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To Evaluate and Compare the Antibacterial Efficacy of Two Sealing Agents at the Implant - Abutment Interface - An In-Vitro Study

Dr. Bhavyashree R[1], Dr. Jnanadev K R[2]

[1] Master of Dental Surgery, Department of Prosthodontics, Vokkaligarasangha Dental College and Hospital, Bengaluru, India
Email: *bhavz.bhavya[at]gmail.com*

[2]Professor, Department of Prosthodontics, Vokkaligarasangha Dental College and Hospital, Bengaluru, India
Email: *drjnanadevkr[at]yahoo.com*

Abstract: ***Background: Colonization of bacteria along the implant-abutment interface micro gap may establish a bacterial reservoir, which can result in soft tissue inflammation, thereby increasing the risk of peri-implantitis and marginal bone loss. Micro gaps along the implant-abutment interface can enlarge under loading conditions and create a" pump like effect" around the bone leading to large amounts of bacteria into the implant. Sealing agents have been reported to reduce the bacterial colonization by filling the micro gaps. Objective: The objective of the study is to compare the antibacterial efficacy of two sealing agents at the implant-abutment interface. Materials and method: A total of 10 implants and abutments were selected for the study and divided into two groups. Sealing agents were applied at the IAI of implant groups. Abutments were connected to the implants, and bacteria were inoculated. Bacterial percolation was evaluated by culturing the specimen from the internal aspect of implants on agar plates after incubation. Efficacy was evaluated by counting the colonies (colony forming units) on the agar plates. Results: Mean of colony forming units for CHX gel was calculated to be 239.80 ± 72.30 cfu, for Loctite 243 was 92 ± 124.09 cfu. In the present study, least value of colony-forming units of bacteria was exhibited by IAIs sealed with Loctite 243. Conclusion: Application of CHX varnish and antibacterial sealing gel can reduce the bacterial leakage through IAI. The application of Loctite 243 provides better sealing than CHX gel. The difference was also statistically significant.***

Keywords: chlorhexidine gel, implant-abutment interface, microleakage, Loctite 243

1. Introduction

Implantology is deemed as the most successful treatment modality for the replacement of missing teeth. There are mainly two established protocols for implant placement, the two-stage and the single-stage procedures. The traditional Branemark's protocol employs a two-stage procedure wherein the fixture is allowed to integrate for a specified duration following which the prosthetic connection involving the abutment and the restoration is carried out.[1]

The connection geometry between the implant and abutment is basically an external or internal connection. To overcome the mechanical problems of external hexagon connection, internal hexagon connection gained importance. Its reduced vertical platform distributes the stress within the long axis of implant, resisting the joint opening.[2]

The implant-abutment interface (IAI) though precisely machined results in a microgap which may establish a bacterial reservoir, which can result in soft tissue inflammation, which subsequently increases the risk of peri-implantitis and marginal bone loss.[3,4] The microgap is found to be 1–49 µm in width, and is wide enough for periodontopathogenic bacteria to pass through.[5] These microgaps have the tendency to increase in size during loading and create a "pump like effect" around the peri-implant bone which can lead bacterial ingress into the implant.[6]

Various studies have been conducted to reduce bacterial leakage in internal connections by using disinfectants and sealants under unloaded conditions.Several materials have been advocated to seal the implant – abutment interface, such as, adhesive, a silicone O – ring, a silicon hermetic washer, chlorhexidine-thymol varnish and 2% chlorhexidine solution.

Gel or varnish forms of chlorhexidine have known to reduce bacterial growth in the internal threads of the implant.[7] Chlorhexidine (CHX) has been considered as the gold standard oral antiseptic for plaque control microbial infection, or superinfection. A recent study also revealed that rinsing with CHX in saline solutionwas efficient to decontaminate implant surfaces following periimplantitis.[8] The CHX in the form of gel(Chlor X gel) is used a disinfectant in the oral cavity and it interacts with the lipophilic cell membrane of bacteria , causing osmotic imbalance and leads to cell death.[9]

The present study also uses Loctite 243 is a dimethacrilate ester combined with maleic acid, used in automotive industry for preventing screw loosenings.

To the best of our knowledge, there are no recently published studies that investigate the abilities of bacteria to penetrate through these new microgap sealants. This study could provide a clinical benefit by providing evidence for use of microgap sealants and if a particular agent shows a beneficial effect, then the same study can be conducted under dynamic loading.

The aim of this study is to evaluate, under static conditions, compare the antibacterial efficacy of two sealing agents at the implant-abutment interface.

2. Materials and Method

In this study, 10 titanium dental implants and 10 titanium abutments were used and divided into two main groups. JD dental implants of 4.0 mm diameter, with an internal connection to the prosthetic abutment were used.

There were 32 test groups formed (with 5 implants in each) for different sealing materials as follows:
1) Chlor X gel (2% chlorhexidine gel)
2) Loctite 243

Preparation of specimens
Before the study, sterilization of the implants and components was accomplished by Gamma irradiation. Additional instruments and materials were autoclaved at 121°C for 20 min. All further steps were carried out in a biosafety cabinet for avoiding contamination. A total of 10 implants and abutments were used in the study to check the efficacy of sealing at the IAI. Implants and abutments were allocated into two groups, of 5 implants and abutments. In the first group, CHX gel was applied at the IAI and served as the control.

The second group comprises implants and abutments that were sealed with the sealing gel, Loctite 243 at the IAI. Application of antibacterial sealing gel was accomplished with provided applicator. Two complete loops of antibacterial sealing gel applied at internal hex junction of implant and abutment before insertion. The implants and abutments of two groups were assembled using a torque wrench at a torque of 20Ncm by stabilizing them in a clamp [Figure 1]. Abutment screws were retightened 10 min after the initial torque application to reduce the settling effect.

Figure 1: Implant stabilization using c-clamp

Microbiologic sampling and examination
Two assembled implants (randomly taken) from each group were incubated in peptone water (one dismantled and other without dismantling). The incubation was carried out for 24 h and absence of turbidity of peptone water ensured complete sterilization of the implant-abutment assembly. The remaining implant-abutment assemblies from each group were then immersed in the test tube containing peptone water, inoculated with Escherichia coli, which is small enough for passage through the microgap, and the dimensions are similar to periodontal pathogens. [Figure 2] Inoculation of E. coli was done with inoculating loop utilizing aseptic technique. Afterward, assemblies in inoculated peptone water were incubated in an incubator for 30 min at 37°C.

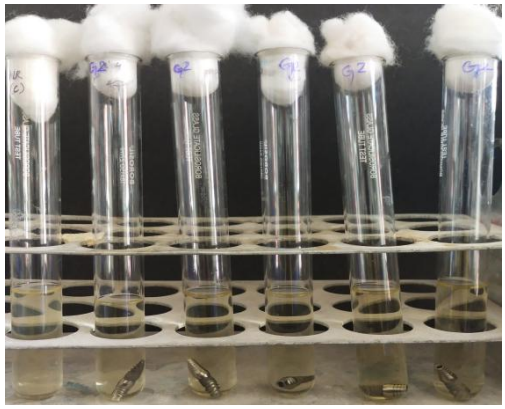


Figure 2: Samples immersed in peptone water inoculated with Escherichia coli.

After incubation, the assemblies were removed from the test tubes and surfaces were washed with 1% freshly prepared hypochlorite solution for 5 min and then with saline solution for 5 min under sterile conditions. To check the efficiency of the surface decontamination procedure, all implants were incubated in sterile peptone water for 24 h and ensured the absence of turbidity of peptone water. Five implants from each group were then dismantled and again placed in sterilized peptone water and well shaken to ensure adequate contact of the broth with the interior of the implants [Fig 3]. Then, 1 ml from each test tube was inoculated on pre-prepared sterileagar plates by drop method using syringe and needle of uniform size and incubated for 24 hours.

Figure 3: Implants are dismantled and then immersed in sterilized peptone water.

Table 1: Depicting the growth of E coli on agar plates.

Groups	N	Minimum	Maximum	Mean	S.D	Mean diff	P value
Group 1	5	139	296	239.80	72.30	147.80	0.05*
Group 2	5	0	294	92.00	124.09		

3. Statistical Analysis

To perform the statistical analysis SPSS (Statistical Package For Social Sciences) version 20. [IBM SPASS statistics (IBM corp. Armonk, NY, USA released 2011)] was used. Inferential statistics like Independent sample t test (based on data distribution) was applied to check the statistical difference of quantitative variables (CFU) between the groups.

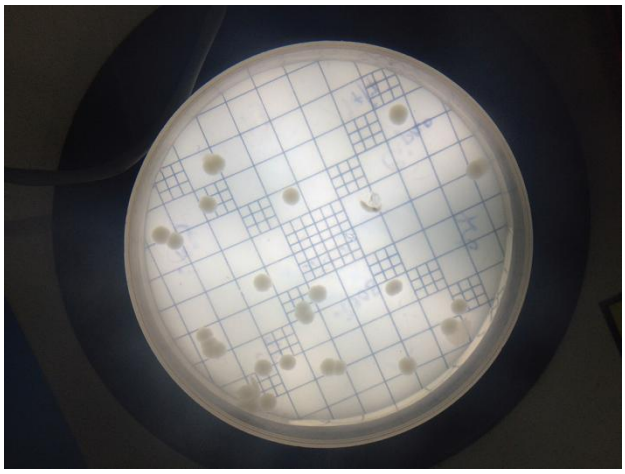


Figure 4: Counting of colony forming units on agar plates using a digital colony counter.

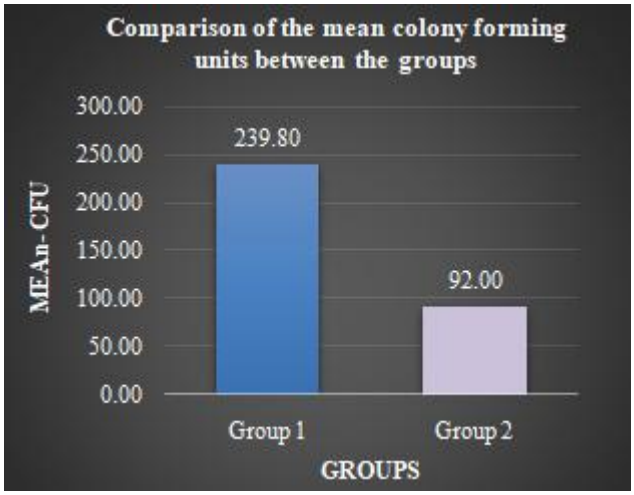
4. Results

Sealing ability of CHX gel and Loctite 243 was tested by assessing the growth of E. coli on agar plates, and values were obtained as colony-forming units [Table 1]. Mean CFU was higher in Group 1- 239.80 ± 72.30 as compared to Group 2- 92 ± 124.09. Independent sample t test was applied to compare the mean CFU between the groups. Independent sample t test showed statistically significant difference between the groups (p=0.05) with a mean difference of 147.80.

5. Discussion

Rehabilitation of partially and completely edentulous arches using dental implants has become a routine and plays a vital role in the general wellbeing of the patients.[10]The successful service of an implant restoration is mainly dependent on the biological and mechanical factors.

The most used implant system is the two-stage implant system with the implant and abutment. Though are different implant connections, internal hex is the most used. Hence, the implant – abutment interface is considered a crucial zone, as it establishes the peri implant health. A weak connection at the interface has an impact on the microgap and the microbial colonization; hence a good connection between the parts is essential. The presence of microgap have shown to produce mechanical and biological complication such as peri-implantitis, screw loosening, wear of components, bone loss, and micro-pump effect which has been validated in some of the studies.[1,11]The tissues which are adjacent to the implant abutment interface revealed a significant infiltration of inflammatory substances.



Graph 1: The least value of colony forming units of bacteria with Loctite 243.

Implant design should be manufactured such that there is an intimate fit between the implant and the abutment component at the interface. But several studies have reported the presence of minimal microgap and misfit values for pre-machined abutments. The bacterial infilteration is due to the misfit between the two components of the implant systems.[12] Martin-Gili et al examined the leakage of fluids and microgap in both internal and external connections of screw-type abutments before and after occlusal loading and concluded that by increasing the number of mechanical cycles, the gap would increase due to the deformation of the titanium alloy.

To reduce the implant-abutment gap, several investigators have examined the effect of mechanical agents as well as disinfectants at the IAI. The mechanical agents used include silicone, O ring, and grease and disinfectants include thymol and CHX.[13]

The current study evaluated the antibacterial efficacy of two sealing agents at the implant-abutment interface. Results showed the presence of bacterial colonies on agar plates of both the groups. This indicates that the complete seal at the IAI is not possible with either of the sealing agents whereas the number of colonies on agar plates of implants with Loctite 243 was significantly less than 2% CHX gel and the difference was statistically significant.

This study highlights that there is some amount of microleakage at the IAI which could be due to the lack of complete wall-to-wall adaptation between implant and abutment. Though, microbial contamination is present in all implant-abutment connections. Among them, internal hex showed less leakage than external hex.[14]

Therefore, this study evaluated microleakage of the internal hexagon connection with titanium abutments, which has lesser mechanical complications. According to the study conducted by Turkyilmaz, there is correlation between the degree of leakage and the closing torque.[15] hence, the abutments were tightened to a torque of 20 Ncm which is also the amount recommended.

To avoid the effect of settling, abutment screws were retightened after 10 min of initial tightening. Sealingability of CHX gel at the IAI was evaluated in this study by comparing with Loctite 243, a screw sealer which has shown marked reduction of microleakage into the implant in previous studies. CHX in the form of gel was used, as they are easy to apply and don't cause discoloration of the adjacent teeth. Results of the present study have shown that CHX gel can be used as an antibacterial sealing gel in preventing percolation through IAI. But better results were shown by Loctite 243, an industrial screw sealer. It is in gel form and could effectively reduce the bacterial infilteration at the IAI. Further studies need to be conducted to determine the toxicity of this material and modifications can be made to this composition. Bacteria used in the study to evaluate efficacy of sealing agents were E. coli, which have dimensions like periodontopathogenic bacteria. Thus, sealing against the periodontopathogenic bacteria can be evaluated indirectly.

The major limitation of the study was that it was conducted in vitro, therefore, the presence of saliva and solubility of sealants could not be assessed. Further evaluation is needed to find the longevity of the sealants and the relation between the quantity of the sealant applied and its sealing ability. Efficacy in preventing percolation was assessed during unloaded conditions; therefore, the effect of masticatory load on microgap at the IAI and bacterial leakage could not be assessed in this study.

Regardless of the studies conducted, clinical relevance of sealing the microgap at the IAI with sealing agents in maintaining inflammation-free marginal mucosa and in achieving clinically successful treatment of peri-implantitis has yet to be determined.

6. Conclusion

Within the limitations of the study, it was concluded that:

- Application of chlorhexidine gel can minimally reduce the bacterial leakage through implant abutment junction, whereas complete seal was not attained with either of the materials.
- The application of Dimethacrylate acrylic (Loctite 243) provides better sealing than Chlorhexidine gel. The difference was also statistically significant.

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