In Vitro Evaluation of the Implant-Abutment Bacterial Seal: The Locking Taper System

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Purpose: *To test in vitro whether the seal provided by the locking taper used in the implant-abutment connection was capable of preventing the invasion of oral microorganisms.* ***Materials and Methods:*** *Twenty-five wide-body implants (5 × 11 mm) and 25 abutments were divided into 2 groups for a 2-phase experiment. The first phase tested the ability of the seal to shield the implant well from outside bacteria; the second phase tested the ability of the seal to prevent bacteria present in the implant well from seeping out. For phase 1, 10 implant-abutment units were immersed in a bacterial broth for 24 hours. The abutments were then separated from the implants and bacterial presence was evaluated using scanning electron microscopy. In phase 2, the tested abutments were inoculated with a droplet of soft agar bacterial gel and assembled with the implant. These units were incubated in a sterile nutrient broth for 72 hours, sampled, and plated to assess bacterial presence.* ***Results:*** *In phase 1, no bacteria were detected in any of the implant wells. In phase 2, no bacteria were detected in the nutrient broth or on the agar plates at 72 hours.* ***Discussion:*** *In implants where a microgap is present, microbial leakage could lead to inflammation and bone loss; thus, it is important to minimize bacterial presence in and around the the implant-abutment junction.* ***Conclusion:*** *The seal provided by the locking taper design has been demonstrated to be hermetic with regard to bacterial invasion in vitro.* Int J Oral Maxillofac Implants 2005;20:732–737

Key words: *bacterial seal, implant-abutment connection*

Dental implants have revolutionized the practice of modern dentistry. Completely or partially edentulous patients are now able to benefit from fixed restorations and not worry about denture stability or comfort. Unfortunately, as useful as they are, implants are not without problems. As the worldwide use of dental implants increases, more practitioners are faced with issues similar to those encountered with natural teeth.[1] Microbial accumulation around dental implants may lead to inflammation and result in a condition known as peri-implantitis. The natural teeth and supporting structures may be affected by a similar condition; however, natural teeth benefit from the buffering power of the junctional epithelium and periodontal ligament. In the natural dentition, the junctional epithelium provides a seal at the base of the sulcus against the penetration of the bacteria and bacterial byproducts. If the seal is destroyed and the epithelial cells are allowed to migrate apically, a periodontal pocket will form.

The other line of defense that is present in the natural dentition, but absent from the endosseous implant structure, is the periodontal ligament. Since no cementum or fibers are present on the surface of an endosseous implant, and therefore no periodontal ligament and space, infection may spread directly into the osseous structures.[2] Peri-implantitis, like periodontitis, can result in bone loss and ultimately implant loss if left untreated.

This problem is further compounded by the implant system utilized. Two general dental implant systems have been available commercially: submerged and nonsubmerged. The submerged system necessitates the placement of the coronal portion of the implant at or below the level of the alveolar crest.

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The nonsubmerged system requires placing the top of the implant above the level of the alveolar crest. In some of the submerged systems, a "microgap" may exist at the level of the alveolar crest where the abutment and implant body meet. This microgap is usually associated with increased inflammation and alveolar crestal bone loss.[3] One of the prevailing hypotheses regarding this phenomenon is that oral bacteria colonize that area during surgery or after placement of the abutment, which can lead to infections over time. These bacteria, which are anaerobic in nature, have been observed growing in the microgap between the implant and the abutment or in the sulcus of implants, especially when sulcus depths are greater than 5 mm.[4] Several studies have documented the microbial contamination of the microgap between the implant and the abutment in the 2-stage implant.[5–7] Scanning electron microscopy (SEM) of failed implants has demonstrated the presence of significant amounts of bacterial accumulation at the implant-abutment interface. It has been suggested that such bacteria are responsible for local inflammation and subsequent bone 1oss.[8] The problem is further compounded by the location of the microgap relative to the alveolar bone crest in the 2-stage implant system. Studies have shown that the closer the location of the microgap to the alveolar crest is , the more bone loss can be expected.[9,10] It has been recommended that a topical antimicrobial agent be used at the time of abutment connection to minimize the risks of infection, since abutments and restorations provide a major surface for bacterial colonization.[1]

Unfortunately the effects of local antimicrobial therapy may be short-lived. Once the concentration of the locally applied agent is no longer bacteriocidal, bacteria will repopulate the implant-abutment interface as well as the implant well if the implant-abutment seal is not hermetic.[1,6,7] The implant well may act as a bacterial reservoir from which microorganisms may seep in and out, perpetuating the infective process that may lead to inflammation and ultimately bone loss.

The purpose of the present investigation was to test the ability of an implant system (Bicon, Boston, MA) with an implant-abutment seal resulting from the use of a locking taper design to withstand a bacterial challenge in vitro.

MATERIALS AND METHODS

In the first phase of the experiment, the ability of the seal to shield the internal well of the implant from outside bacteria was tested. Ten wide-body implants (5 × 11-mm uncoated implants with 3.0-mm wells) and 10 abutments (5 × 6.5 mm abutments with 3.0-mm posts) were used.

All experiments were carried out by the same investigator (MFS) in the sterile environment of a cell culture hood (Nuaire, Plymouth, MN). The abutments were seated on the implant bodies according to the guidelines given by the manufacturer. The implant-abutment units were then immersed individually in glass culture tubes containing 10 mL of a bacterial mixture (*Actinobacillus actinomycetemcomitans* serotype b ATCC strain 43718, *Streptococcus oralis* ATCC strain 35037, and *Fusobacterium nucleatum* ATCC strain 10953 at optical density 1) in brain-heart infusion broth (Gibco, Rockville, MD). The 10 implant-abutment units were incubated for 24 hours in an anaerobic chamber (BBL; Becton, Dickinson and Co, Franklin Lakes, NJ) at 37°C. After 24 hours, each glass culture tube was removed from the chamber, the bacterial broth was discarded, and the implant-abutment units were washed twice in sterile phosphate buffer saline (PBS) and fixed with 4% formalin overnight and prepared for SEM viewing. At that point, the abutments were separated from the implant bodies using 2 carbon-coated forceps, and the inside well was analyzed for bacterial presence using SEM (JEOL 6400; JEOL USA, Peabody, MA).

In the second phase of the experiment, the ability of the seal to prevent bacterial seepage from the implant well was tested. This experiment was repeated 3 times to assess reproducibility. Wide-body implants and abutments were used again. A 2% bacterial agar mix (100 µL of the same bacteria described for phase 1, in 100 µL of 4% soft agar [Gibco]) was prepared and kept in a liquid form at 45°C in a water bath; 1 µL of trypan blue (Gibco) was added to the mix for coloring purposes (total volume 201 µL). One tenth of a microliter of the 2% bacteria-agar solution was deposited at the apical end of 4 of the locking taper abutment posts. Three of these abutment posts were carefully inserted in 3 implant wells and tapped into place. One was not inserted into an implant; this abutment post was used as a positive control. Another abutment post was left bacteria-free and inserted into an implant well to serve as a negative control. All tested abutments were seated carefully on the implants according to the manufacturer's guidelines as soon as the agar droplet had solidified. The implant-abutment units were immersed individually in 5 glass culture tubes containing 10 mL of sterile brain-heart infusion broth and incubated in the anaerobic chamber at 37°C for 72 hours. At the end of the first 24 hours, 20 µL of the broth from the glass culture tubes containing the implants were pipetted out and individually plated

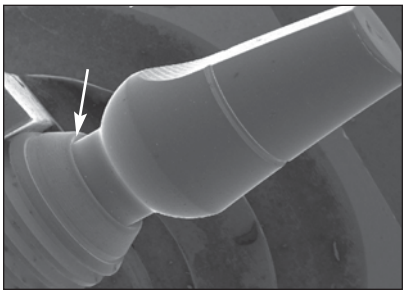


Fig 1a SEM of the assembled unit. Arrow indicates the site of implant-abutment interface, which is shown at higher magnification in Figs 1b and 1c.

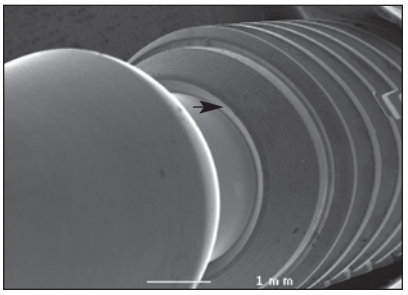


Fig 1b The assembled implant-abutment unit at a higher magnification. The arrow indicates the gap.

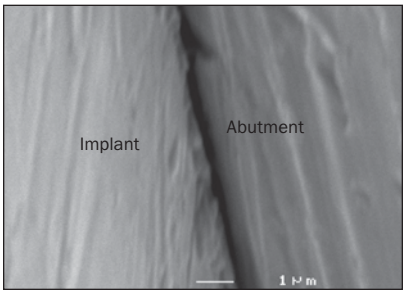


Fig 1c The implant-abutment unit at a higher magnification. The gap between the 2 components is less than 0.5 µm. The space is smaller than the diameter of bacteria.

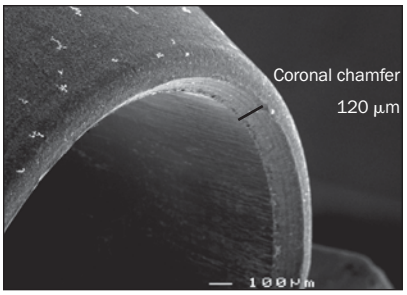


Fig 2a SEM of the coronal chamfer of an implant.

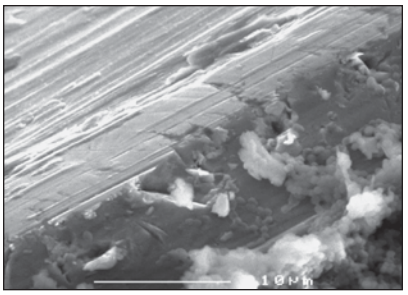


Fig 2b Bacteria on the collar of an implant. Note that the bacteria do not penetrate the well region of the implant. No bacteria were seen along the walls or bottom of the well in any of the specimens.

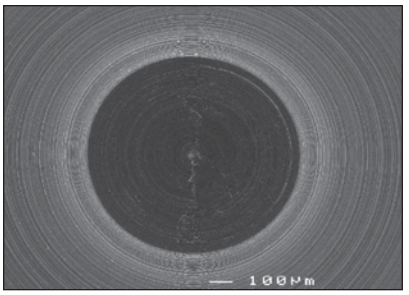


Fig 2c An SEM view of an implant well free of bacteria.

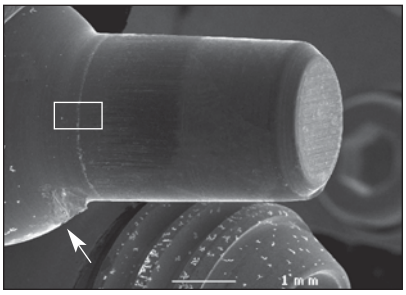
on agar plates (TSBY agar plates, Northeast Laboratories, Watersville, ME) and incubated in an anaerobic chamber for an additional 5 days. The same procedure was repeated at 48 and 72 hours.

RESULTS

Phase 1: Outside-in Experiment

The assembled implant-abutment units were tapped into place and examined by SEM (Fig 1a) before being immersed in the bacterial solution. Overall, the assembled units generally appeared clean and free of debris. The implant-abutment interface was examined at high magnification (525) (Fig 1b). A small gap was observed between the implant body and the abutment post. This is explained by the presence of a 120-µm-wide chamfer present in the coronal portion of the implant (Fig 2a) that creates a "crevice" when assembled with the abutment post. The bottom of that crevice as seen by SEM is less than 0.5 µm (Fig 1c) and does not allow for any bacterial invasion.

After assembly, the 10 units were incubated in a bacterial culture broth. The implant was clamped into a vise, and forceps were used to remove the abutment. The separate components were then examined by SEM to address the extent of bacterial penetration. As indicated in Fig 1c, the gap between implant and abutment was too small for bacteria to penetrate. The bacteria were only able to adhere to and colonize the coronal chamfer ("crevice") of the implant and the external surfaces of the implant under these experimental conditions. There was no evidence of bacterial presence in the implant well, as can be seen in the micrographs obtained (Figs 2a to 2c). This was true for all 10 tested samples. The bacterial presence seemed to stop at approximately 200 µm from the implant-abutment junction (Figs 3a and 3b). Interestingly, the "real" implant-abutment junction apparently providing the seal created by the locking taper design is located on the internal wall, approximately 200 µm below the edge of the coronal chamfer (ie, the bottom of the crevice); this area was a bacteria-free zone. The 1.5-degree tapered post of the abutment locks into the implant with friction. It is the metal-to-metal cold welding of the post against the implant wall that creates the impenetrable seal.[11] To test the veracity of this hypothesis, 3 different bacterial sizes were used. The microorganisms were divided into small (*A actinomycetemcomitans*), medium (*S oralis*), and medium-large sizes (*F nucleatum*). *A actinomycetemcomitans* is a facultatively



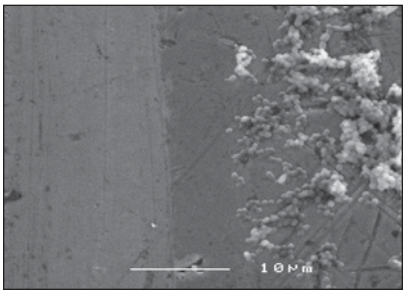


Fig 3a *(Left)* The stalk portion of the abutment after separation from the implant. The arrow points to the mark left by the forceps used to separate the unit. The rectangle indicates the area shown in Fig 3b.

Fig 3b *(Right)* An area of the abutment presumably not covered by the implant at a high magnification. Note the straight margin that separates the bacteria from the bacteria-free zone.

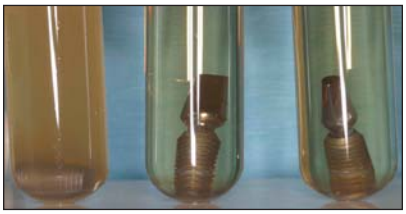


Fig 4a *(Left)* The left glass tube shows disassembled implant abutment with inoculant in nutrient broth (positive control). Notice cloudiness of broth as a sign of bacterial growth. The middle and right glass tube show assembled implant abutments with inoculant in clear nutrient broth (no bacterial growth).

Fig 4b *(Right)* An assembled sterile implant-abutment unit in nutrient broth. The broth is clear and shows no evidence of bacterial contamination (negative control).

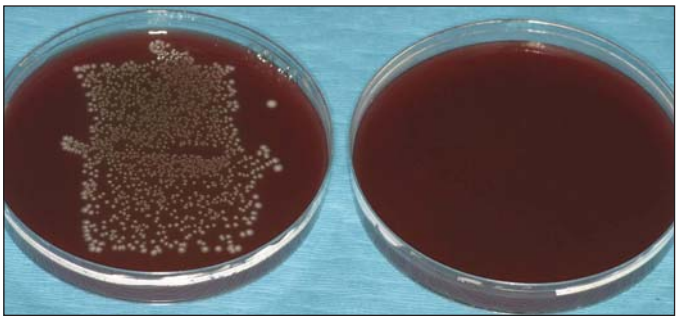


Fig 5 Culture plates after plating of 20 µL of broth from test and control glass tubes (72 hours). The left plate shows bacterial colonies (positive control), while the right plate shows no bacterial growth (test group).

anaerobic gram–negative microorganism whose size is approximately 0.4 × 1.0 µm[4]; *S oralis* is a facultatively anaerobic gram–positive microorganism whose size[15] is less than 2 µm in diameter[15]; and *F nucleatum* is a gram–negative anaerobic microorganism that ranges from 0.4 to 0.7 µm wide and from 3 to 10 µm long.[15] The SEM photographs show these microorganisms colonizing the implants and abutments. None of the 10 tested samples showed microbial presence past the implant-abutment junction. The inside wells of all implants as well as the bottoms of the abutment tapers appeared to be free from microorganisms (Fig 2c).

Phase 2: Inside-Out Experiment

In the second phase of the experiment, conventional microbial culturing techniques were used to test the resistance of the seal to bacterial seepage. The goal was to assess the capability of oral microorganisms to leave the implant well and seep into the environment. All of the 9 test samples (assembled implant-abutment units containing 0.1 µL of bacterial gel) showed a clear broth at the end of the 3 days of incubation. The 3 positive controls (unassembled implants and abutments with 0.1 µL of bacterial gel) showed cloudy broths, which confirmed the viability of the microorganisms throughout the experiment (Fig 4a). The last 3 samples, "noninfected" implant-abutment units (ie, the negative controls), were used to check for microbial cross-contamination during the experiment. These samples also had clear broths (Fig 4b). From each of the 15 glass culture tubes containing the test and control samples, 20 µL of broth were sampled at 24, 48, and 72 hours. These broth samples were individually plated on tryptic soy broth yeast (TSBY) plates and incubated in the anaerobic chamber at 37°C for 5 days. The 9 test samples and the 3 negative controls showed no evidence of bacterial presence at 72 hours as could be observed on the agar culture plates (Fig 5). The broth from the 3 positive controls exhibited heavy bacterial presence when plated (Fig 5).

DISCUSSION

Microbial and occlusal factors are generally regarded as the 2 main reasons for implant failure.[12,13] These failures can be further divided into early and late failures. Early implant failure has been associated with inappropriate surgical technique, premature loading, poor bone quality, and infection.[14] Late failures, which occur after implant restoration, have been associated with bacterial infection and biomechanical overload.[12] While the occlusal factor may be controlled with careful prosthetic planning, the microbial factor is more elusive. The presence of a microgap in some submerged implant systems has prompted researchers to speculate that the initial bone loss seen on radiographs after implant restoration is the result of bacterial presence at the implant-abutment interface.[16] In implants where a microgap is present, microbial leakage and persistent bacteria at this peri-implant location could lead to inflammation. This sustained activation of inflammatory cells has been shown to promote osteoclast formation and activation, which can result in alveolar bone loss.[3] Therefore, it becomes important to ensure minimal bacterial presence in or around the implant-abutment junction.

In the present study the bacterial seal provided by the locking taper design was tested. No attempt was made to compare the various implant-abutment connections available commercially. No bacteria were seen on any of the units below the chamfer of the implant. There was also a bacteria-free zone from where the chamfer ended to the site of the true cold weld on the abutment. Apparently the gap above the cold weld between the implant and the abutment was too narrow for bacterial penetration (Fig 1c), since most bacteria are more than 0.5 µm in diameter and the gap was measured to be less than 0.5 µm. Other studies have compared microbial leakage and marginal fit of the implant-abutment interface using different implant systems.[17–20] Tests were conducted in vivo as well as in vitro, and in all instances, the authors concluded that bacterial leakage occurred. They also stated that because of the physical space, fluids containing bacteria, bacterial byproducts, and nutrients could pass through the interface gap into the implant well, contributing to malodor and peri-implantitis.[18] The degree of leakage found was dependent on the closing torque; there was an inverse correlation between the degree of closing torque and the severity of the leakage. The higher the torque intensity, the less leakage was observed.[18] In some of the in vitro studies,[16,17] the seal of the implant-abutment connection was tested by exposing the microgap to the penetration of bacteria such as *Escherichia coli.*

Jansen and associates tested 13 implant-abutment connections (conical, flat, flat and slightly angulated, flat and conical at the inside, and flat with and without silicone washer).[17] They inoculated the tip of each abutment screw with 0.5 µL of *E coli* (ATCC 25922), assembled the systems, and bathed the units in a nutrient solution that covered the implant-abutment margins. Bacterial leakage was determined by looking at the cloudiness of the solution and was later confirmed by cultural methods. The authors reported that all implant systems tested presented leakage; however, for 1 system, leakage was reduced when a silicone washer device was used.[19] This finding was confirmed in vivo when the seal efficacy of the implant-abutment interface with and without an o-ring silicone washer device was evaluated. The authors concluded that the addition of a silicone ring washer would reduce bacterial contamination. In the present study, 3 different bacterial sizes were used: small (*A actinomycetemcomitans*), medium (*S oralis*), and medium-large (*F nucleatum).* It was understood that if a small microorganism such as *A actinomycetemcomitans* could not penetrate the seal, then any more sizable microorganisms, such as *E coli,* which is 1.1 to 1.5 µm wide and 2.0 to 6.0 µm long, would not. *A actinomycetemcomitans, S oralis,* and *F nucleatum* were also used because they can be found in the oral cavity.

External surface contamination by the inoculant during the "inside-out" phase of the experiment was circumvented by the use of a soft agar bacterial gel instead of a droplet of bacterial broth. The latter had been used initially in conjunction with exposure to ultraviolet light for sterilization with a certain degree of unpredictability and was, therefore, abandoned. The use of a gel proved to be highly predictable in controlling the "overflow" of the inoculant from the internal well of the implant to the external surface and did not require the use of an ultraviolet light.

The size of the locking-taper wide-body implants' internal well was determined by the manufacturer to be 1 mm^3. After several trials, a volume of 1 µL was determined to be the ideal amount of inoculant. Under these experimental conditions, and with the limitations related to a small sample size, there was no communication between the inside of the implant and the outside environment. These findings seem encouraging, as they point toward a system (the locking taper) that does not allow oral microorganisms to colonize the implant-abutment interface. This in turn may reduce the possibility of pert-implant inflammation and infection.

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