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TO ASSESS THE ADEQUACY OF ANTIMICROBIAL SEALING GEL AND O-RING AT THE IMPLANT-ABUTMENT INTERFACE TO PREVENT THE MICROLEAKAGE - AN IN VITRO STUDY.

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Abstract:

Peri-implantitis is an inflammatory process which occurs around an osseointegrated implant, resulting in pocket formation and bone loss. Most implant system consist of two pieces; an implant fixture and an abutment, the microgap which exist between them is referred as implant-abutment interface. The aim of this study was to evaluate the adequacy of sealing materials on microleakage at implant-abutment interface.

Key words: Microleakage, Implant-abutment interface, periimplantitis

Introduction

Peri-implantitis is an inflammatory process which occurs around an osseointegrated implant, resulting in pocket formation and bone loss[1]. Most implant system consist of two pieces; an implant fixture and an abutment, the microgap which exist between them is referred as implant-abutment interface[2]. This gap at implant-abutment interface offer shelter to the accumulated biofilm which contain bacteria leading to bacterial colonization and peri-implantitis[3].

Microleakage has been considered to occur in both directions from an external source to the inner area of an implant and vice versa. The gap between the implant and abutment facilitates the microleakage[4]. During function, bending forces act on the implant component which losses the screw joint, thereby increasing the gap. It also produces the pumping effect to transport the bacteria, allowing for microleakage[5]. Various measures have

been used to prevent microleakage at implant-abutment interface using sealing material, shape memory alloy and different connection geometries[4]. The aim of this study was to evaluate the adequacy of sealing materials on microleakage at implant-abutment interface.

Materials and Methods

Experimental Groups

In this study, 120 titanium dental implant, standard, internal hexagon, 3.5mm diameter, and 10mm length were utilized to assess the adequacy of different sealing materials at IAI (implant-abutment interface). The samples were divided into three groups containing forty samples of each group:

Group I: Titanium dental implant with internal hexagon were connected with straight, titanium abutment 3mm with a torque of 25Ncm according to manufacturer instructions, without the application of sealing material at IAI,

Group II: Titanium dental implant with internal hexagon were connected with straight, titanium abutment 3mm with a torque of 25Ncm according


FIG 1(a)

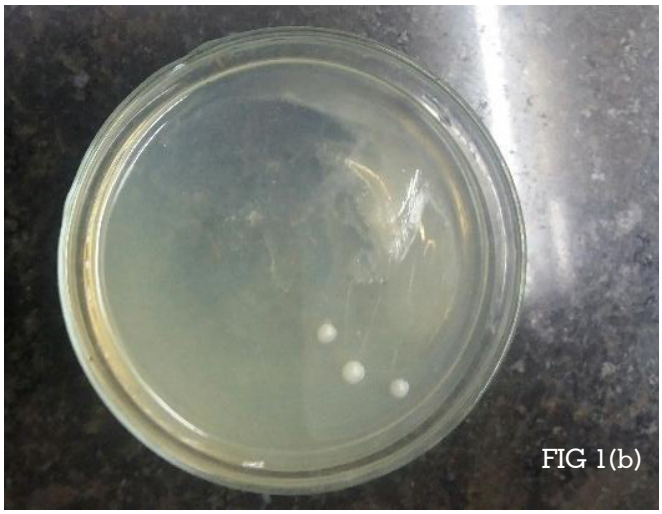
FIG 1(b)

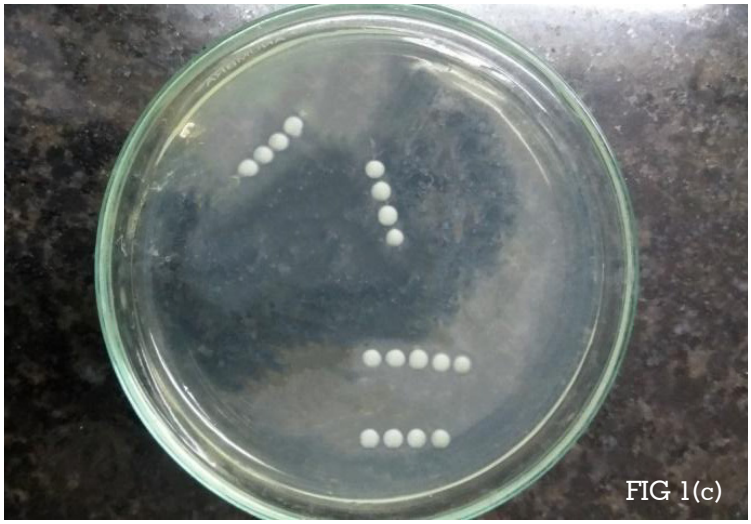
FIG 1(c)

Figure 1 (a), (b), (c): Nutrient agar plates indicating the resultant colonies in Group I, II and III

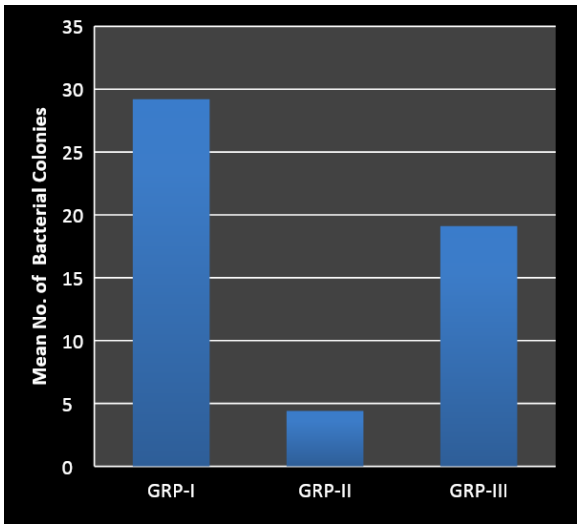

Figure 2: Graphical representation of mean number of bacterial colonies in three groups

to manufacturer instructions with the application of antimicrobial sealing gel (Gapseal) at IAI.

Group III: Titanium dental implant with internal hexagon were connected with straight, titanium abutment 3mm with a torque of 25Ncm according to manufacturer instructions with the application of O-ring at IAI.

Preparation of the samples

Under sterile conditions, dental implants and abutments were removed from commercial packaging. These samples were cultured for another 24 hours in a sterile nutrient solution, to ensure complete sterilization. The sample that fulfilled the criteria was selected for the study.

Revival of staphylococcus aureus from freeze-dried culture powder

Freeze-dried culture powder of staphylococcus aureus (MTCC 3160) was revived by incubating the culture powder in nutrient broth for 24 hours under a sterile environment. 50µl of this suspension were transferred on Tryptic Soy Agar plate using a sterile loop. The bacteria were streaked across the plate from left to right and top to bottom and the plates were incubated for 12 to 16 hours at 37^{0}C to obtain isolated colonies of staphylococcus aureus.

Preparation of inoculum

The cultures of Staphylococcus aureus (MTCC 3160) onto Tryptic Soy agar were used to prepare a bacterial suspension of about 1 x 108 colony forming units (CFU/ml) in nutrient broth by adjusting turbidity to 0.5

Experimental procedure

The experimental procedure was carried out under aseptic conditions. The working area was disinfected with 70% ethanol before starting the procedure. The aseptic conditions were maintained by following routine measures such as using sterile gloves, sterile equipment, eye protection, Bunsen burner, and laminar flow cabinet. The implant and abutment from each group were attached and immersed into 3ml of bacterial suspension inoculated with Staphylococcus aureus that covered the IAI. These samples were further incubated at 37^{0}C for 24 hours. Later, the assemblies were removed from the bacterial suspension and the external surface is decontaminated with a 2% solution of sodium hypochlorite for 30 minutes. The residual sodium hypochlorite was removed with normal saline.

To check the adequacy of the external surface decontamination strategy, the assemblies were

TABLE 1 Kruskal-Wallis Rank Sum Test

(for Overall Testing of Equality of the Three Groups):

Test Statistic	Degrees of Freedom	p-Value	Remark
105.762***	2	< 0.0001	Highly Significant

TABLE 2 Number of Bacterial Colonies in Each Group

***: Significance at 0.1 % probability level

Group	Sample Size	Mean ± SD	SEm	CV (%)	Confidence Interval	
					95%	99%
I	40	29.20 ± 2.79	0.44	9.57	28.31 - 30.09	28.00 – 30.40
II	40	4.40 ± 1.58	0.25	35.95	3.89 – 4.91	3.72 - 5.08
III	40	19.10 ± 3.64	0.57	19.04	17.94 – 20.26	17.54 - 20.66

additionally placed in sterile nutrient solution and incubated for 24 hours at 37^{0}C.

After decontamination, the implant and abutment assemblies were disassembled and submerged into sterile nutrient solution in the test tubes. The test tubes were agitated so that nutrient solution sufficiently contacts the inner surface of the implant and abutment assemblies, allowing the bacteria to flow into the solution. Nutrient agar plates were divided into four quarters and were inoculated with 100μl of nutrient solution (containing staphylococcus aureus). The nutrient agar plates were then incubated for 24 hours at 37^{0}C. The resulting colonies were identified and quantified (FIGURE 1).

Statistical Analysis

Statistical analysis was performed using customized R programming software. The data obtained was subjected to Kruskal Wallis analysis of variance (Table 1). The level of significance was set at $p \leq 0.05$. Statistically, a significant difference was found between the three groups (P-value <0.001). The Mann-Whitney U-test was applied to evaluate differences between the three groups with respect to the mean number of bacterial colonies.

Results

Microleakage occurs in all the groups with or without sealing material. In group, I maximum bacterial count was observed, ranged from 25 to 33 (mean, 29.20; standard deviation (SD) +- 2.79). In group III average bacterial count ranged from 13 to 25 (mean 19.10; SD,+-3.64). However, group II exhibit the maximum resistance to microleakage, observing the least bacterial count, ranged from 2 to 6 (mean, 4.40; SD+-1.58) (TABLE 2).

There existed highly significant differences among the three groups understudy for the average number of bacterial colonies (FIGURE 2). Consequently, it becomes imperative to make post-hoc comparisons among their performance, using the Mann-Whitney U test. Member groups in all the three paired comparisons showed highly significant differences (each at 0.1 percent probability level) concerning the mean number of bacterial colonies (TABLE 3).On average, the number of bacterial colonies was the minimum (= 4.4) in Group-II, followed by that (= 19.1) in Group-III and the maximum (= 29.2) in Group-I.

Discussion

The present study was conducted to assess the adequacy of sealing material at the implant-abutment interface to prevent microleakage. The results showed that bacterial infiltration of staphylococcus aureus occurs in all three groups, however, the least amount of bacterial infiltration was observed with Gapseal followed by O-ring. Furthermore, the study was conducted under static conditions, which revealed that the presence of sealing material help to reduce the microleakage, but a reliable seal is not obtained at the interface. The presence of gapseal helps to reduce the leakage by its antimicrobial properties or its sealing ability. Gapseal is a highly viscous silicone material, which allows it efficiently seal the interstitial spaces, maintaining a complete seal. It also has hydrophobic properties, which ensure high retention and prevent it from being washed away[6]. Several studies have shown the same results[7-9].

Paolantonia et al. found that filling the internal cavity with 1% chlorhexidine gel; significantly reduce bacterial colonization over a period of 6 months[7]. The sealing ability of chlorhexidine varnish and silicone sealant was tested by Duarte et al. In vitro, both materials could prevent some bacterial leakage for a period of 45 to 63 days[8]. Nayak et al. recommended the use of gapseal to enhance the sealing capability, the viscous nature of the gel allows it to flow easily throughout the interfaces[2]. Zarbakhsh et al. reported that gapseal reduces the microgap and prevents the

microleakage under cyclic loading[9].

In group III microleakage occurs because the O-ring prevents the abutment from complete seating, resulting in increased microleakage at the implant-abutment interface. Furthermore, rubber can also deteriorate over time, leading to increase leakage. Without sealing material microleakage occurs in group I, which was most likely owing to the lack of complete wall adaptation between the implant and abutment assembly[2].

Several investigations have revealed bacterial leakage along with the implant-abutment interface of systems with varied connection arrangements[10]. Quirynen et al. found that microbe infiltration occurs into the internal part of the implant which could be a result of abutment installation or unscrewing[5]. Jansen et al. stated that microleakage occurs at the implant-abutment interface, even if the size of microgap was less than 10μm [11].

The Rationale to use colonies of Staphylococcus aureus for the present investigation was the biological role that, this aerobic bacterium has, during the initial phase of biofilm development on the titanium implant surface. It is an initial colonizer with a strong affinity to attach to other pathogenic bacteria as well as to any type of titanium surface[12].

Limitation

Cyclic loading of the implant may also contribute to microleakage. One limitation of the present in vitro study is that cyclic loading was not implemented to mimic masticatory stress. Steinebrunner et al. investigated bacterial leakage at the implant-abutment interface, following the use of dynamic loading, which significantly improved in various implant systems[13]. According to Nascimento et al. human saliva can penetrate the implant-abutment interface under loaded and unloaded conditions[14]. Thus, it's vital to substantiate or contrast the current study findings with different loading conditions.

Conclusion

Considering the limits of the present in-vitro study it was concluded that Gapseal was effective in preventing microbial leakage at implant-abutment interface followed by O- ring. Further evaluation is needed about the longevity of the antibacterial sealing gel.

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