

# Microleakage and Bacterial Adhesion with Three Restorative Materials Used to Seal Screw-access Channels of Implant Abutments: An *In vitro* Study

Firas Khalid AlQarawi, Baneen Mansour AlShammasi, Zainab Ahmed AlBasry, Heba Zaki AlAwami, Doaa Mostafa AlEraky<sup>1</sup>, Reem Yussuf AlJindan<sup>2</sup>, Hamad Saleh Alrumaih, Faris Abdullah Alshahrani

Departments of Substitutive Dental Science, <sup>1</sup>Biomedical Dental Science, College of Dentistry, Imam Abdulrahman Bin Faisal University, <sup>2</sup>Department of Microbiology, College of Medicine, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

## Abstract

**Background:** Proper sealing of screw-access channels against microbial microleakage is advisable for the long-term success of screw-retained implant prosthesis.

**Objective:** This study aimed to compare the bacterial adhesion and microleakage with three restorative materials, namely, composite resin, acrylic resin and bis-acryl, that are used to cover the access channels of screw-retained implant prostheses, using polytetrafluoroethylene tape as a spacer material.

**Materials and Methods:** In this *in vitro* study, 18 titanium straight abutments (Hex-lock® Zimmer) were torqued into implant analogs, which were then subdivided into three groups. The samples of each group were filled with polytetrafluoroethylene tape and sealed with the three restorative materials (Group A: composite resin; Group B: acrylic resin; Group C: bis-acryl). Measurements of surface bacterial adhesion and internal microleakage were then recorded. The results were statistically analyzed using Kruskal–Wallis and Chi-square tests.

**Results:** No significant difference was found between the investigated materials in terms of their sealing effectiveness against microbial microleakage ( $P = 0.06$ ). Regarding bacterial adhesion, composite resin showed the highest number of surface adhesion, but there was no significant difference between the three materials ( $P = 0.081$ ).

**Conclusion:** The results of this study suggest that composite resin, acrylic resin and bis-acryl materials could be used alternatively in sealing the implant access channel owing to no significant differences in terms of microleakage and bacterial adhesion.

**Keywords:** Bacterial adhesion, implant abutment, microleakage, sealing ability, spacer materials, titanium abutment

**Address for correspondence:** Dr. Firas Khalid AlQarawi, Department of Substitutive Dental Science, College of Dentistry, Imam Abdulrahman Bin Faisal University, P. O. Box 1982, Dammam 31441, Saudi Arabia.

E-mail: fkalqarawi@iau.edu.sa

Submitted: 26-Feb-2020 Revised: 17-Oct-2020 Accepted: 01-Jul-2021 Published: 04-Sep-2021

Access this article online	
Quick Response Code:	Website: www.sjmms.net
	DOI: 10.4103/sjmms.sjmms_76_20

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

**For reprints contact:** WKHLRPMedknow\_reprints@wolterskluwer.com

**How to cite this article:** AlQarawi FK, AlShammasi BM, AlBasry ZA, AlAwami HZ, AlEraky DM, AlJindan RY, *et al.* Microleakage and bacterial adhesion with three restorative materials used to seal screw-access channels of implant abutments: An *in vitro* study. Saudi J Med Med Sci 2021;9:241-7.

## INTRODUCTION

Screw-retained implant-supported prostheses have an advantage over those that are cement retained, in that they allow retrieval of the restorations when indicated.<sup>[1,2]</sup> The lack of need for cement in screw-retained prosthesis adds to the benefit of reducing the risk of peri-implant disease development.<sup>[3]</sup> Nevertheless, a proper sealing of the access channel is recommended for the long-term stability and success of the screw-retained implant prosthesis.<sup>[4]</sup> Pathogenic and nonpathogenic bacterial microleakage and colonization of the internal implant system have been linked to malodor and progression of peri-implant inflammation, which, in turn, might lead to bone loss and eventual implant failure.<sup>[5,6]</sup> Multiple studies have investigated the effectiveness of different materials to seal the access channels of screw-retained implant prostheses.<sup>[4,7]</sup>

Studies have suggested that the use of polytetrafluoroethylene (PTFE) tape, gutta-percha, wax, vinyl polysiloxane and cavitation spacer materials were more favorable than cotton and endo-frost cotton pellets, in which bacterial and fungal adhesion has been reported.<sup>[4,7]</sup> The available literature supports the superiority of PTFE tape use to seal the screw channel mainly due to the less load of microbial density and volume associated with it, which greatly impacts the long-term stability of the implant system.<sup>[8,9]</sup> Other studies also reported easier manipulation, sterilization and retrievability of PTFE tape when needed.<sup>[10]</sup>

The effectiveness of different materials in covering the access channel of screw-retained implant prosthesis has been widely studied. However, most of these studies investigated the effectiveness of the internal filling materials rather than that of the restorative materials used to cover the coronal part of the screw-access hole. One study aimed to measure the microbial loading that occurred on the use of a combination of spacer materials to close the entire screw-access channel showed that the least counts of microbial species were with the use of PTFE tape combined with resin composite ( $2.81 \pm 0.38$ ) or gutta-percha ( $3.41 \pm 0.38$ ). On the other hand, the use of cotton pellet combined with light-cured provisional composite was associated with the greatest number of microbial colonies ( $17.45 \pm 1.67$ ).<sup>[9]</sup>

Composite resin is one of the most commonly used dental restorative materials nowadays. It has been also used as the conventional material to seal the coronal part of the screw-access channel of the implant-supported prosthesis.<sup>[11]</sup> Composite resin materials are mostly used for

their good mechanical and esthetic properties. However, the major drawback is the polymerization shrinkage and the resultant internal stresses that might lead to microcracks formation, and hence bacterial microleakage.<sup>[12]</sup> Assessment of the integrity of composite resin and the occurrence of bacterial microleakage has been experimented on human teeth under different application methods, and no statistically significant difference was found between methacrylate- and silorane-based composite resins.<sup>[13]</sup>

An *in vivo* study has compared the integrity of nanohybrid composite resin and a modified 4-methacryloxyethyl trimellitate anhydride/methyl methacrylate-tri-n-butyl borane-based resin (M4M) as filling materials of the screw-access channel with respect to marginal deterioration over a 12-month period. The results showed no statistically significant difference between the two groups in terms of the depth of marginal discrepancy ( $P = 0.58$ ), but the mean angle of marginal discrepancy was significantly lower with M4M than the composite resin ( $P < 0.0001$ ). Evaluation of other aspects that included surface area changes and discoloration showed no significant difference between the materials.<sup>[14]</sup>

Polymethyl methacrylate acrylic (PMMA) is one of the most commonly used materials in prosthodontics dentistry for the fabrication of provisional fixed prosthesis. PMMA is a cost-effective material that can produce good marginal adaptation and polish. Yet, the main disadvantages involve the high polymerization shrinkage and wear susceptibility.<sup>[15]</sup>

Bis-phenol A glycidyl methacrylate (Bis-GMA) was later introduced for similar purposes.<sup>[16]</sup> A comparative study has shown superior properties of Bis-GMA material over PMMA, as the results reported better marginal adaptation and less polymerization shrinkage, and thus reduced bacterial microleakage.<sup>[17]</sup> The inorganic filler content of Bis-GMA adds to its abrasion resistance.<sup>[15]</sup>

Given that there is a lack of reported evidence regarding the different types of restorative materials used to seal the coronal part of the access channel of screw-retained implant prosthesis against increased bacterial penetration and proliferation, this *in vitro* study was conducted with the aim of comparing the sealing effectiveness of three different materials used to fill the access channel of screw-retained implant prosthesis, using PTFE tape as a spacer material. The null hypothesis of this study is that composite resin, acrylic resin and bis-acryl materials used over PTFE tape for sealing the coronal part of screw-access channels will have no statistically significant difference in their susceptibility to bacterial adhesion and microleakage.

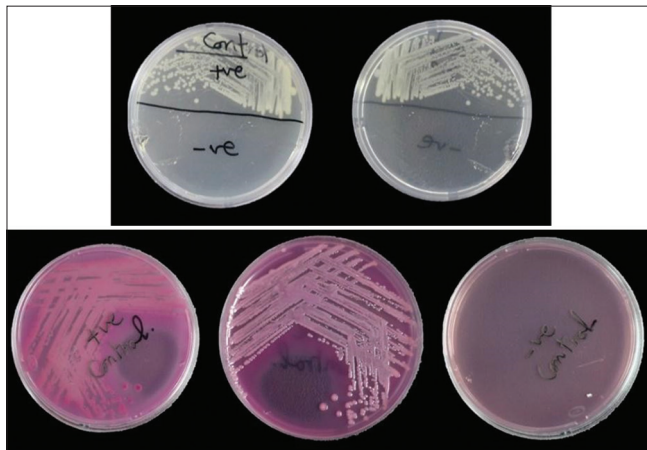
## MATERIALS AND METHODS

The study's samples consisted of 18 implant analogs of 4.5-mm diameter (Zimmer Biomet Co., Indiana, USA) that resembled the dental implants and 18 straight titanium abutments (Hex-lock® Zimmer of an internal connection system with a diameter of 4.5 mm and cuff height of 3 mm). The samples were divided into three groups. Each group consisted of six samples assigned to different restorative materials. Under aseptic conditions, the titanium abutments were hand torqued to the analogs using a screwdriver. Each analog was then partially embedded in autopolymerizing acrylic resin molds (BMS Dental, Capannoli, Italy).

A 45-mm sterilized polytetrafluoroethylene tape was twisted and packed using a sterilized plugger. A 3-mm coronal, height measured using a sterilized UNC 15 periodontal probe, was left to be filled with the restorative material of each group. Group A was restored with condensable composite resin (Filtek™ Bulk Fill, Bis-GMA; 3M/ESPE, St. Paul, MN, USA), Group B with light-cured acrylic resin (PMMA; UNIFAST™ LC, GC Corp., Tokyo, Japan) and Group C using bis-acryl (Protemp™ 4, 3M/ESPE, St. Paul, MN, USA).

### Thermocycling test

The samples were immersed in a thermocycling machine in alternating hot and cold bath tanks to simulate the oral environment temperature changes. The machine was set according to a standardized protocol at 5°C and 55°C for cold and hot water baths, respectively.<sup>[18]</sup> Each cycle lasted 30 s in each bath tank. The samples were subjected to 2000 cycles for 2 weeks, which is equal to almost 2.5 months intraorally.<sup>[18]</sup> Upon completion of the thermocycling test, the condition of the restorative materials was evaluated for each sample of all groups.



**Figure 1:** Positive and negative control on agar plate and (bottom row) MacConkey agar plate

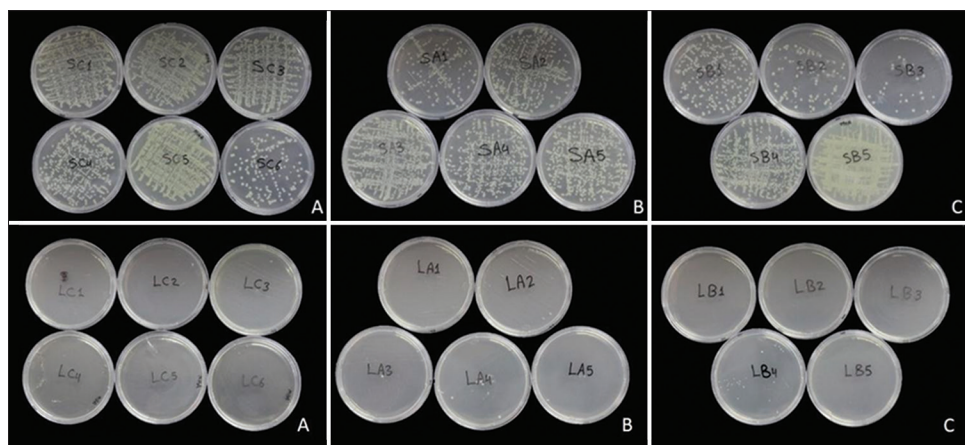
### Microbiology

Each sample was coded with two characters: a number and a letter (A, B or C) corresponding to the respective group of the samples. The specimens were immersed separately in containers filled with a contaminant solution of *Escherichia coli* in Luria-Bertani broth so that each restorative material was fully exposed to the solution. Baseline optical density was standardized by DensiChek Plus device for all samples at 0.5 McFarland unit. A positive control sample was prepared in a similar condition to ensure *E. coli* growth through solution turbidity during the entire investigation period. A negative control counterpart sample was used and confirmed through transparency of the solution under the same incubation conditions to validate the results. The specimens were then incubated for 7 days at 37°C. Optical density was validated in all samples after 7 days.

For measuring bacterial surface adhesion, phosphate-buffered saline was used to double wash the samples to remove the nonadherent bacterial cells. A swab from the surface of each sample was taken and then inoculated on an agar plate for overnight growth at 37°C. Each plate was coded with the number of the sample, its respective group letter and (S) referring to surface swab (Group A: SA1–SA6; Group B: SB1–SB5; Group C: SC1–SC5). After completion of the incubation period, the covering restorations were removed using a high-speed handpiece and a round carbide bur, leaving the PTFE tape intact inside the analogs. Then, samples were collected from inside the screw-access channel using a micropipette and cultured on MacConkey and nutrient agar mediums for 24 h [Figure 1]. Finally, the total number of colony-forming units per ml (CFU/ml) was counted for each specimen [Figure 2].

### Statistical analysis

Statistical data were analyzed using SPSS-20.0, IBM product of Chicago (USA). Numeric data of bacterial counts were presented by using descriptive statistics such as mean, standard deviation, median and interquartile range. Kruskal–Wallis test was used to compare the two main aspects: average bacterial counts and bacterial growth among the three groups (A, B and C). Later, this numeric variable was stratified based on bacterial count concentration (<1000 and >1000) and bacterial growth (negative and positive). Chi-square test was performed to compare the proportion of <1000/>1000 bacterial counts and negative/positive bacterial growth between the three groups. A statistically significant result was set at  $P \leq 0.05$ .



**Figure 2:** Bacterial surface adhesion. Group A: Composite Resin, Group B: Acrylic Resin, Group C: bis-acryl. (Bottom row): Bacterial microleakage. Group A: Composite Resin, Group B: Acrylic Resin, Group C: bis-acryl

## RESULTS

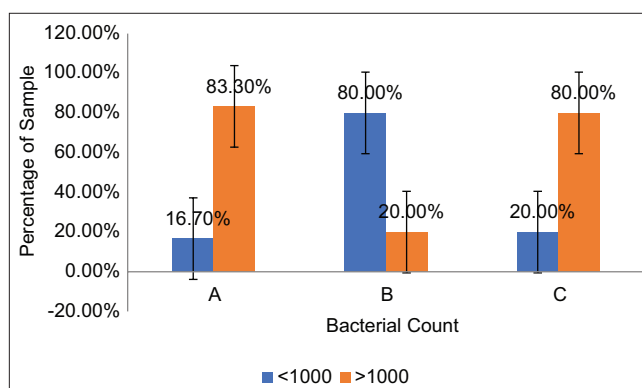
### Thermocycling test

After 2 weeks of thermocycling, all the samples were intact in Group A. However, one restoration was completely detached and lost in both Groups B and C. Hence, a total of two samples with lost restorations were excluded from the microbiological analysis.

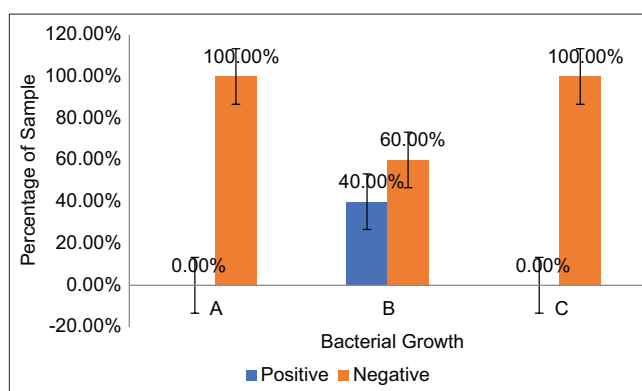
### Microbiology

The results of microbiological analysis were measured on two scales: bacterial adhesion on the surface of the restorations and bacterial microleakage of the internal cavity of the abutments. The results of bacterial surface adhesion are presented in Table 1. Composite resin showed the highest bacterial adhesion of >1000 bacterial units (83.3%). In contrast, the light-cured acrylic resin showed the least surface adhesion of >1000 bacterial units (20.0%). However, bis-acryl had the highest bacterial adhesion of <1000 bacterial units (80%). The mean and median bacterial counts are shown in Table 1. No statistical significance was noted between the three groups in terms of bacterial concentration and count ( $P = 0.06$  and  $0.086$ , respectively).

Bacterial microleakage was assessed by measuring both bacterial counts [Figure 3] and growth [Figure 4]. No bacterial microleakage could be detected in the composite resin and bis-acryl groups (i.e., 100% negative results). Two samples of the light-cured acrylic resin showed bacterial microleakage (40%) with a mean of  $2.6 (\pm 4.77)$ . There was no statistically significant difference among the three groups in terms of bacterial count proportion ( $P = 0.81$ ) [Table 2].



**Figure 3:** Relation between bacterial count and percentage of the samples



**Figure 4:** Relation between bacterial growth and percentage of the samples

## DISCUSSION

This study intended to assess the effectiveness of three restorative materials in sealing the coronal access of screw-retained implant prostheses against microleakage. The results supported the null hypothesis that light-cured

**Table 1: Bacterial surface adhesion after 7 days of incubation**

Factors	Group A (n=6)	Group B (n=5)	Group C (n=5)	P
Bacterial count concentration, n (%)				
<1000	1 (16.7)	4 (80.0)	1 (20.0)	0.060 <sup>a</sup>
>1000	5 (83.3)	1 (20.0)	4 (80.0)	
Bacterial counts				
Mean±SD	862.2±337.6	440.0±454.2	843.2±350.6	0.086 <sup>b</sup>
Median (IQR)	>1000 (207)	>1000 (872)	>1000 (392)	

<sup>a</sup>Nonsignificant difference of proportions of bacterial count among the three groups (Chi-square test); <sup>b</sup>Nonsignificant difference of average bacterial count among three groups (Kruskal–Wallis test). SD – Standard deviation; IQR – Interquartile range

**Table 2: Bacterial microleakage after 7 days of incubation**

Factors	Group A (n=6)	Group B (n=5)	Group C (n=5)	P
Bacterial growth, n (%)				
Negative (=0)	6 (100)	3 (60)	5 (100)	0.081 <sup>a</sup>
Positive (>0)	0	2 (40)	0	
Bacterial counts				
Mean±SD	0	2.6±4.8	0	0.096 <sup>b</sup>
Median (IQR)	0	0 (6.5)	0	

Nonsignificant difference of proportions of bacterial count among the three groups (Chi-square test); Nonsignificant difference of average bacterial count among three groups (Kruskal–Wallis test). SD – Standard deviation; IQR – Interquartile range

acrylic resin and Bis-GMA materials can be used as alternatives to composite resin for sealing the access channel, with no significant difference in terms of microbial microleakage.

Microbial microleakage and anaerobic byproducts that occur through the implant-abutment interface with the repeated use of implants can cause peri-implant tissue inflammation.<sup>[8]</sup> Restorative material used to cover the screw channel is another risk factor for peri-implant diseases. Hence, the integrity of the filling material is crucial in maintaining the long-term functionality of the implant prosthesis.

Composite resin has been the most commonly used conventional material for covering the access channel. Yet, only a few studies have evaluated the integrity of composite resin as a sealing material in comparison to other different materials. Tanimura and Suzuki, in their study that compared the mechanical and esthetic properties of composite resin and M4M resin-based by assessing surface area changes, marginal discrepancy and photographical analysis material, found no significant changes in both the materials over a 12-month period.<sup>[14]</sup>

A case–control study evaluated the esthetic and mechanical properties of ceramic inlay used for covering the access channel and compared it to composite resin.<sup>[11]</sup> Occlusal wear (in  $\mu\text{m}$ ) was measured at baseline, 1 year and 2 years of follow-up for both the groups. The study concluded that ceramic inlays are more predictable esthetically and mechanically over the traditionally used composite resin ( $P < 0.001$ ).<sup>[11]</sup>

Our results showed that bacterial adhesion was mostly related to composite resin restorations. This can be attributed to the surface and other properties that would influence bacterial adhesion on each material.<sup>[19]</sup> In a study where *E. coli* adhesion and colonization on composite resin restorations were measured (based on the chemical and physical characteristics of three types of composite resins) at different time intervals over a 72-hour period, the highest bacterial adhesion was found to occur between 0 and 24 h, with no significant difference between the different groups in the amount of bacterial colonization, noted after that time period. This study suggested that bacterial adhesion and biofilm development were mostly related to the size of composite resin particles rather than other properties.<sup>[20]</sup>

A study that compared bis-acryl (3M ESPE Protemp<sup>TM</sup>4) and PMMA, in terms of surface roughness and its relation to bacterial adhesion, found a significant difference between unpolished surfaces of PMMA and bis-acryl ( $P = 2.2 \times 10^{-16}$ ). Bacterial adhesion was higher in PMMA than bis-acryl, although the latter had a rougher surface without polishing. Surface roughness differed significantly after polishing the two materials ( $P < 0.05$ ). It was concluded that a direct relationship was found between bacterial adhesion and surface roughness in the PMMA groups, while it was not directly related in the groups of bis-acryl.<sup>[21]</sup>

It can be perceived that bacterial adherence can be variable depending on multiple factors, either related to the properties of the material or bacterial pathogen. However, it cannot be determined if bacterial adhesion can significantly affect the long-term survival of the

implant system. Despite our results and those of other studies<sup>[20,22]</sup> showing that composite resin can demonstrate increased bacterial counts adhered to its surface, it does not show a significant amount of bacterial microleakage that can directly affect the implant success rate. Hence, the ineffectiveness of a specific material on sealing the implant internal cavity cannot be determined based on the bacterial adhesion alone.

The samples with the lost restorations following thermocycling were excluded because of the loss of the coronal restorative seal that was intended to be assessed regardless of the type of spacer material used. Other studies suggested that loading forces can greatly enhance bacterial adhesion and penetration.<sup>[23,24]</sup> However, this was not tested in our study, which is one of the limiting factors that must be considered in future studies. Although a variety of other bacterial strains could have been tested, *E. coli* has a vital impact on the progression of dental infection in the oral environment.<sup>[25]</sup> Nevertheless, other bacterial strains such as *Staphylococcus aureus* and *Streptococci* microleakage have been investigated at the implant-abutment interface thoroughly in the literature.<sup>[26,27]</sup>

A study showed that artificial saliva contamination did not affect the shear bond strengths of restorative materials or their degree of microleakage;<sup>[28]</sup> however, Nair *et al.*, from a review of the literature, concluded that saliva could impair the bond quality of the adhesive materials.<sup>[29]</sup> Therefore, this study was designed to eliminate possible adverse effects of the saliva and standardize the method for all restorative materials. However, further studies to test the influence of saliva on restorative materials should be considered.

Within the limitations of this present *in vitro* study, it is recommended that further investigations are to be conducted on a broader array of oral bacterial strains and fungal species. Testing the effects of other factors that would influence bacterial proliferation and penetration and that can closely simulate the oral environment should be taken into consideration in future studies.

## CONCLUSION

Based on the results of this *in vitro* study, it can be concluded that composite resin, acrylic resin and bis-acryl materials can be used alternatively to seal the access channel of the screw-retained implant prostheses, as no significant statistical difference could be detected between the three materials in terms of microleakage and bacterial adhesion. Further studies are required to confirm these findings.

## Peer review

This article was peer reviewed by three independent and anonymous reviewers.

## Acknowledgment

The authors would like to thank Mr. Roberto Caravana (Office of the Vice President for Postgraduate Studies and Scientific Research, College of Dentistry) and Mr. Intisar Siddiqui (Department of Dental Education, College of Dentistry, Imam Abdulrahman bin Faisal University), for their assistance with thermocycling and statistical analysis.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

## REFERENCES

- Jivraj S. Screw versus cemented implant restorations: The decision-making process. *J Dent Implant* 2018;8:9.
- Garg A, Shenoy K, Shetty S. Principles of screw-retained and cement – Retained fixed implant prosthesis: A critical review. *J Interdiscip Dent* 2014;4:123-9.
- Wittneben JG, Joda T, Weber HP, Brägger U. Screw retained vs. cement retained implant-supported fixed dental prosthesis. *Periodontol* 2000 2017;73:141-51.
- Raab P, Alamanos C, Hahnel S, Papavasileiou D, Behr M, Rosentritt M. Dental materials and their performance for the management of screw access channels in implant-supported restorations. *Dent Mater J* 2017;36:123-8.
- Jervøe-Storm PM, Jepsen S, Jöhren P, Mericske-Stern R, Enkling N. Internal bacterial colonization of implants: Association with peri-implant bone loss. *Clin Oral Implants Res* 2015;26:957-63.
- Mishra SK, Chowdhary R, Kumari S. Microleakage at the Different Implant Abutment Interface: A Systematic Review. *J Clin Diagn Res* 2017;11:E10-5.
- Park SD, Lee Y, Kim YL, Yu SH, Bae JM, Cho HW. Microleakage of different sealing materials in access holes of internal connection implant systems. *J Prosthet Dent* 2012;108:173-80.
- Schoenbaum TR, Wadhvani C, Stevenson RG. Covering the implant prosthesis screw access hole: A biological approach to material selection and technique. *J Oral Implantol* 2017;43:39-44.
- do Nascimento C, Pita MS, Calefi PL, de Oliveira Silva TS, Dos Santos JB, Pedrazzi V. Different sealing materials preventing the microbial leakage into the screw-retained implant restorations: An *in vitro* analysis by DNA checkerboard hybridization. *Clin Oral Implants Res* 2017;28:242-50.
- Moráquez OD, Belser UC. The use of polytetrafluoroethylene tape for the management of screw access channels in implant-supported prostheses. *J Prosthet Dent* 2010;103:189-91.
- Mihali S, Canjau S, Bratu E, Wang HL. Utilization of Ceramic Inlays for Sealing Implant Prostheses Screw Access Holes: A Case-Control Study. *Int J Oral Maxillofac Implants* 2016;31:1142-9.
- Kim EJ, Lee KB, Jin MU. Evaluation of marginal leakage of bulk fill flowable composite resin filling with different curing time using micro-computed tomography technology. *J Dent Rehabil Appl Sci* 2016;32:184-93.
- Moosavi H, Afshari E, Forghani M, Ghazvini K, Velayati F. Bacterial leakage assessment for different types of resin-based dental restorations

- applied using various placement methods. *JDMT* 2014;3:158-65.
14. Tanimura R, Suzuki S. Comparison of access-hole filling materials for screw retained implant prostheses: 12-month *in vivo* study. *Int J Implant Dent* 2017;3:19.
  15. Singh A, Garg S. Comparative evaluation of flexural strength of provisional crown and bridge materials – An *in vitro* study. *J Clin Diagn Res* 2016;10:C72-7.
  16. Akhshan V. Marginal integrity of provisional resin restoration materials: A review of the literature. *Saudi Dent J* 2015;6:33-40.
  17. Arora SJ, Arora A, Upadhyaya V, Jain S. Comparative evaluation of marginal leakage of provisional crowns cemented with different temporary luting cements: *In vitro* study. *J Indian Prosthodont Soc* 2016;16:42-8.
  18. Morresi AL, D’Amario M, Capogreco M, Gatto R, Marzo G, D’Arcangelo C, *et al.* Thermal cycling for restorative materials: Does a standardized protocol exist in laboratory testing? A literature review. *J Mech Behav Biomed Mater* 2014;29:295-308.
  19. Al-Aaskari S, Ariffin Z, Husein A, Reza F. Comparison of microbial adherence to polymethylmethacrylate for maxillo-facial prostheses. *World Appl Sci J* 2014;31:2115-9.
  20. Azam M, Khan A, Muzzafar D, Faryal R, Siddiqi S, Ahmad R, *et al.* Structural, surface, *in vitro* bacterial adhesion and biofilm formation analysis of three dental restorative composites. *Materials* 2015;8:3221-37.
  21. Lara-Jara NU, Romo-Ramírez GF, Goldaracena-Azuara MD, Aragón-Piña A, Girón CB-T, Méndez-González MV, *et al.* Effect of polishing procedure on the roughness and bacterial adhesion of provisional restorative materials. *Microsc Res* 2018;06:9-18.
  22. Derchi G, Vano M, Barone A, Covani U, Diaspro A, Salerno M. Bacterial adhesion on direct and indirect dental restorative composite resins: An *in vitro* study on a natural biofilm. *J Prosthet Dent* 2017;117:669-76.
  23. Erdilek D, Dörter C, Koray F, Kunzelmann KH, Efes BG, Gomec Y. Effect of thermo-mechanical load cycling on microleakage in class II Ormocer Restorations. *Eur J Dent* 2009;3:200-5.
  24. Soares GP, Ambrosano GM, Lima DA, Marchi GM, Correr-Sobrinho L, Lovadino JR, *et al.* Effect of light polymerization time, mode, and thermal and mechanical load cycling on microleakage in resin composite restorations. *Lasers Med Sci* 2014;29:545-50.
  25. Faria R, May L, Vasconcelos DD, Volpato CM, Bottino M. Evaluation of the bacterial leakage along the implant-abutment interface. *J Dent Implants* 2011;1:51.
  26. do Nascimento C, Ikeda L, Pita M, Pedroso e Silva R, Pedrazzi V, de Albuquerque Junior R, *et al.* Marginal fit and microbial leakage along the implant-abutment interface of fixed partial prostheses: An *in vitro* analysis using Checkerboard DNA-DNA hybridization. *J Prosthet Dent* 2015;114:831-8.
  27. Guindy J, Besimo C. Bacterial leakage into and from prefabricated screw-retained implant-borne crowns *in vitro*. *J Oral Rehabil* 1998;25:403-8.
  28. Shimazu K, Karibe H, Ogata K. Effect of artificial saliva contamination on adhesion of dental restorative materials. *Dent Mater J* 2014;33:545-50.
  29. Nair P, Hickel R, Ilie N. Adverse effects of salivary contamination for adhesives in restorative dentistry. A literature review. *Am J Dent* 2017;30:156-64.