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Comparative evaluation of the microbial leakage at two different implant abutment interfaces using a new sealant

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To evaluate the microbial leakage at two different implant abutment interfaces using a new sealant. Forty implants of the size 4.2 Dx11.5 L (Adin, Israel), internal hex, were selected. Twenty Titanium abutments (Adin, Israel) with twenty titanium implants were used in Group 1, Twenty CAD/CAM Zirconia abutments with twenty titanium implants were used in Group 2. Each group was subdivided into two subgroups, Subgroup A- Control without sealant and Subgroup B- with new sealant. Implants were immersed in brain heart infusion broth (Accumix Microxpress, India) and a poly microbial solution (ATCC 10556 Streptococcus sanguis, Himedia, India and ATCC 90030 Candida glabrata Himedia, India) was introduced into the eppendorf tubes (Tarson, India) containing the implant assemblies of the four subgroups and incubated for 14 days. Then samples from implant wells were taken using sterile absorbent paper points (Diadent, Korea) and cultured in Mueller Hinton agar (HimediaIndia) .The colonies formed were counted using digitalcolony counter. The results obtained were tabulated and statistically analysed. Mann Whitney U test was used to compare the values between the two groups. There was a significant difference in microbial leakage in Titanium abutments with and without the new sealant for both microorganisms ($P<0.05$). There was a significant difference in microbial leakage in Zirconia abutments with and without the new sealant for both microorganisms ($P<0.05$).There was no statistically significant difference in microbial leakage between Titanium and Zirconia abutments with new sealant for both the microrganisms ($P>0.05$). There was significant reduction in microbial leakage for both microorganisms on application of this new sealant in both Titanium and Zirconia abutments over Titanium implants. The new sealant used has both antibacterial and antifungal efficacy on usage with both types of abutments over Titanium implants. (JOURNAL OF DENTAL IMPLANT RESEARCH 2021;40(2):35-47)

Key Words: Dental implants, Dental implant abutment design, Dental leakage, Microbiology, Titanium, Zirconia

INTRODUCTION

Titanium is the ideal material in implant dentistry due to its excellent biocompatibility. However in cases with thin gingival biotype, usage of Titanium causes greyish hue to surrounding soft tissues affecting the esthetic outcome, Zirconia abutments are preferred in such cases[1-3]. In contrast to Titanium abutments, Zirconia abutments have natural tooth like color, high translucency, exceptional tissue-compatibility and fracture strength[4]. Early adhesion/colonization of bacteria on Zirconia surfaces was significantly less compared to Titanium, which proves that Zirconia and its derivatives have the capacity to reduce plaque on implant and tissues, favouring better soft tissue healing and implant success at bone level[5-7].

Zirconia abutments enhance peri -implant bone health by reducing inflammation and less bleeding on probing as compared to Titanium abutment[1]. Despite the high success rates for dental implant-supported prosthesis,peri-implant pathology exists and a thorough knowledge regarding it is not available. The presence of a microgap between implant fixture and abutment might be a possible etiological factor[8-11].

Definition of Microgap is given as the microspace that exists between the implant fixture and abutment. This gap is located at the alveolar crest level and is generally in microns[8,10]. This microgap acts as a reservoir for microorganisms which leads to release of by products and

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may induce inflammatory reaction in both soft and hard tissues around the implants[10,12-15].

The microgap of the Titanium-to-Titanium interface has been widely documented in terms of precision of fit, whereas the Zirconia-to-Titanium interface has not been well documented in the scientific literature. The size of the microgap is influenced by factors like the precision of the milling method and the correct torque of the screw that joins the two pieces (implant and abutment) together and implant connection designs.

All connection designs between the implant and its respective abutment may be classified in two different types: external connections and internal connections. These differ in many ways, including surface and precision of fit[16]. In two-stage implants microgap is present near the crestal bone whereas in one-stage implants the microgap between the abutment and the implant is near the gingival crest[17].

Lack of precise fit at the implant abutment interface near the crestal bone may result in marginal microgap causing microleakage that will lead to inflammation and bone loss[17-20].

Micromotion in apparently stable implant screw joints is because of loading clinically. This micromotion may contribute to screw loosening and prosthesis failure.Thus, the more precise the fit, the less micromotion will occur, there by enhancing the longevity of the prosthesis[17].

Several in vitro and in vivo studies have evaluated the ability of different species of microorganisms to penetrate into the IAI with different types of abutments and different types of implant geometry[10,12,14,21-23].

The ability of Streptococcus sanguinis to penetrate into and along the implant abutment fixture was proved by Quirynen and Von Steenberg in 1993 in their in vivo study[8]. Streptococcus sanguinis is a small size gram-positive coccus, measuring between 0.5 and 1 μm, and a commonly found organism in the biofilm formed on the implants[12,13,24]. Streptococci strains are reported to have the unique ability to bind to other microbial species (coaggregation) and to each other (aggregation)[12,25].

These bacteria are frequently associated with Candidal organism, a commensal fungal species often found in biofilms of peri-implant areas that infect mucosal and gum tissues only when the host defenses are weak[12,25].

Candida species are an important resident microbial organism of the oral microbiome. In the formation of biofilm on implantable medical devices including dental implants these organisms are frequently seen. The artificial materials introduced into the body are accompanied by the ability of microorganisms, including Candida species, to colonize them and form biofilms[25]. This biofilm protect them from antibiotic diffusion and host defenses, leading to persistent infections[12]. Candida glabrata represents an emerging species of nonalbicans Candida and is a smal fungi (2.0~4.03×3.0~5.5 μm in size) is a common organism in the oral cavity and is known for its ability to colonize medical devices[12]. There are very limited number of studies in which the ability of fungi in coaggregation to Streptococci sanguinis to colonize the implant abutment interface microgap had been studied.

Many methods for analysis of microbial leakage like nutrient medium turbidity, DNA checkerboard, Real time Quantitative reverse transcription polymerase chain reaction (q RTPCR), gram-staining, biochemical reactions, colony forming units are employed in different studies[26]. The widely used gold standard method is Colony Forming Units (CFU) counting on plates.

In this study we have counted the colony forming units using digital colony counter for both Streptococcus sanguinis and Candida glabrata. Only viable bacteria are counted in this particular method, which excludes dead bacteria and debris and any number of bacteria using dilution can be counted[27]. Numerous efforts were put in, to seal the microgap between the implant and the abutment for preventing microleakage at the implant-abutment interface. Several materials have been used to seal the implant – abutment interface, such as, adhesive, silicone O – ring, a silicon hermetic washer, chlorhexidine-thymol varnish, and 2% chlorhexidine solution, some commercial antiseptic sealents like Gapseal, Keiroseal are also available[28-33].

In this study a new antiseptic sealent Viruseal (Polydimethysiloxane, Aerosol 380, Thymol, DSI, Israel) has been used[34], with both Titanium and Zirconia abutments connected to internal hexagon type of connection of Titanium implants. Currently, there is very limited data available on possible microleakage problems related to CAD/CAM milled Zirconia abutments after application

Fig. 1. Viruseal antibacterial sealant (DSI, Israel).

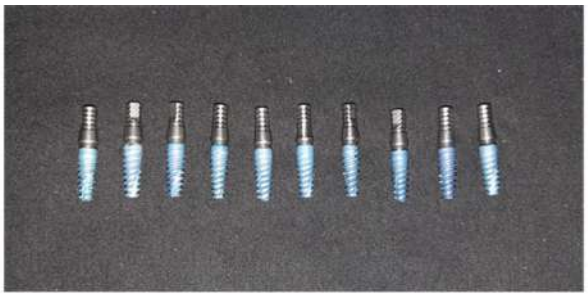

Fig. 2. Subgroup 1A (n=10)-Titanium abutments-Titanium implants-control-unsealed.

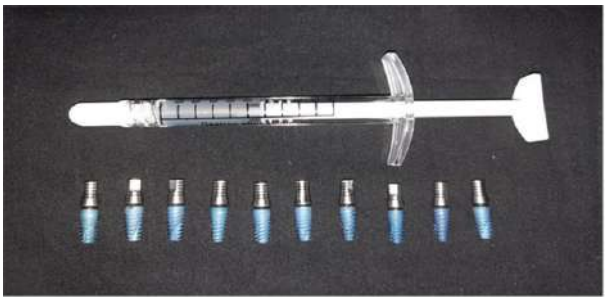

Fig. 3. Subgroup 1B (n=10)-Titanium abutment-Titanium implant-Sealant applied at IAI.

Fig. 4. Subgroup 2A (n=10)-CAD/CAM Zirconia abutment-Titanium implants-control-unsealed.

of a sealant.

The aim of this study was to determine the microbial leakage of 2 different types of organisms (Streptococcus sanguinis, Candida glabrata) in 2 groups of implants with different abutments.

Null-hypothesis adopted for this study was that there will be no differences between stock Titanium abutments and CAD/CAM Zirconia abutments in preventing microbial leakage at implant abutment interface after application of a new antiseptic sealant.

MATERIALS AND METHODS

1. Methodology

Forty premachined Titanium implants and twenty Titanium abutments (Adin, Israel) were purchased. Preparation of another twenty Zirconia abutments with Zirconia blocks were carried out. Standard platform straight CAD/CAM milled twenty Zirconia abutments were fabricated using EXOCAD software for designing, and WorkNC Software for milling Zirconia blocks (DG STAR, Unbreakable solid zirconia, DENTGALLOP USA) using Yenadent, 5 AXIS CAD/CAM milling machine and sintered using Vita ZYRCOMAT 6000 MS Sintering machine. Fit of these forty abutments were checked on to the implants. Autoclave (Unident Imported Sun B Class Autoclave, Hyvin Care, New Delhi) was used to sterilize the implants and abutments to ensure that they are not contaminated (121C- 20 minutes, at 15 lb pressure).

The new sealant (Fig. 1) used in this study was applied on top of twenty implants. Ten Titanium abutments and ten Zirconia abutments were torqued to manufactures recommended torque value (35 Ncm) using hexdriver and torque wrench without application of sealant. Ten Titanium abutments and ten Zirconia abutments were torqued to manufactures recommended torque value (35 Ncm) using hexdriver and torque wrench after the application of sealant on top of implants. Forty Titanium implants were divided into 2 groups of twenty each (Group 1 and Group 2), in which GROUP 1 were divided into 2 subgroups based on the application of sealant. SUBGROUP 1A (control-Unsealed) (Fig. 2) and SUBGROUP 1B (VIRUSEAL applied) (Fig. 3). GROUP 2 were divided into 2 Subgroups based on the application of sealant SUBGROUP 2A (Control-unsealed) (Fig. 4) and

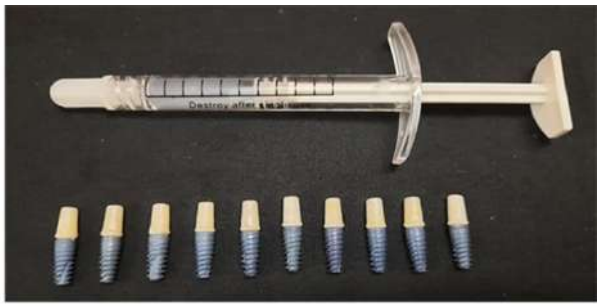

Fig. 5. Subgroup 2B-Zirconia abutments-Titanium implants-Sealant applied at IAI.

Fig. 6. ATCC 10556 Streptococcus sanguinis (Himedia, Mumbai, India).

SUBGROUP 2B (VIRUSEAL applied) (Fig. 5). The test bacterial strain, ATCC 10556 Streptococcus sanguinis (Fig. 6) and fungi ATCC 90030 Candida glabrata (Fig. 7) (Himedia, Mumbai) were revived from glycerol stock maintained at −20°C. The overnight young subculture of the both test strains were suspended in 2 ml Brain heart infusion broth (BHIB) (Himedia, Mumbai) for preparing the polymicrobial broth suspension.

Thirty seven grams of Brain heart infusion broth powder was suspended in one litre of distilled water and mixed well. The prepared Brain heart infusion broth was autoclaved at 15lb pressure, 121° centigrade for 15 minutes. Both Group I (twenty Titanium- Titanium abutment implants) and Group II (twenty Zirconia abutment-Titanium implants) assemblies were immersed in fresh Brain heart infusion broth, a little above the implant

Fig. 7. ATCC 90030 Candida glabrata (Himedia, Mumbai, India).

abutment interface in forty eppendorf tubes individually to avoid contamination through the access holes, under the Laminar air flow chamber to maintain the aseptic condition. The implant abutment assemblies in the forty eppendorf tubes were inoculated with 2 µl polymicrobial suspension at the end of the tubes (Fig. 8), into the Brain heart infusion broth. All the assemblies were incubated in Ausco selec 3303 incubator (Ashok United Scientific co, TAMIL NADU) at 37°C for 14 days with intermittent BHIB refreshment for every 48 hrs for maintaining the viability of the organism.

After the incubation period of 14 days all the implant assemblies were removed from polymicrobial suspension and were subjected to thorough rinsing with diluted 1% sodium hypochlorite followed by saline wash to avoid contamination from the surface of implant fixtures to implant wells. The Zirconia and Titanium abutments were detorqued using Adin hex drive from the implants aseptically under the laminar air flow chamber. Sample collection was done by insertion of absorbent paper points (DIADENT, Korea) from the implant wells. The samples taken from the implant wells were plated using Spread plate - pure culture technique on MUELLER HINTON AGAR and incubated for 24 hrs and organisms were differentiated based on colony morphology (Fig. 9, 10). The colonies were counted using a digital colony counter and the total colony forming unit was calculated using the formula:

Total colony forming unit, CFU/ml=
Total number of colony counted/Dilution factor

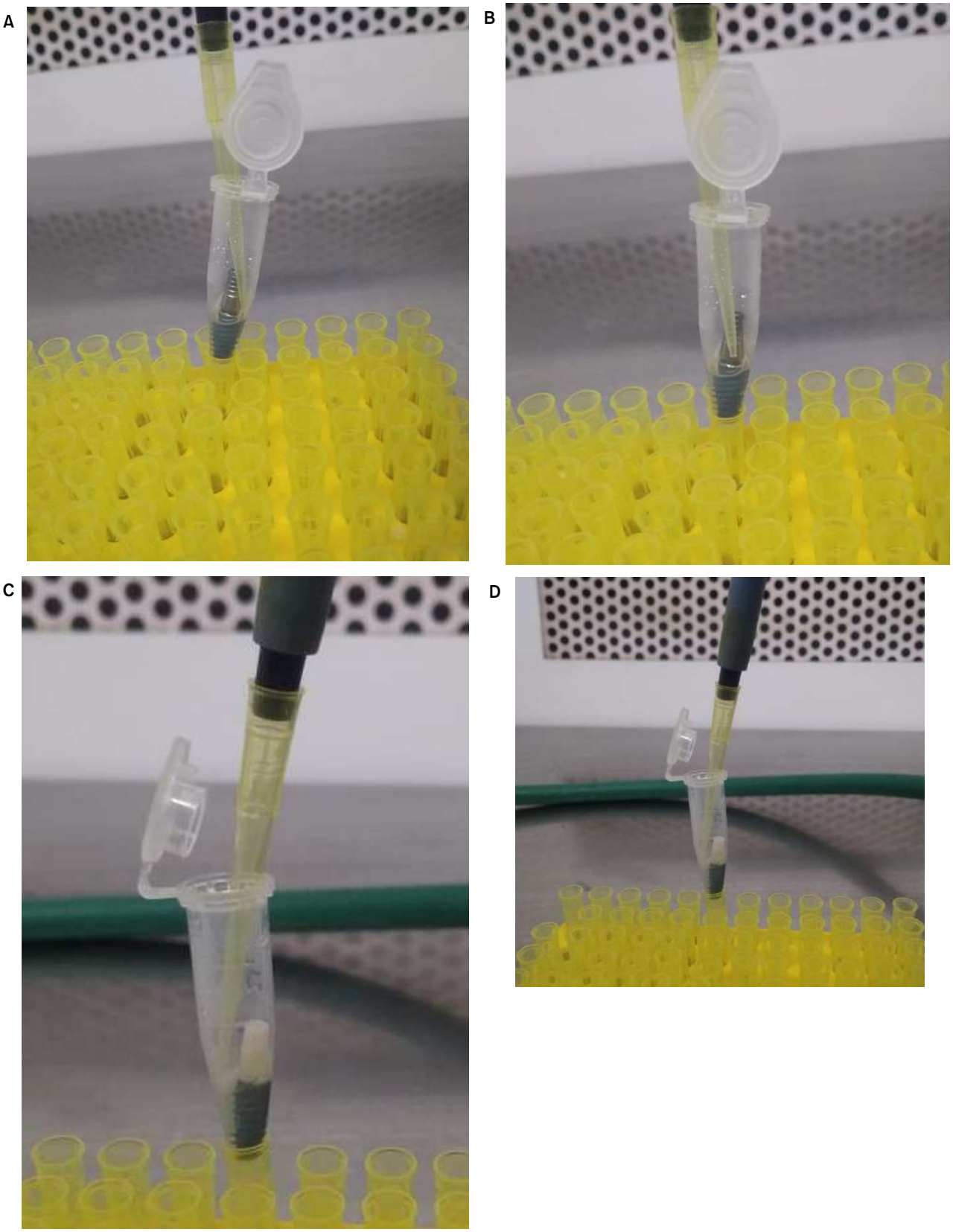

Fig. 8. Polymicrobial inoculation (A) Subgroup 1A. (B) Subgroup 1B. (C) Subgroup 2A. (D) Subgroup 2B.

The number of colonies formed were counted in all the four subgroups tabulated using Microsoft Excel 10 (Microsoft, USA) and median were calculated by taking the CFUS obtained from each group samples. The data were subjected to statistical analysis using SPSS software for windows 10.0.5 (SPSS Software Corp, Germany).

RESULTS

All the implants were immersed in brain heart infusion broth and a poly microbial solution (S. sanguinis and Candida glabrata) was introduced into the eppendorf tubes with implant assemblies and incubated for 14 days. At the end of 14 days the specimens from implant wells were taken and cultured and the formed colonies were

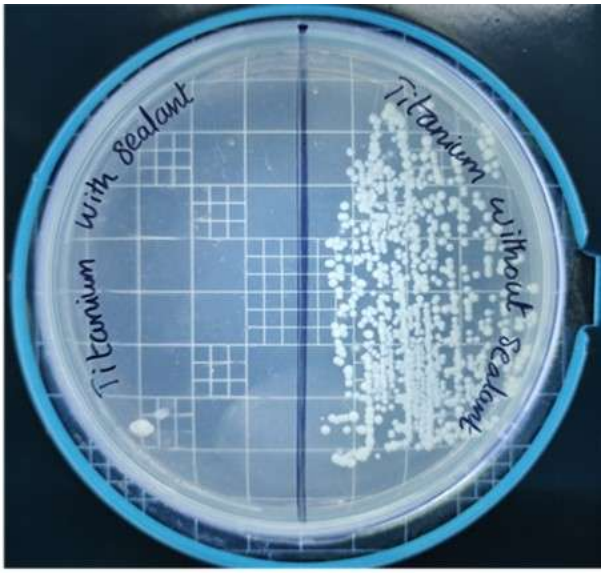

Fig. 9. Petri dish showing colony forming units in Titanium abutments with and without sealant using colony morphology.

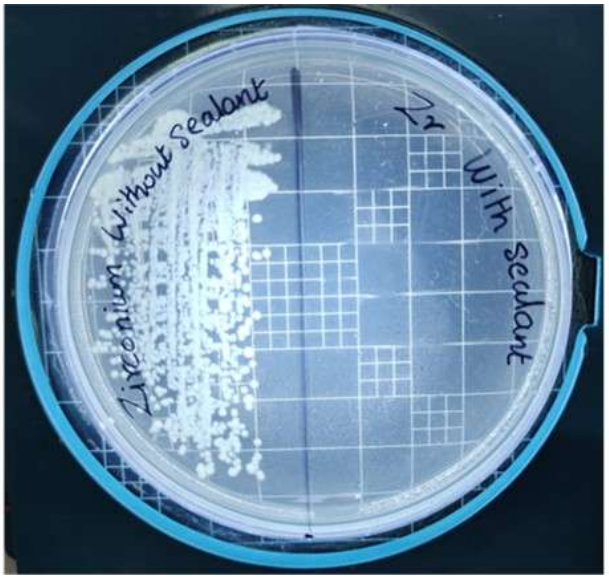

Fig. 10. Petri dish showing colony forming units for Zirconia abutments with and without sealant using colony morphology.

Table 1. To compare microleakage between (Titanium abutments) subgroup 1A and 1B for Streptococcus sanguinis with and without sealant

	Subgroup 1AS (CFUs/ml)	Subgroup 1BS (CFUs/ml)
Sample 1	10^8	2×10^2
Sample 2	10^8	Growth inhibited
Sample 3	10^8	Growth inhibited
Sample 4	10^8	Growth inhibited
Sample 5	10^8	3×10^2
Sample 6	10^8	Growth inhibited
Sample 7	10^8	Growth inhibited
Sample 8	10^8	Growth inhibited
Sample 9	10^8	Growth inhibited
Sample 10	10^8	Growth inhibited

Organism tested	Group 1	Median	P-value
Streptococcus sanguinis	#Without sealant (subgroup 1AS)	8.0000	0.002*
Streptococcus sanguinis	#With sealant (subgroup 1BS)	2.3891	

#Mann Whitney U test to determine difference in microbial leakage for S. sanguinis between 1AS and 1BS.
The cell density of the inoculated polymicrobial suspension is 10^8 CFUs/ml.
*P-value <0.05 considered significant.
Subgroup 1AS: Titanium abubments without sealant,checked for Streptococcus sanguinis.
Subgroup 1BS: Titanium abutments with sealant,checked for Streptococcus sanguinis.

Table 2. Comparision of microbial leakage between subgroup 1A and 1B for Candida glabrata (Titanium abutments) with and without sealant

	Subgroup 1AC (CFUs/ml)	Subgroup 1BC (CFUs/ml)
Sample 1	10^8	Growth inhibited
Sample 2	10^8	Growth inhibited
Sample 3	10^8	Growth inhibited
Sample 4	10^8	Growth inhibited
Sample 5	10^8	Growth inhibited
Sample 6	10^8	Growth inhibited
Sample 7	10^8	3×10^2
Sample 8	10^8	Growth inhibited
Sample 9	10^8	Growth inhibited
Sample 10	10^8	Growth inhibited

Organism tested	Group 1	Median	P-value
Candida glabrata	#Without sealant (subgroup 1AC)	8.0000	0.002*
Candida glabrata	#With sealant (subgroup 1BC)	2.3010	

#Mann Whitney U test to determine difference in microbial leakage for Candida glabrata between 1AC and 1BC.
The cell density of the inoculated polymicrobial suspension is 10^8 CFUs/ml.
*P-value<0.05: Statistically significant.
Subgroup 1AC: Titanium abutments without sealant checked for Candida glabrata.
Subgroup 1BC: Titanium abutments with sealant checked for Candida glabrata.

Table 3. To compare microbial leakage between subgroup 2A and 2B for Streptococcus sanguis with and without sealant (Zirconia abutments)

	Subgroup 2AS (CFUs/ml)	Subgroup 2BS (CFUs/ml)
Sample 1	10^8	Growth inhibited
Sample 2	10^8	2×10^2
Sample 3	10^8	Growth inhibited
Sample 4	10^8	Growth inhibited
Sample 5	10^8	Growth inhibited
Sample 6	10^8	Growth inhibited
Sample 7	10^8	Growth inhibited
Sample 8	10^8	Growth inhibited
Sample 9	10^8	Growth inhibited
Sample 10	10^8	Growth inhibited

Organism tested	Group 2	Median	p-value
Streptococcus. sanguinis	#Subgroup 2AS (without sealant)	8.0000	0.002*
Streptococcus. sanguinis	#Subgroup 2BS (with sealant)	2.3010	

#Mann Whitney U test to determine difference in microbial leakage for Streptococcus sanguinis between 2AS and 2BS.
The cell density of the inoculated polymicrobial suspension is 10^8 CFUs/ml.
P-value<0.05: Statistically significant.
Subgroup 2AS: Zirconia abutments without sealant for Streptococcus sanguinis.
Subgroup 2BS: Zirconia abutments with sealant for Streptococcus sanguinis.

counted using digital colony counter. The results obtained were tabulated and statistical analysis was done using Mann Whitney U test (SPSS V 20, USA).

The test revealed that the microbial leakage for Streptococcus sanguinis and Candida glabrata between Titanium and Zirconia abutments without sealant has no statistically significant difference (P>0.05). On application of sealant both Titanium and Zirconia abutments showed statistically significant difference in microbial leakage when compared without application of sealant for both organisms (P<0.05) (Tables 1-4).

The test revealed that the microbial leakage for Streptococcus sanguinis between Titanium and Zirconia abutments has no statistically significant difference

Table 4. To compare the microleakage at IAI between subgroups 2AC and 2BC for Candida glabrata with and without sealant

	Subgroup 2AC (CFUs/ml)	Subgroup 2BC (CFUs/ml)
Sample 1	10^8	Growth inhibited
Sample 2	10^8	$2x10^2$
Sample 3	10^8	Growth inhibited
Sample 4	10^8	Growth inhibited
Sample 5	10^8	Growth inhibited
Sample 6	10^8	Growth inhibited
Sample 7	10^8	Growth inhibited
Sample 8	10^8	Growth inhibited
Sample 9	10^8	Growth inhibited
Sample 10	10^8	Growth inhibited

Organism tested	Group 2	Median	P-value
Candida glabrata	#Subgroup 2AC (without sealant)	8.0000	0.002*
Candida glabrata	#Subgroup 2BC (with sealant)	2.3010	

#Mann Whitney U test to determine difference in microbial leakage for Candida glabrata between 2AC and 2BC.
The cell density of the inoculated polymicrobial suspension is 10^8 CFUs/ml.
*P-value<0.05: Statistically significant.
Subgroup 2AC: Zirconia abutments without sealant checked for Candida glabrata.
Subgroup 2BC: Zirconia abutments with sealant checked for Candida glabrata.

Table 5. Comparison of microleakage between group 1 (subgroup 1B) and group 2 (subgroup 2B) for Streptococcus sanguinis (Titanium and Zirconia abutments) with sealant

	Group 1 subgroup 1BS (CFUs/ml)	Group 2 subgroup 2BS (CFUs/ml)
Sample 1	2×10^2	Growth inhibited
Sample 2	Growth inhibited	2×10^2
Sample 3	Growth inhibited	Growth inhibited
Sample 4	Growth inhibited	Growth inhibited
Sample 5	3×10^2	Growth inhibited
Sample 6	Growth inhibited	Growth inhibited
Sample 7	Growth inhibited	Growth inhibited
Sample 8	Growth inhibited	Growth inhibited
Sample 10	Growth inhibited	Growth inhibited

The cell density of the inoculated polymicrobial suspension is 10^8 CFUs/ml.
Subgroup 1BS: Titanium abutments with sealant checked for Streptococcus sanguinis.
Subgroup 2BS: Zirconia abutments with sealant checked for Streptococcus sanguinis.

Table 6. Comparison of micro bialleakage between group 1 (subgroup 1B) and group 2 (subgroup 2B) for Candida glabrata (Titanium versus Zirconia abutments) with sealant

	Group 1 subgroup 1BC (CFUs/ml)	Group 2 subgroup 2BC (CFUs/ml)
Sample 1	Growth inhibited	Growth inhibited
Sample 2	Growth inhibited	2×10^2
Sample 3	3×10^2	Growth inhibited
Sample 4	Growth inhibited	Growth inhibited
Sample 5	Growth inhibited	Growth inhibited
Sample 6	Growth inhibited	Growth inhibited
Sample 7	2×10^2	Growth inhibited
Sample 8	Growth inhibited	Growth inhibited
Sample 9	Growth inhibited	Growth inhibited
Sample 10	Growth inhibited	Growth inhibited

The cell density of the inoculated polymicrobial suspension is 10^8 CFUs/ml.
Subgroup 1BC: Titanium abutments with sealant checked for Candida glabrata.
Subgroup 2BC: Zirconia abutments with sealant checked for Candida glabrata.

(P>0.05) (Table 5). The test revealed that the microbial leakage between Titanium and Zirconia abutments for Candida glabrata has no statistically significant difference (P>0.05) (Table 6).

On comparison among Streptococcus sanguinis and Candida glabrata, the colony forming units formed in Titanium abutments -Titanium implant interface with sealant following microbial leakage shows statistically no significant difference (P>0.05). The colony forming units formed in Zirconia abutments -Titanium implant interface with sealant following microbial leakage shows statistically no significant difference (P>0.05) (Table 7, 8).

DISCUSSION

Biofilm formation due to the accumulation of microorganisms at the implant abutment interface in two piece implants will lead to microbial leakage into the implant wells through the microgap between implant and abut-

Table 7. Comparision of microbial leakage titanium and Zirconia abutments with sealant using Mann Whitney U test

Organisms tested	Groups	P-value
Streptococcus sanguinis	Group 1 (Titanium abutments) 1BS	0.480*
Streptococcus sanguinis	Group 2 (Zirconia abutments) 2BS	
Candida glabrata	Group 1 (Titnaium abutments) 1BC	1.000*
Candida glabrata	Group 2 (Zirconia abutments) 2BC	

*P-value >0.05: statistically no significant difference.
Subgroup 1BS: Titanium abutments with sealant checked for Streptococcus sanguinis.
Subgroup 2BS: Zirconia abutments with sealant checked for Streptococcus sanguinis.
Subgroup 1BC: Titanium abutments with sealant checked for Candida glabrata.
Subgroup 2BC: Zirconia abutments with sealant checked for Candida glabrata.

Table 8. Comparision of microbial leakage between Streptococcus sanguinis and Candida glabrata in Titanium and Zirconia abutments with sealant using Mann Whitney U test

Type of abutment	Organism	P-value
Titanium abutments (Group1)	Streptococcus sanguinis Candida glabrata	0.684*
Zirconia abutments (Group 2)	Streptococcus sanguinis Candida glabrata	1.000*

*P-value >0.05: statistically no significant difference.

ment, thereby causing inflammation of the peri-implant tissues, which affects the success of osseointegrated implants in a long term[9,12,14].

It has been reported that bacteria found around implants from the oral cavity of patients with clinical signs of periimplantitis present a microbiota pattern similar to that of natural teeth affected with periodontitis[12,35,36].

In this study we have inoculated Streptococcus sanguinis and Candida glabrata to evaluate the microbial leakage at the implant abutment interface as the dimensions of these organisms are smaller, and can easily pass through the microgap in the implant abutment interface[9,12,13,37]. In studies without sealant application, these organisms were seen inside the implant wells following 14 days of incubation at 37 degree centigrade.

Candida glabrata is dimensionally smaller than C. albicans. Silva et al. reported that C. glabrata exhibits a degree of hydrophobicity comparable with that of C. albicans, although only a few studies have evaluated the role and pathogenicity of C. glabrata in the formation of biofilm on medical devices[37]. The ability of C. glabrata to enter and arrange a mixed biofilm in coaggregation with Streptococcus sanguinis in implant systems had been stated by Baggi et al.[12]. In this study we have evaluated Candida glabrata in Titanium implant systems with CAD/CAM Zirconia abutments. The existence of bacterial leakage is not surprising if one compares the diameter of oral microorganisms (less than 10 mm) with the passive fit between implant components.

The present study analyzed the microbial contamination within the implant surfaces, in a manner similar to previous studies except for the use of CAD/CAM zirconia abutments[8,21]. Quirynen et al. performed an in vitro study using only Branemark implant system to evaluate their resistance against bacterial penetration at implant–abutment interface[9].

Findings from similar in-vitro studies have documented that bacteria infiltration may occur both from an external source to the inner area of an implant and in reverse. This migration of bacteria is probably facilitated through the unavoidable presence of microgaps between the fixture and the abutment components of the assembled system[3,8,11,21,28,38]. The larger the microgap, larger the bacterial accumulation, leading to periimplant pathology. Under clinical loading, these microgaps may be further widened when bending forces during function loosen the screw joint[16,40]. The microgap between the implant and the abutment ranges from 0.1 to 5.6 μm. Scarano et al.have stated that microgap in screw retained implant–abutment system is critical for bacterial colo-

nization and the microgap size will be much larger in vivo than seen in vitro[3].

Rotational freedom between the implant and the abutment is a critical factor related to the stability of the implant-abutment connection. The implant- abutment connection is most stable when the degree of rotational freedom is less than 2°, and significant torque loss occurs when the degree is greater than 5°. Anti-rotation design of the abutment can limit the rotational freedom, thereby maintaining the stability of the joint when the degree is greater than 5 degrees[41]. The parafunctions such as bruxism, excessive occlusal force, bad chewing habits, and others which might be risk factors of screw loosening, should cause concern as screw loosening will result in increase of microgap between abutment and implant, thereby increasing microleakage[42]. The antirotational property of internal hexagon connection in providing stability and preventing screw loosening under loaded conditions was proved in previous studies[43,44].

Attempts to minimize the bacterial leakage through the implant abutment interface have led to constant modifications of implant connection designs. In previous in vitro studies, evaluating the microleakage in different types of connections, it was found, in all cases, a much lesser degree of bacterial leakage in internal conical connections[22,23,45-49]. A recent review of the literature reported that external hexagon implants had the greatest bacterial leakage, followed by internal trilobe, internal hexagon, and internal taper configuration. Internal Morse Cone conical connection Implants showed an absolute congruity without any microgaps between implant and abutment[14,46,50-52]. In this study we have evaluated the microbial leakage using internal hexagon implants with Titanium and CAD/CAM milled Zirconia abutments.As stated by Quirynen and van Steenberghe, hermetic sealing is capable of causing the death of microorganisms[9].

Motosfy, Zarbaksh concluded that the use of GapSeal decreased the microgap and microleakage in internal hex implant-abutment connection[30,53].

Nayak et al. proved that using a gel easily throughout the Implant abutment interface, leading to a better seal than the O-ring. Unlike the low viscosity gel, the O-ring's body prevents complete seating of the abutment. The rubber can deteriorate over time, which may increase leakage[21].

Podhorsky et al. concluded that usage of setting, non setting sealing agents and disinfectant agents reduced bacterial load inside the implant well. He also stated that setting compounds were difficult to remove and usage of greasy sealing agents has shown promising outcome preventing microleakage in his study[33].

Zekiy concluded that an antiseptic sealant and a nanocoated implant- abutment interface will provide better osseointegration and stability to the implants with better outcomes[31].

In this study a new sealant (Viruseal, DSI, ISRAEL) was used which proved to be effective in controlling microbial leakage at both titanium and zirconia abutments. This sealant provides a reliable seal and fills the hollow spaces in the implants. It is a highly viscous silicone matrix which ensures a reliable sealing and prevents the ingress of organisms. The principle of this material is that as the place is already occupied, there will be no nutrition for vital activity of organism to grow and develop. The addition of thymol ensures better antifungal and antibacterial activity. The material did not get dried off, maintaining its viscocity and preventing formation of new microgaps. Thin silicone film effectively prevents adhesion of plaque on the supporting implants. The leakage without a sealing agent was probably due to the lack of complete wall-to-wall adaptation between abutment and implant[34].

The microbial leakage of Titanium abutments (Group 1) for Streptococcus sanguinis and Candida glabrata without sealant was found to be significantly higher than the Titanium abutments with sealant and the difference was found to be statistically significant ($P<0.05$). The microbial leakage of Zirconia abutments (Group 2) for Streptococcus sanguinis and Candida glabrata without sealant was found to be significantly higher than the Zirconia abutments with sealant and the difference was found to be statistically significant ($P<0.05$). In this present study, Mann Whitney U test revealed that there was there was no significant differences ($P>0.05$) in microbial leakage for Streptococcus sanguinis and Candida glabrata, among the two abutment types. This explains that the new sealant was effective in exhibiting both antibacterial and antifungal effect, irrespective of the type of abutments used

in the present study. Factor of film thickness of this material and its possible interference with machining tolerance of the implant system should be considered. Here, the new sealant effectively sealed the microgap as evidenced from the various results, but whether tolerance was affected is a question requiring further research and necessitates a continuation study.clinically, Viruseal doesn't have any seting time and forms effective seal around the microgap and can be easily removed with alcohol swab in case of abutment removal for any reason and can be reapplied again. distortion in plane of contact between implant and abutment may be considered stress reducing[55].

Therefore the null hypothesis was accepted. This present study had some limitations, Streptococcus sanguinis and Candida glabrata were the only two microorganisms considered whereas the oral cavity harbours many organisms. Only internal hexagon connection design was evaluated. The study was conducted under unloaded conditions, whereas masticatory loads are present in oral cavity. Future studies should evaluate the marginal sealing efficacy of this new sealant with other available sealants. long term studies are required to evaluate its efficacy and the need for reapplication of the sealant should also be evaluated.

CONCLUSION

The new sealant was effective in exhibiting both antibacterial and antifungal effect, irrespective of the type of abutments and type of organism used in the present study.The new sealant used in this study was effective in reducing microbial leakage.

CLINICAL SIGNIFICANCE

This new sealant can be used clinically with both Titanium and Zirconia abutments over Titanium implants.

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