



**EVALUATION OF BACTERIAL PENETRATION THROUGH THE
IMPLANT-ABUTMENT INTERFACE FOLLOWING PLACEMENT OF
IMPLANT SUPPORTED CEMENT RETAINED AND SCREW
RETAINED METAL CROWNS: AN IN-VITRO STUDY**

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ABSTRACT

Justification: Peri-implantitis is a cause for failure in any type of implant restoration; microgap between implant and abutment is a contributing factor. The present study was designed to identify microbial penetration at implant-abutment junction in cement retained and screw retained implant restorations.

Objectives: To evaluate and compare the bacterial penetration through implant-abutment interface between screw retained and cement retained restorations and penetration rate with three different types of bacteria.

Material and methods: Bacterial penetration between implant-abutment interface in cement retained and screw retained restoration with three types of bacteria (*Staph aureus*, *A.actinomycete mcomitans* and *P.gingivalis*) was studied. A total of 20 implants (n=20) were taken.10 implants (n=10) received cement retained restorations and another 10 implants (n=10) received screw retained restorations. All the implants assembly were sterilized using autoclave, the bacterial penetration assay was performed

using assay method. The broth that showed cloudiness was noted and the optical density values are recorded using micro plate reader. After assay method the crowns were removed using automated crown remover and the abutments were removed using hex drive from all implant assembly. A plain broth was added in the inner part of implants and the samples were collected in micro centrifuge tube and values are calculated using colony forming unit method. The samples were removed and dried to confirm the bacterial presence in both restoration using SEM analysis. Mann-Whitney test was used for statistical analysis.

Results: Descriptive statistics for broth turbidity values reveal that mean values for screw retained group was 0.131(SD±0.132) and cement retained group was 0.3 (SD±0.016). Mann Whitney test mean ranking showed $p=0.015$ ($P<0.05$). When the Colony forming unit values were compared for the microbial species individually, Mann Whitney test mean ranking in screw retained and cement retained restorations for *Staphylococcus aureus* were 9.80 and 11.20 respectively ($p=0.591$, $p>0.05$), for *Aggregatibacter actinomycetem comitans* were 9.90 and 11.10 ($p=0.644$, $p>0.05$) respectively and for *Porphyromonas gingivalis* were 8.50 and 12.50 respectively ($p=0.119$, $p>0.05$).

Conclusions: Screw retained restorations resulted in lesser bacterial penetration as compared to the cement retained restoration and there is no significant difference in penetration rate of bacteria in both the restorations.

Keywords: Bacterial penetration, Implant, Screw retained, Cement retained, Microorganisms

INTRODUCTION

Implant restorations have become one of the most successful rehabilitation techniques among various replacement methods available. Over the past thirty years the long term success rate of implants has been more than 90% and the predictability of osseointegrated oral implants has been well documented. In spite of the excellent success rates in osseointegrated implant rehabilitation, failures have been reported in the literature particularly related to mechanical and microbiological factors [1]. Earlier, mechanical failures were considered to be the main cause for implant loss. Presently, failures of dental implants are understood

better and due consideration is given to bacterial infection of the peri-implant tissues as a cause for loss of implants [2]. Bacterial penetration into the implant body is nearly unavoidable, when prosthetic abutment is attached to the subgingival implant. The submerged implant is surrounded by peri-implant tissue. Peri-implant diseases caused by bacteria are of two types peri-implant mucositis and peri-implantitis [1,2].

The common causative organisms associated with peri-implantitis are *Porphyromonas gingivalis*, *Prevotella intermedia*, *Aggregatibacter actinomycetem comitans*, *Staphylococcus*

aureus, *Streptococcus sanguinis* [3], etc. The factors favouring bacterial colonization are poor oral hygiene, implant design, implant topography and pre-existent periodontal disease [4]. Periodontitis close to implants and pathogenic bacteria around the peri-implant tissues are considered the risk factors for implant failure [5]. The implant-abutment surfaces are machined, whereas the prosthetic structures are casted or milled [6]. Marginal discrepancy of restoration leads to micro leakage. The presence of a micro gap between implant and abutment with a possible contamination of the internal portion of the implants leads to crystal bone loss around dental implants [7]. There are few evidence based studies to understand the failure of implants due to peri-implantitis in cement retained and screw retained prosthesis. Hence the present in vitro study was designed to evaluate the bacterial penetration between implant-abutment interface following placement of cement retained and screw retained metal crowns and Penetration rate among three different types of bacteria namely, *Staphylococcus aureus*, *Aggregatibacter actinomycetem comitans*, *Porphyromonas gingivalis*.

MATERIAL AND METHODOLOGY:

The implant (Genesis, India) of dimension 3.75 x 11.5mm was embedded in an autopolymerizing resin (DPI cold cure, Mumbai) block made from stainless steel (SS) mould of inner dimension 1 x 1 x 1inch to render the desired dimension of 1inch x 1inch x 1inch block. The SS mould was stabilized using putty polyvinyl siloxane on a glass slab, then the autopolymerizing resin was mixed in a porcelain jar and poured into the SS mould and it was cured under pressure in pressure pot (Vertex BV Multi cure, Netherland) at 20 psi for 30 minutes. A dental surveyor (Bego, Germany) was used to position the implant perpendicular to the base. The implant platform [fig.1a] was set at 1mm above the resin level to allow the abutment connection.

After resin polymerization the abutments were connected to the implant stabilized in an acrylic base which was firmly held on a bench during abutment tightening. The abutment was torqued at 32N/cm as per manufacturer's specifications (Genesis Implants, India).

The fabrication of milled metal crown was done using CAD-CAM method. For the cementable and screw retained restorations, standard abutment with the mounted block were scanned with Imetric LM1 scanner. After scanning, upper first molar crown morphology was designed using EXOCAD

software and the designed crown is exported to the CAM (Hyperdent classic) software and the toolpath calculation was prepared. The NC file was shifted to the milling machine (Roboice RF3+, Istanbul, Turkey) for further processing. The crowns were fabricated with Cobalt-Chromium alloy and the crowns were finished with silicone rubber wheel. The final polishing is done with felt wheel and universal polishing paste.

The same procedures were repeated for the remaining implants and a total of 10 screw retained metal crowns and 10 cement retained metal crowns [fig1b] were fabricated. The cement retained crowns are cemented with type I Glass Ionomer Cement (GC Corporation, Tokyo, Japan) and screw retained crowns with hex drive.

The bacterial penetration at the implant-abutment junction was analysed using

1. Assay method
2. Colony forming unit method
3. Scanning Electron Microscope analysis

1. Assay method:

For the preparation of bacterial penetration assay, the each assembly (milled metal crown, abutment, implant mounted block) was placed inside the 20ml test tube and were sterilized using autoclaving at 121°C for 15 minutes at 15 lbs. After sterilization, thioglycollate broth was poured until the crown implant assembly was completely

immersed. Three different micro-organisms were used namely *P.gingivalis*, *A.A.comitans*, and *Staph.aureus*. 10-20µl of bacterial suspension mixture of all three organisms was added in the test tube containing the implant assembly with the thioglycollate broth [8]. The suspension was adjusted to 3×10^8 cfu /ml [McFarlandstandard]. The assembly was incubated in the anaerobic jar at 37°C and 10% CO₂ for 72 hrs[3,8]. The broth was changed every 24 hrs.

After an incubation period of 72 hrs the test tubes were removed from the anaerobic jar .The broth which showed an increase in turbidity [fig2] confirmed the bacterial growth. This was noted and the broth was discarded. Each assembly was removed from the test tube, carefully dried and the cement retained metal crown was removed using automated crown remover (Topdent, Bangalore) following which the abutment was removed using hex drive (Genesis implants, surgicalkit, India). In the screw retained metal crowns the GIC on the screw access hole was removed and the crowns were removed using hex drive. Plain broth was added in the inner surface of the implant holding the implant assembly with a sterile gloved hand on the Petri dish. After proper rinsing, the samples(penetrated microorganism and plain broth) was collected using 2ml

syringe and added in the 100µl of plain thioglycollate broth in the sterile micro centrifuge tube. The tubes were placed in Vortexer, BR-2000(BIO-RAD) mixed and the samples were placed in the Microplatereader [fig 3],(i-MARK, BIO-RAD) and the bacterial penetration was assessed.

2. Colony Forming Unit Method:

The broth that showed bacterial penetration was cultured on four blood agar plates. Each agar plate was made into 5 wells and 20µl of solution from the micro centrifuge tube was added to each well. Then the agar plates were cultured anaerobically at 37°C and 10% CO₂ for 2days to check the viability of each organism(fig4a&b) through colony forming unit[9] and the values were calculated using Optical density (OD Values) .The presence of organisms were analysed by Spectrophotometry. *P.gingivalis* appeared as greyish brown, *A.A.comitans* as clear and translucent and *Staph.aureus* as bluish-pink.

3. Scanning Electron Microscopic Analysis:

After bacterial penetration assay, the abutments were prepared for scanning electron microscope (SEM) analysis [10] to verify the presence of bacteria on the abutment surface. The abutments were washed with Phosphate Buffer Solution

(PBS) & fixed in 2% gluteraldehyde for 2hrs and rinsed with distilled water and air-dried. The samples were placed on the aluminium holder stub using a double sided carbon tape and the stub was placed in the mounting holes and screws were tightened. After 30-45mins, high vacuum $<5 \times 10^{-5}$ was achieved and acceleration voltage was kept at 15KV. The internal hex of the cement retained and screw retained abutments were magnified at 60x, 700x and 1000x and the images were scanned to confirm the presence of bacteria on the abutment surface [fig5a&b].

The data thus obtained were subjected to statistical analysis using SPSS Software (IBM SPSS Statistics for windows, Version 22.0). The variables do not follow normal distribution, therefore to analyse the data non parametric methods applied. To compare the values between cement retained and screw retained groups Mann Whitney test is applied and the significance level is fixed as 5% ($p<0.05$).

RESULTS

Descriptive statistics for broth turbidity values reveal that mean values for screw retained group was 0.131(SD±0.132) and cement retained group was 0.3 (SD±0.016) [tab1]. Mann Whitneytest mean ranking showed screw retained and cement retained restorations were 7.34 and 13.7 respectively with a value of

P=0.015(P<0.05) (tab2). When the Colony forming unit values were compared for the microbial species individually, Mann Whitney test mean ranking in screw retained and cement retained restorations for *Staphylococcus aureus* were 9.80 and 11.20 respectively (P=0.591, P>0.05) (tab3), for *Aggregatibacter actinomycetum*

Comitans were 9.90 and 11.10 respectively (P=0.644, P>0.05) [tab4] and for *Porphyromonas gingivalis* were 8.50 and 12.50 respectively (P=0.119, P>0.05)[tab5]. We can infer that the values were not statistically significant in all three species.

Table I Descriptive Statistics for Broth Turbidity Value of Screw retained and Cement retained

		Group	
		Screw retained	Cement retained
Broth Turbidity Value	N	10	10
	Mean	.0131	.0300
	Std. Dev	.0132	.0163
	Median	.009	.035
	1st Quartile	.005	.014
	3rd Quartile	.012	.043

Table II Mann-Whitney Test to compare values between Screw and Cement retained

Variable	Group	N	Mean Rank	p-Value
Broth Turbidity Value	Screw retained	10	7.30	0.015
	Cement retained	10	13.70	

Table III Mann-Whitney Test to compare values between Screw and Cement retained

Variable	Group	N	Mean Rank	p-Value
<i>Staphylococcus aureus</i> (CFU)	Screw retained	10	9.80	0.591
	Cement retained	10	11.20	

Table IV Mann-Whitney Test to compare values between Screw and Cement retained

Variable	Group	N	Mean Rank	p-Value
<i>Aggregatibacter actinomycetemcomitans</i> (CFU)	Screw retained	10	9.90	0.644
	Cement retained	10	11.10	

Table V Mann-Whitney Test to compare values between Screw and Cement retained

Variable	Group	N	Mean Rank	p-Value
<i>Porphyromonas gingivalis</i> (CFU)	Screw retained	10	8.50	0.119
	Cement retained	10	12.50	



Fig 1a, b: Implant placed 1mm above the resin level and milled CR and SR Crowns



Fig 2: Implant assembly showing broth turbidity

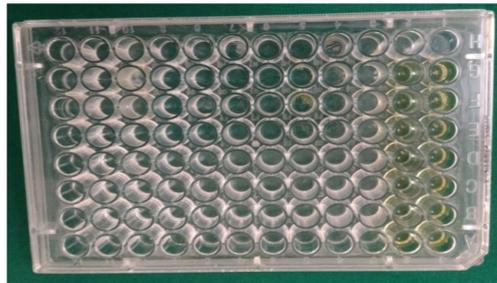


Fig 3. Microplate Reader



Fig 4: Bacterial culture of SR and CR restorations

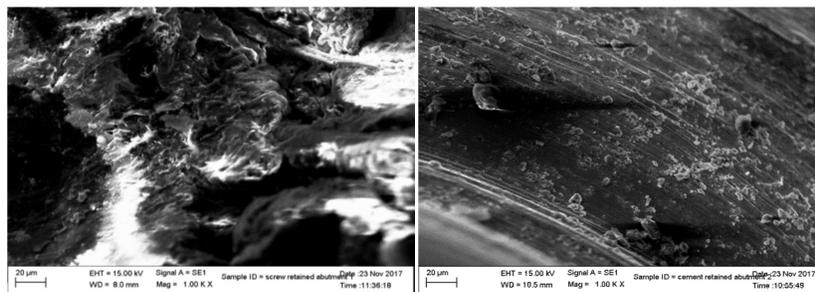


Fig 5: SEM Magnification of SR & CR 1000X

DISCUSSION

The emergence of implants in the field of dentistry has brought about a paradigm shift in treatment planning for partially and completely edentulous situations [11]. The titanium oral implants have become one of the most successful treatment procedures and implants are being used increasingly in

place of various treatment procedures like removable and fixed dental prosthesis for partial and complete edentulism [12]. Implant restorations have exhibited a success rate of over 90%. The successes of oral implants have been well documented; at the same time failures do occur. Earlier failure of dental implants was mainly

attributed to premature loading, excessive loading, poor bone support and occlusal trauma [13]. Presently with the help of many studies we have an understanding that the presence of bacteria in the oral cavity especially those found in periodontal disease conditions result in failure of dental implants [14]. This has led to the introduction of a maintenance therapy for long term success of implant supported restorations during the treatment planning phase [15]. Periodontitis is an inflammatory disease of the oral cavity which affects the soft and hard tissues surrounding the teeth. It involves progressive loss of the alveolar bone around the teeth and if not treated leads to tooth loss. The causes for periodontitis, apart from several other factors, include microorganisms, predominantly gram negative anaerobes like *A.actinomycetemcomitans*, *P.gingivalis*, *P.intermedia*, *E.nodatum*, *B.forsythus* and *Treponema* species [1,3]. The microorganisms responsible for the failure of an implants are Gram negative anaerobes like *Prevotellaintermedia*, *Porphyromonas gingivalis*, *Aggregatibacter actinomycetem comitans*, *Bacteriodes* etc. according to another study. Failure of dental implants due to microorganisms leads to peri-implantmucositis and peri-implantitis. Both the above mentioned studies have *Porphyromonas gingivalis*,

Prevotella intermedia and *A.actinomycetem comitans* in common. According to McCrea [16], “peri-implantitis is characterized as an inflammatory reaction that affects the hard and soft tissue, which results in loss of supporting bone and pocket formation surrounding the functioning osseointegrated implant” [17]. Periodontitis in close proximity to implants and periodontal pathogenic bacteria present in the peri-implant sulci are considered risk factors for the success of dental implants. Another factor responsible for peri-implantitis is the presence of marginal discrepancies between the abutments and prosthetic crowns. The assessment of these discrepancies depends on the material used for making the crowns, the types of restorations (cement retained and screw retained) and the material used for cementation. Most of the present implant systems use a two stage protocol. Hence, they contain two parts, the abutment and the implant fixture connected by screws. The implant-abutment interface is placed at the gingival level or bone level depending on the type of system used [18, 19]. The size of the micro gap present between the abutment and implant depends on the hex design of the implants system which can act as reservoir for pathogenic bacteria leading to inflammation of tissues around the implants. Numerous in-vitro and in-

vivo studies have reported leakage of bacteria through implant-abutment junction from external surfaces to inner part of implants or vice versa. [20] Identifying the presence of microorganisms inhabiting peri-implant sulci and the inner parts of implants becomes relevant for the successful outcome of dental implant procedures. [21] Bacterial culture is considered as a classical method for identifying the microbiota and this is most commonly used in microbiological examination. Several newer studies have shown appropriate sample collection technique and specific results for bacterial sensitivity like molecular diagnostic method, checkerboard DNA-DNA hybridization and polymerase chain reaction based techniques [22]. But most of the studies explaining the microbial leakage through the implant-abutment interface are based on conventional culture method results. [1,23] In the present study, penetration of bacteria was evaluated between the abutment-implant fixture in cement retained and screw retained restorations by Assay method, Colony forming unit method and the presence of bacteria was confirmed by SEM analysis also. Three different microorganisms were tested for viability viz., *Staphylococcus aureus*, *Aggregatibacter actinomycetum comitans* and *Porphyromonas gingivalis*. In

the assay method, after sterilization using an autoclave, the implant crown assemblies were inoculated with thioglycollate broth containing the three microorganisms and placed in an anaerobic jar at 37°C and 10% CO₂ for 72 hrs. The cloudiness of broth confirmed bacterial penetration by broth turbidity. In colony forming unit method, the abutments were removed and a plain broth was added to the inner surface of implants and the samples were cultured in blood agar plates. The values thus obtained showed the viability of three different microorganisms tested. Finally the abutments were removed and air dried; SEM analysis was done at a magnification of 60x, 700x, 1000x for all the samples to confirm the bacterial presence on the abutment surface. This study was also done in the conventional method, but only few in-vitro studies have been done with a restoration like a crown over the implant-abutment assembly. [24] Two types of restorations studied were cement retained and screw retained crowns. The present study evaluated the bacterial penetration between abutment-implant interface in screw retained and cement retained restorations mainly because there were conflicting findings in the various studies in literature. The results of this study showed that screw retained restorations had lesser bacterial penetration when compared to

cement retained restorations when broth turbidity method was used for testing. Statistical analysis results showed that there was statistical difference between the cement retained and screw retained restorations as the $p=0.015$ was less than $p<0.05$. As this is an in-vitro study done for 72 hrs, additional in-vitro studies and in-vivo studies for longer periods of time would be needed for a better understanding of the role of bacterial penetration on long term success of implant supported restorations. When the colony forming unit method was used for testing the bacterial penetration, it was found that *Staphylococcus aureus* was the numerous microorganism present followed by *A. Actinomycetem comitans* and this was followed by *P.gingivalis*. The viability of the organisms was in the above order for cement retained and screw retained restorations. However, a statistical analysis of the results showed that there was no significant difference between the two types of restorations. When a Scanning electron microscopic analysis was done for both types of restorations, there was bacterial penetration in both at 60x700x and 1000x magnification. The results have conclusively shown that irrespective of the method tested all of the restorations showed bacterial penetration.

The limitations of the study can be that only three microorganisms were tested. More periodontal pathogens can be included for further evaluation of bacterial penetration. This study was done in an in vitro set up. A clinical study can provide more information on the bacterial penetration in these restorations. The viability of the microbes can also be checked using more modern methods of evaluation.

CONCLUSION

Within the limitations of the study, the following conclusions can be drawn.

1. Bacterial penetration in implant-abutment interface in cement retained restorations showed higher penetration compared to screw retained restoration.
2. *Staphylococcus aureus* was the most numerous microorganism present followed by *Aggregatibacter actinomycetum comitans* and *Porphyromonas gingivalis* by colony forming method. *Staphylococcus aureus* was more viable, though the values are not statistically significant.
3. SEM analysis is done to confirm the presence of bacteria in both screw retained and cement retained crowns.

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