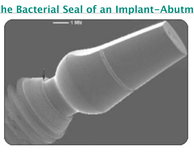
Literature & Publications

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Evaluating the Bacterial Seal of an Implant-Abutment Connection



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ABSTRACT

Background: In the submerged implant design, the quality of the implant-abutment connection is thought to play a critical role in preserving the alveolar crest bone levels from loss due to oral bacteria. Oral microorganisms that would colonize that structure are suspected to initiate inflammation and promote bone loss over time.

Methods: In order to test the efficacy of an implant-abutment seal to the bacterial challenge in vitro, a 2 phase experiment was devised. *Phase 1:* testing the ability of the seal to shield the implant well from outside bacteria. 10 Abutments were seated on 10 wide body implants and then immersed in culture tubes containing a bacterial mixture. They were incubated for 24 hours anaerobically at 37˚C. The abutments were then separated from the implant, carbon coated, and the inside well analyzed for bacterial presence using a scanning electron microscope. *Phase 2:* testing the ability of the seal to prevent bacterial seepage out from the implant well. 0.1µl of a bacterial mix was deposited at the apical end of abutment posts, which were then carefully inserted into implant wells. The implant/abutment units were immersed, individually, in culture tubes containing sterile broth and incubated anaerobically at 37˚C for 72 hrs. The broths were then plated on agar plates to check for bacterial growth.

Results: *Phase 1:* Scanning electron microscopy did not show any evidence of bacterial presence into the implant wells. *Phase 2:* There was no evidence of bacterial growth on the agar plates.

Conclusion: The tested implant abutment seal was hermetic to oral bacteria in vitro, with no microorganisms transiting from the "outside-in" or the "inside-out".

INTRODUCTION

Microbial accumulation around dental implants may lead to inflammation and result in a condition known as peri-implantitis. This condition is similar to the one affecting the natural teeth, and supporting structures, when exposed to the microbial challenge. The difference is that 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 by-products. If the seal is destroyed the epithelium migrates apically forming a periodontal pocket. The other natural line of defense absent from the endosseous implant structures is the periodontal ligament. Since there is no cementum or fibers inserted on the surface of an implant, and therefore no periodontal ligament and space, infection will spread directly into the osseous structures. Peri-implantitis, like periodontitis, if left untreated could result in bone loss and ultimately implant loss. This problem is further compounded by the implants system utilized. Two main dental implant designs have been on the market; submerged and non-submerged. The submerged design necessitates the placement of the coronal portion of the implant at or below the level of the alveolar crest. The non-submerged design requires placing the top of the implant above the level of the alveolar crest. In some of the submerged designs, 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. One of the prevailing hypothesis regarding this phenomenon is that oral bacteria colonize that area, during surgery or after placement of the abutment, hide and lead to infections over time. The implant well may act as a bacterial reservoir, from which microorganisms may seep in and out perpetuating the infective process that will lead to inflammation and ultimately bone loss.

The purpose of the present investigation was to test the efficacy of the Bicon (Boston, MA, USA) implant-abutment seal, resulting from the use of a locking taper design, to the bacterial challenge in vitro. The experiment was divided in two phases. The first phase would test the ability of the seal to shield the internal well from the outside bacteria (outside-in experiment), whereas the second phase would test the ability of the seal to prevent bacteria present in the implant well to seep out, into the outside environment (inside-out experiment).

MATERIALS & 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 Bicon implants (5x11mm Uncoated Implant 3.0mm well), and 10 abutments (5x6.5mm 0˚ Abutment 3.0mm post) were used. All experiments were carried out in a sterile environment, under a cell culture hood. The abutments were seated on the implant bodies according to the guidelines given by the manufacturer. The implant abutment units (IAU) 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. The 10 IAUs were incubated for 24 hours in an anaerobic chamber at 37˚C. After that time each glass culture tube was removed from the anaerobic chamber, the bacterial broth discarded, the IAUs washed twice in sterile PBS and fixed with 4% formalin overnight and prepared for scanning electron microscopy (SEM) viewing. At that point the abutments were separated from the implant bodies and the inside of the well was analyzed for bacterial presence by SEM.

In the second phase, the ability of the seal to prevent bacterial seepage out from the implant well was tested. This experiment was repeated 3 times, in order to assess reproducibility. Wide body Bicon implants (5x11mm Uncoated implant 3.0mm well) and abutments (5x6.5mm Abutment 3.0mm post) were used again. A bacterial mix of 2% soft agar was prepared and kept in a liquid form at 45˚C in a water bath; 1µl of Trypan blue was added to the mix for coloring purposes, and 0.1 µl of the 2% soft 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 and was used as positive control. Another abutment post was left bacteria free and inserted into an implant well to serve as a negative control. All the tested abutments were seated very carefully, on the implants according to the manufacturer's guidelines as soon as the soft agar droplet had solidified. The IAUs were immersed, individually, in 5 glass culture tubes containing 10 ml of sterile Heart-Brain infusion broth and incubated into an anaerobic chamber at 37˚C for 72 h. At the end of the first 24 h, 20 µl of the broth from the glass culture tubes containing the implants were pipeted out and individually plated on agar plates and incubated in an anaerobic chamber for 5 days. The same procedure was repeated at 48 and 72 h.

RESULTS

Phase 1: Outside-in experiment. The IAUs were tapped in place and examined in the SEM. The overall appearance of the assembled unit was generally clean and free of debris. The abutment/implant interface was examined at high magnification **(Fig. 1A)** and showed the presence of a small gap 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. That "crevice" as seen by SEM is less then 0.5 µm **(Fig. 1B)** and does not allow any bacterial invasion. Ten assembled units were incubated in bacterial culture broth. After removing the abutment, the separate components were examined by SEM to determine where the penetration of bacteria stopped. In **Fig. 1B**, the gap between implant and abutment is too small for bacteria to penetrate and they can only adhere to and colonize the coronal chamfer ("crevice") of the implant as well as all external surfaces under experimental conditions. There was no evidence of bacterial presence into the implant well **(Figs. 2A, B, C)**. This was true for all 10 tested samples. All bacterial presence seem to stop at a certain distance of the implant-abutment junction **(Figs. 3A, 3B)**. It is of interest to notice that the "real" implant abutment junction providing the seal due to the locking taper design is up to 200 µm below the bottom of the crevice, this area is a bacteria free zone. This seal when in place seems to perfectly unify the implant and abutment. The 1.5 degree tapered post of the abutment, locks into the implant with friction. The metal to metal cold welding of the post against the implant wall creates the impenetrable seal. To test this hypothesis three bacterial sizes were used: small (A. actinomycetemcomitan 0.4 ± 0.1 x 1.0 ± 0.4 µm), medium (S. oralis, 2 µm) and medium-large sizes (F. nucleatum, 0.4-0.7 x 3-10 µm). The SEM photographs show these bacteria colonizing the implants and abutments. None of the 10 tested samples show microbial presence passed the implant/ abutment junction. The inside well of all implants as well as the bottom of the abutments' taper appear to be free from microorganisms **(Fig. 2C)**.

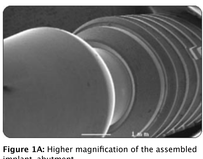


Figure 1A: Higher magnification of the assembled implant-abutment.

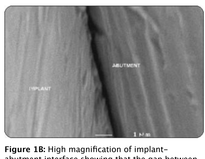


Figure 1B: High magnification of implant-abutment interface showing that the gap between the 2 components is less then 0.5 micron. The space is smaller than the diameter of bacteria.

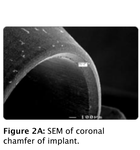


Figure 2A: SEM of coronal chamfer of implant.

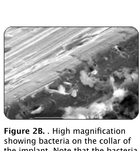


Figure 2B. . High magnification showing bacteria on the collar of the implant. Note that the bacteria do not penetrate the well region of the implant. No bacteria were seen along the walls or bottom of the well in any of the specimen.

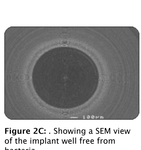


Figure 2C: . Showing a SEM view of the implant well free from bacteria.

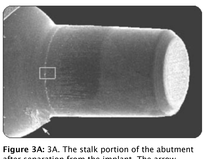


Figure 3A: 3A. The stalk portion of the abutment after separation from the implant. The arrow points to the mark left by the forceps used to separate the unit. The rectangle indicates the area shown in figure 3B.

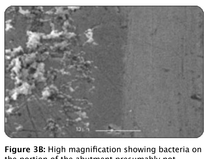


Figure 3B: High magnification showing bacteria on the portion of the abutment presumably not covered by the implant. Note the straight margin that separates the bacteria from the bacteria free zone.

Phase 2: Inside-Out experiment. In the second phase of the experiment, standard microbiology culturing techniques were used to test the resistance of the seal to bacterial seepage. The goal was to assess the capabilities for oral microorganisms to leave the implants' well and seep out into the environment. All of the 9 test samples (assembled IAUs containing 0.1µl of bacterial gel) showed a clear broth at the end of the 3 days of incubation in an anaerobic chamber at 37˚C. The 3 positive controls (unassembled implant/abutments, with 0.1µl of bacterial gel) showed cloudy broths, which confirmed the viability of the microorganisms **(Fig. 4)**. The last 3 samples ("non-infected" IAUs) that were used to check for microbial cross-contamination (negative controls) during the experiment had a clear broth. From each of the 15 glass culture tubes containing the tests and controls, 20 µl of broth were sampled at 24, 48 and 72 h, and individually plated on TSBY agar. The plates were incubated in an anaerobic chamber at 37˚C for 5 days. The 9 test samples and the 3 negative controls showed no evidence of bacterial presence at 72h as could be seen when looking at the agar culture plates. On the other hand the broth from the 3 positive controls exhibited heavy bacterial presence when plated **(Fig. 5)**.

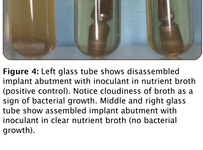


Figure 4: Left glass tube shows disassembled implant abutment with inoculant in nutrient broth (positive control). Notice cloudiness of broth as a sign of bacterial growth. Middle and right glass tube show assembled implant abutment with inoculant in clear nutrient broth (no bacterial growth).

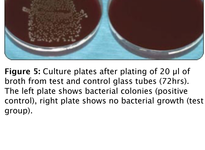


Figure 5: Culture plates after plating of 20 µl of broth from test and control glass tubes (72hrs). The left plate shows bacterial colonies (positive control), right plate shows no bacterial growth (test group).

CONCLUSIONS

Under these experimental conditions 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 peri-implant inflammation and infection.