was used to measure the optical density. At 0.5 McFarland standard concentration, optical density must be 0.3–0.8. This condition was met in all test tubes. Implant-healing abutment complexes were immersed in the respective test tubes individually and incubated at 37°C for 24 hours. Next, they were removed from the test tubes and rinsed with saline to eliminate bacteria on the implant surface. Healing abutments were then removed by a sterile screwdriver and samples were taken from inside the implants using a sterile sampling needle. Samples were transferred to erosin methylene blue culture medium and incubated at 37°C for 24 hours. Green metallic sheen indicated presence of *E. coli* colonies. To ensure that the proliferated bacteria were *E. coli*, colonies were also cultured in differential culture media including triple sugar iron agar, urea, citrate, and indole and incubated for 24 hours. Positive test results confirmed the presence of *E. coli*.

Two implants out of 10 in each system served as negative controls to ensure no accidental contamination. Negative control implants were immersed in test tubes containing sterile brain heart broth instead of microbial suspension.

Healing abutments were screwed to implants again with 10 Ncm torque and immersed in microbial suspension and incubated for 7 and 14 days. At the aforementioned time points, healing abutments were removed and samples were obtained from inside the implants as explained earlier and cultured. Number of grown *E. coli* colonies was counted and reported as colony forming units per 1 mL. The same procedures were repeated with healing abutments torqued to 20 Ncm as well.

All of the data were compared with a repeated-measures analysis of variance. The significance level of the statistical test was determined at $\alpha = 0.05$. A P value was set at .05 with the Bonferroni correction for multiple comparisons.

Data were analyzed using SPSS version 23. The methodology was reviewed by an independent statistician.

Results

In this study, we torqued the healing abutment to 10 and 20 Ncm, which are commonly applied in the clinical settings. In application if 10 Ncm torque, no significant difference was noted in microleakage among the 4 groups after 1, 7, and 14 days but in application of 20 Ncm torque, significant differences were noted in microleakage in the fourth group after 1 and 7 days. These results showed that microbial leakage depended on both passage of time and torque of healing abutments, and in all groups except for group 3, microleakage decreased by an increase in torque. Also, microleakage occurred in all specimens of all groups after 24 hours in application of both 10 and 20 Ncm torque.

1. *At 1 day:*

All specimens in all four groups showed microleakage (Table 1). The mean microleakage was the highest in group 3 and the lowest in group 2. Microleakage was not significantly different among the 4 groups ($P > .05$).

2. *At 7 days:*

Group 2 showed the highest and group 1 showed the lowest microleakage. Some specimens in the 4 groups showed no microleakage. The difference in microleakage was not significant among the 4 groups ($P > .05$).

3. *At 14 days:*

Maximum microleakage was noted in group 2 (Table 1). All groups had specimens without microleakage. The mean microleakage in group 2 was higher than that in other groups. The mean microleakage in group 1 was lower than that in other groups. The differences in microleakage among the 4 groups were not significant ($P > .05$).

Table 1

Microleakage (mean number of CFUs) in the 4 groups (n = 8) with healing abutments torqued to 10 Ncm after 1, 7, and 14 days*

Time	Minimum CFUs	Maximum CFUs	Mean CFUs	Standard Deviation
1 day				
Group 1	10 000	80 000	40 625/00	29 330/323
Group 2	10 000	80 000	36 875/00	28 149/791
Group 3	20 000	100 000	61 250/00	26 958/469
Group 4	10 000	80 000	38 125/00	280 276/873
7 days				
Group 1	0	50 000	10 750/00	20 253/747
Group 2	0	80 000	27 625/00	30 789/319
Group 3	0	65 000	213 125/00	27 766/565
Group 4	0	45 000	15 875/00	16 269/494
14 days				
Group 1	0	16 000	5250/00	7324/128
Group 2	0	80 000	31 250/00	34 408/263
Group 3	0	4000	8500/00	14 372/593
Group 4	0	80 000	22 500/00	31 052/950

*CFUs: colony forming units. Group 1: Double hexagon, flat to flat; Group 2: Internal hexagon, Morse Taper; Group 3: Internal octagon, external bevel; Group 4: Internal hexagon internal bevel.

1. *At 1 day:*

No microleakage was noted in any specimen in group 4. The highest mean microleakage was noted in group 3 (Table 2). Significant differences were noted between groups 1 and 3 ($P = .002$), 2 and 3 ($P = 0$), and 3 and 4 ($P = 0$).

2. *At 7 days:*

Leakage-free samples were noted in all four groups. Maximum microleakage was noted in group 3. Minimum microleakage was noted in groups 1 and 3. Significant differences were noted in microleakage between groups 1 and 3 ($P = .004$), 2 and 3 ($P = .008$), and 3 and 4 ($P = .032$).

3. *At 14 days:*

Leakage-free samples were noted in all 4 groups. Maximum mean microleakage was noted in group 4 and minimum mean microleakage was noted in group 1 (Table 2). No significant difference was noted in microleakage among the 4 groups ($P > .05$).

Figures 1 and 2 compare the microleakage of the 4 systems with healing abutments torqued to 10 Ncm and 20 Ncm. Figure 3 compares microleakage among the 4 systems after 1, 7, and 14 days.

Discussion

This study evaluated microbial leakage at the implant-healing abutment interface and showed that microleakage occurred in all 4 groups with no significant difference among them.

Several connection designs have been designed by implant manufacturers to minimize microleakage at the implant-abutment interface. The connection type in the 3i system is the internal hexagon. This implant system uses a system for precise implant-abutment connection, which contains 3 main

Table 2

Microleakage (mean number of CFUs) in the 4 groups (n = 8) with healing abutments torqued to 20 Ncm after 1, 7, and 14 days*

Time	Minimum CFUs	Maximum CFUs	Mean CFUs	Standard Deviation
1 day				
Group 1	0	50 000	21 250/00	24 748/737
Group 2	0	14 000	1750/00	4949/747
Group 3	10 000	110 000	66 250/00	35 431/020
Group 4	0	0	0/00	0/000
7 days				
Group 1	0	20 000	2500/00	7071/068
Group 2	0	25 000	6000//00	9456/668
Group 3	0	100 000	55 000/00	40 708/020
Group 4	0	100 000	13 500/00	35 063/208
14 days				
Group 1	0	10 000	1250/00	3535/534
Group 2	0	50 000	12 250/00	17 580/427
Group 3	0	30 000	6500/00	11 747/340
Group 4	0	50 000	15 000/00	21 380/899

*CFUs: colony forming units. Group 1: Double hexagon, flat to flat; Group 2: Internal hexagon, Morse Taper; Group 3: Internal octagon, external bevel; Group 4: Internal hexagon internal bevel.

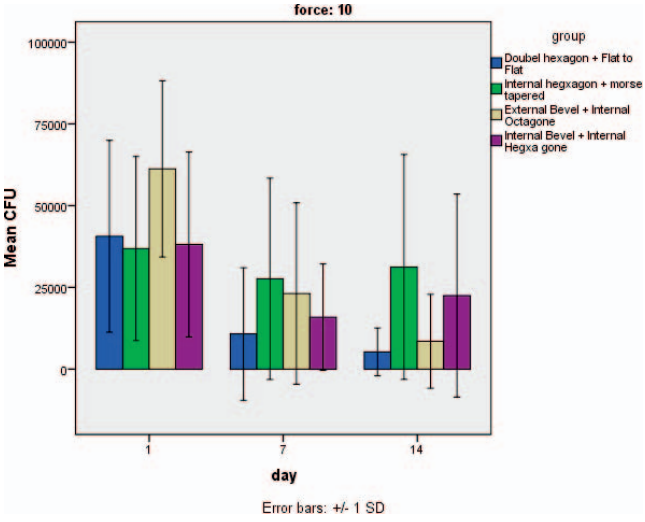

Figure 1. Microleakage of the 4 systems with healing abutments torqued to 10 Ncm after 1, 7, and 14 days.

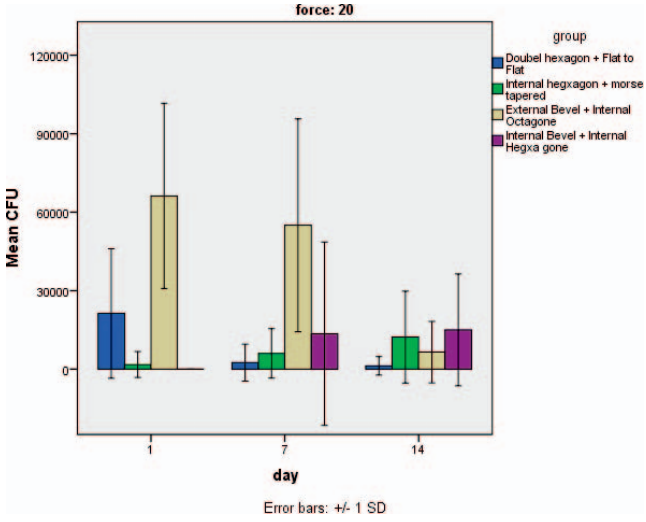

Figure 2. Microleakage of the 4 systems with healing abutments torqued to 20 Ncm after 1, 7, and 14 days.

components of hex (which is similar to other internal systems), double hex (which minimizes misfit according to the manufacturers), and click zone (hearing the clicking sound ensures complete seating of abutment in its proper location).[29] The Swiss Plus implant system has an internal octagon connection. Only small size implants in this system with 3.7 mm diameter have internal hexagon. The manufacturer of this implant system claims that by development of friction-fit technology (cold soldering of implant and abutment), adaptation of abutment to internal implant walls increases and the gap at the interface is minimized. The connection system in IDI implants is Morse Taper. According to the manufacturer, this connection confers strength to prosthetic components due to the engraved prosthetic grooves.

To the best of the authors' knowledge, no previous study has compared implant-healing abutment microleakage of different implant systems. Thus, we compared the results with studies on microleakage at the implant-abutment interface. Binon et al[30] measured the implant-abutment gap in several connection systems and reported it to be in the range of 20–49 μm. Thus, microbial leakage can occur through this gap as the diameter of many oral microorganisms is less than 10 μm. However, King et al[31] believes that actual size of implant-abutment gap has no significant effect on crestal bone loss. They stated that manufacturers' attempts to minimize this gap have insignificant effects on occurrence of peri-implant biological complications and concluded that the stability of the implant-abutment interface greatly affects biological incidents occurring in peri-implant tissues adjacent to the gap. In other words, shape of components participating in implant-abutment connection, irrespective of the size of gap, relates to biological changes.

Gross et al[14] evaluated microleakage through the implant-abutment interface in 5 implant systems and attached the abutments to implants with various torques. Microleakage was noted in all systems but the amount of microleakage was variable depending on the applied torque. Increasing the torque from 10 to 20 Ncm significantly decreased the microleakage. Applying the torque recommended by the manufacturers decreased the microleakage in all systems. Thus, they concluded that screwing the abutments by the torque recommended by the manufacturers can significantly decrease microbial leakage and its adverse consequences such as halitosis, soft tissue inflammation, and peri-implant bone loss. The same results were obtained in our study and increasing the torque from 10 to 20 Ncm decreased microleakage.

Dibart et al[2] in an in vitro study evaluated the Bicon implant system and the efficacy of locking taper (Morse Taper) in prevention of microbial leakage through the implant-abutment interface. They concluded that the seal provided by locking taper design in Bicon implants was resistant to microbial leakage. Their results were in contrast to ours since in our study, all specimens showed microleakage even with 20 Ncm torque. Since their study is the only one showing no microleakage, it

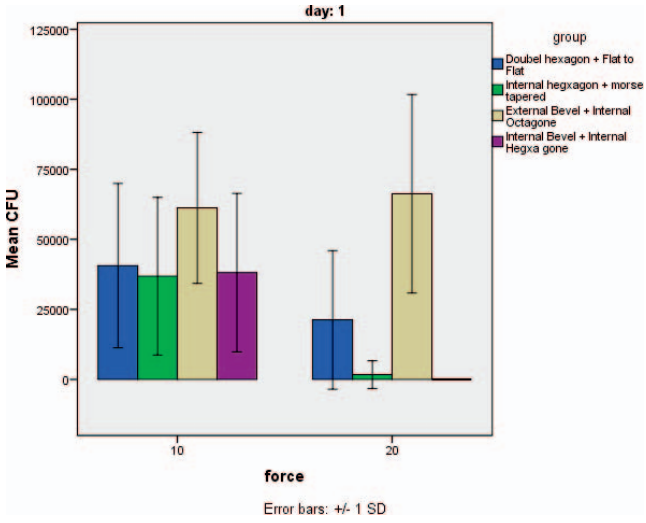

Figure 3. Comparison of microleakage among the 4 systems after 1, 7, and 14 days.

appears that similar studies are required to better elucidate this topic.

Aside from the implant-abutment fit, the microenvironment between components and the applied torque also affect the amount of microbial leakage. Moreover, height of contact area at the interface is also responsible for the variability in bacterial leakage. By an increase in ratio of interlocking tubes (abutment and implant) at the interface to the height of connection area, structural integrity of the site increases and microenvironment and subsequently its pumping effect (microleakage during small movements of components) decrease between internal implant surface and periodontal tissues.[23]

A reduction in microleakage occurs as the result of a firm fit, which occurs by an increase in torque.[32] Gross et al[14] showed that microleakage significantly decreased by increasing the torque from 10 to 20 Ncm and eventually to the torque recommended by the manufacturer. They stated that when abutments are screwed with the torque recommended by the manufacturer, microleakage in abutments with taper connection was higher than that in abutments with flat connection. They recommended that irrespective of the implant type, small movements of components are associated with higher microleakage while optimal fit of components, minimal abutment microenvironment, proper prosthetic design, optimal occlusion, and gradual increase of load are associated with decreased microleakage.

E. coli has been extensively used for in vitro assessment of microleakage[27] since it can be easily cultured and it proliferates in a short period of time. Since at least 24 hours are required for proliferation of *E. coli*, we obtained the first samples after 24 hours of immersion in microbial suspension. In a study by Jansen et al,[27] all specimens showed microleakage after 24 hours except for Frialit-2 implant supplied with a silicon washer. The difference in microbial leakage after 7 and 14 days was not significant. Their findings were similar to ours.

Jansen et al[27] stated that even implant systems with optimal fit between components cannot perfectly prevent bacterial leakage. Based on their results and ours, the connection between implant and abutment and healing abutment must be modified to obtain a perfect seal. However, it should be noted that despite the presence of gap at the implant-healing abutment interface, these systems are routinely used worldwide with high success rates. Such a high rate of success despite the presence of gap may be attributed to rinsing of the internal implant space after removing the healing abutments as well as the soft tissue attachments to implants after their placement. Epithelial attachment to implant is equal to establishment of biologic width and serves as a biological seal protecting the crestal bone against leakage of the bacteria and their products.[27] On the other hand, health of peri-implant tissues is an impotent prerequisite to benefit from the protective effects of this biological seal.

Our study had some limitations including small sample size and suboptimal conditions for growth of anaerobic bacteria. Surface contamination occurred in some implants and resulted in elimination of some samples Moreover, microbial leakage in some samples was so high that it complicated accurate counting of colony-forming units.

The bacterial microleakage was assessed in this evaluation; however, it is possible that bacterial products with much smaller size than the bacteria could penetrate the inner space of implants. It is recommended to evaluate molecular microleakage in further studies.

We evaluated inward microleakage to implant internal space. It is suggested that assessment of outward bacterial microleakage could also be performed.

With a wide variety of types of connections available, where each manufacturer advocates positioning the implant at different levels, it is difficult to compare between studies. The preventive maintenance of rehabilitations related to dental and foreign implants, for example, through their peculiarity of residence, in contact with the environment, with favorable conditions for the development of infections, and also subject to eating habits, hygiene, and individual vices of each patient, are among the most important factors. Thus, component accuracy, proper torque, and preventive maintenance are factors that work together and, when not controlled, contribute to the failure of the implants.

In presence of healthy peri-implant tissue, microbial leakage would result in mild inflammation with no apparent clinical manifestations.[13] To minimize microleakage through the implant-healing abutment interface:

1. The implant-healing abutment gap should not be at the level of alveolar bone crest. In other words, non-submerged placement of implants is preferred.
2. Use of silicon washers recommended by the manufacturers may help minimize microleakage.[27]
3. After removing the healing abutment and prior to placing the abutment, the internal implant area must be thoroughly rinsed with saline or chlorhexidine and dried.
4. Application of antimicrobial agents such as antibiotic gels to the internal implant surface after removing the healing abutment and prior to placing the abutment may be helpful.[2]

The efficacy of these measures in the clinical setting is still questionable and in need of further investigation. Last but not least, precise oral hygiene by patients plays an important role in long-term success of dental implants.

Conclusion

In vitro microbial leakage through the implant-healing abutment gap occurs in almost all implant systems. Thus, in one-stage implant placement, healing abutments should be preferably torqued to 20 Ncm to minimize microleakage. Further clinical studies are needed to investigate this topic for definite conclusion in practical use.

Note

The authors declare no conflict of interest.

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