



# A novel retentive type of dental implant prosthesis: marginal fitness of the cementless double crown type implant prosthesis evaluated by bacterial penetration and viability

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**PURPOSE.** This study aims to compare the marginal fitness of two types of implant-supported fixed dental prosthesis, i.e., cementless fixation (CL.F) system and cement-retained type. **MATERIALS AND METHODS.** In each group, ten specimens were assessed. Each specimen comprised implant lab analog, titanium abutment fabricated with a 2-degree tapered axial wall, and zirconia crown. The crown of the CL.F system was retained by frictional force between abutment and relined composite resin. In the cement-retained type, zinc oxide eugenol cement was used to set crown and abutment. All specimens were sterilized with ethylene oxide, immersed in *Prevotella intermedia* culture in a 50 mL tube, and incubated with rotation. After 48 h, the specimens were washed thoroughly before separating the crown and abutment. The bacteria that penetrated into the crown-abutment interface were collected by washing with 500  $\mu$ L of sterile saline. The bacterial cell number was quantified using the agar plate count technique. The BacTiter-Glo Microbial Cell Viability Assay Kit was used to measure bacterial adenosine triphosphate (ATP)-bioluminescence, which reflects the bacterial viability. The t-test was performed, and the significance level was set at 5%. **RESULTS.** The number of penetrating bacterial cells assessed by colony-forming units was approximately 33% lower in the CL.F system than in the cement-retained type ( $P < .05$ ). ATP-bioluminescence was approximately 41% lower in the CL.F system than in the cement-retained type ( $P < .05$ ). **CONCLUSION.** The CL.F system is more resistant to bacterial penetration into the abutment-crown interface than the cement-retained type, thereby indicating a precise marginal fit. [J Adv Prosthodont 2020;12:233-8]

**KEYWORDS:** Implant-supported fixed dental prosthesis; Cementless fixation system; Marginal fitness; Bacterial penetration; Cement-retained type

## INTRODUCTION

The fitness of a dental prosthesis is a critical factor for function and long-term maintenance.<sup>1</sup> In the restoration of

an implant crown, fitness is generally classified into passive fit, which provides a stable and strain-free structure in the absence of an external load, and marginal fit.

Retaining type of the implant-supported fixed dental prostheses are largely classified into screw-retained type and cement-retained type. For the screw-retained type prosthesis, where the crown margin is not inherent, only passive fit is considered. This type has no cement layer that provides compensation for misfit-induced strain. Hence, a passive fit in the oral cavity is more difficult to achieve when the screw-retained type is used compared with the cement-retained type. In addition, screw holes cause a loss of occlusal table integrity, thereby ultimately limiting the function of the prosthesis as well as inducing difficulty in control of occlusion, particularly in the crowns with small occlusal sur-

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Received March 4, 2020 / Last Revision June 30, 2020 / Accepted July  
10, 2020

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face in posterior sites.<sup>2,3</sup> On the other hand, cement-retained type is advantageous for passive fit and occlusal integrity can be achieved. However, it also has limitations in terms of removing excessive cement remnants and retrieving the prosthesis.<sup>4,6</sup> To overcome these problems, a combination type, which is a cement-retained type prosthesis with a screw hole, has been introduced. However, the problems associated with the use of both retention types are still not solved.

The cementless fixation (CL.F) system is a novel retentive type of dental implant prosthesis, which is structurally similar to the cement-retained type. However, the composite resin is relined between the crown and abutment, and the retention of the crown portion is achieved by a static frictional force between the abutment and the relined composite resin on the intaglio surface of the crown. In the oral cavity, the retention of the CL.F system is increased due to the relative expansion of the composite resin because the coefficient of thermal expansion of the relined resin is greater than that of the titanium abutment, and the temperature in the oral cavity is higher than the temperature *in vitro*. The internal air pocket aids in stress distribution (Fig. 1).<sup>7</sup> The retentive principle of the CL.F system facilitates the precise fit of the margin area of the crown, and the marginal fitness of the crown affects plaque deposition, infiltration of microorganism, and development of peri-implantitis.

The present study aimed to compare the marginal fitness of the CL.F system versus the cement-retained type. For this purpose, we evaluated the colony forming units (CFUs) of the bacteria that penetrated the abutment-crown interface of each type of prosthesis, and assessed the viability of the bacterial cells by measuring adenosine triphosphate (ATP)-bioluminescence.

**MATERIALS AND METHODS**

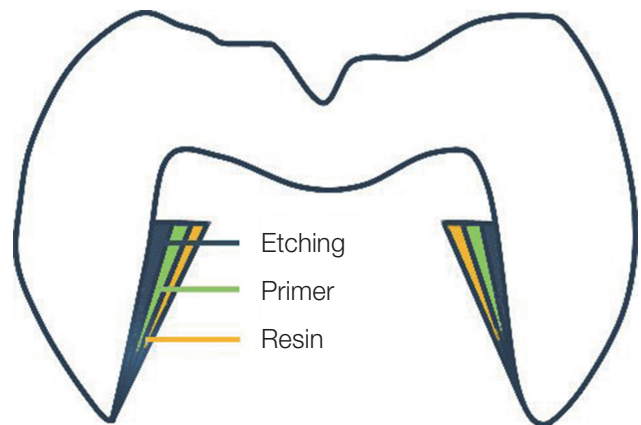
For the CL.F system, ten specimens were prepared. Each

specimen comprised implant lab analog (GSTLA400; Osstem, Seoul, Korea), titanium abutment, and zirconia crown. Titanium abutments were fabricated with a 2-degree tapered axial wall using the computer-aided design (CAD) and computer-aided manufacturing (CAM) system with the dental CAD software (Exocad; Exocad GmbH, Darmstadt, Germany) and milling machine (ARUM 5X-200; Doowon, Daejeon, Korea). Zirconia crowns were designed, milled from zirconia block (AUTOcera; Auto Industrial Co., Incheon, Korea) using the milling machine (ARUM 5X-200), and sintered at 1600°C. All lab analogs and abutments were tightened using a torque wrench at 30 N·cm torque. The inner surface of each zirconia crown was sandblasted with 50 μm of aluminum oxide (Al<sub>2</sub>O<sub>3</sub>), was etched (Freedent Etching; LaboTech, Seoul, Korea), and a zirconia primer (MKZ Primer; Bredent GmbH & Co. KG, Senden, Germany) was applied (Fig. 2). Then, the composite resin (Crea.lign; Bredent GmbH & Co. KG, Senden, Germany) was relined between the inner surface of each zirconia crown and the abutment, and was light cured after removing the excess resin (Fig. 3A). Each zirconia crown and each abutment were assembled only with finger pressure at a room temperature of 23°C. All specimens were stored in distilled water for 24 h and were disinfected with ethylene oxide gas.

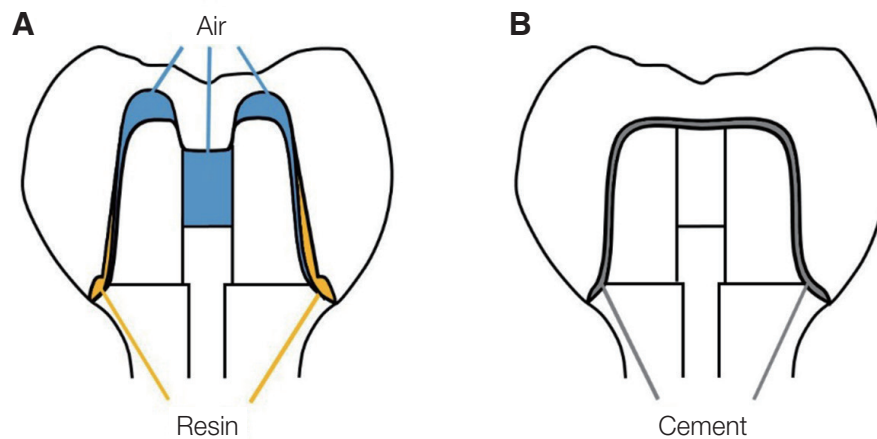
In the cement-retained type group, ten implant lab analogs, titanium abutments, and zirconia crowns were prepared, as described above. Using the CAD software (Exocad), the cement space was set at 10 μm around the margin, and additional cement space starting at 1 mm above the finish line of the abutment was set at 60 μm (Fig. 3B). Before the cementation of crown and abutment, the screw access hole of each abutment was filled with polytetrafluoroethylene tape and sealed with a provisional restorative material (Fermit N; Ivoclar Vivadent AG, Schaan, Liechtenstein). Vaseline was thinly applied over the external marginal contour of each crown to reduce cement adhesion to the external surface of the crowns and to facilitate the removal of



**Fig. 1.** Section of the dental implant prosthesis of the CL.F system.

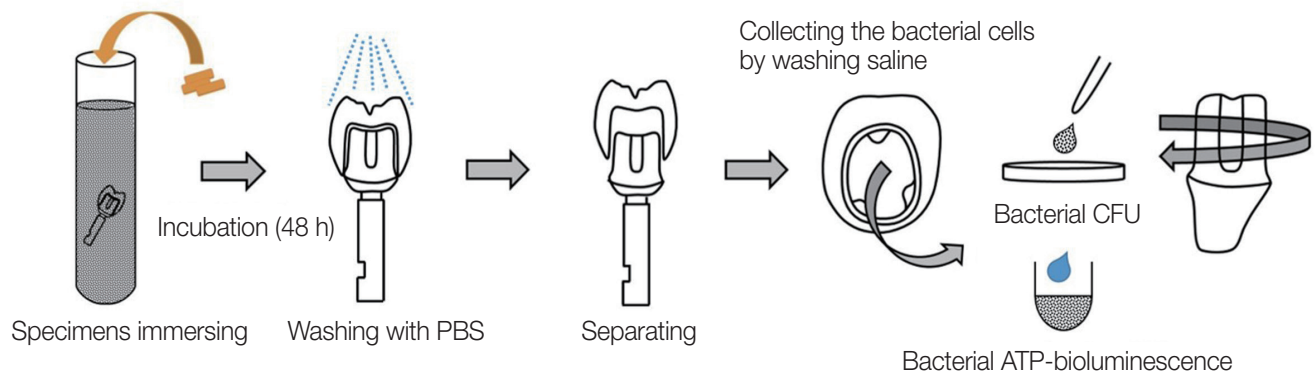


**Fig. 2.** Surface treatment of the intaglio surface of the crown of the CL.F system.



**Fig. 3.** Diagram of the dental implant prostheses. (A) CL.F system, (B) Cement-retained type.

*P. Intermedia* ATCC49046



**Fig. 4.** Schematic illustration of the bacterial penetration test.

excessive cements. Zinc oxide eugenol cement (Temp-Bond™; Kerr Corporation, MI, USA) was mixed according to the manufacturer's instructions, then applied thinly onto intaglio surface of the crown using a microbrush (Microbrush International, Grafton, WI, USA) to minimize the amount of extruded cement. After the cement had set, the excess cement was completely removed. The cementation process was performed at a room temperature of 23°C. All specimens were disinfected as described above.

*Prevotella intermedia* ATCC49046 was grown at 37°C anaerobically (85% N<sub>2</sub>, 10% H<sub>2</sub>, and 5% CO<sub>2</sub>) in brucella agar (Becton Dickinson, Franklin Lakes, NJ, USA) supplemented with 5% sheep blood or in brucella broth. Both media were supplemented with 5 µg/mL of hemin (Sigma Chemical Co., St. Louis, MO, USA) and 1 µg/mL of vitamin K<sub>1</sub> (Sigma Chemical Co., St. Louis, MO, USA). The bacterial culture growing exponentially was adjusted to an optical density at 600 nm (OD<sub>600</sub>) of approximately 0.5, and was used in bacterial penetration experiments. The schematic illustration of the experiment is shown in Fig. 4. The ster-

ilized specimens were immersed in bacterial suspension using a 50 mL tube and were incubated anaerobically with rotation. The specimens were exposed to exponential- and stationary-phase cells ( $\geq 10^9$  cells/mL) of *P. intermedia* for 48 h. Then, the specimens were removed from the bacterial culture and washed with phosphate-buffered saline before separating each crown and the assembled abutment. The retention of the CL.F system specimens exposed to a temperature lower than that of the oral cavity decreased due to abutment and composite resin shrinkages, so the crown and abutment were separated using a crown remover. The cement-retained type specimens were also separated using a crown remover. The bacterial cells were collected from the interface between the crown and abutment by washing with 500 µL of sterile saline (0.85% NaCl). The abovementioned procedure was independently carried out four times (1<sup>st</sup> time: 2 specimens, 2<sup>nd</sup> time: 2 specimens, 3<sup>rd</sup> time: 3 specimens, and 4<sup>th</sup> time: 3 specimens) using same procedure. The number of bacterial cells in the saline (100 µL) was determined by counting the colony-forming units (CFUs) after

growth at 37°C for 72 h on brucella blood agar. The ATP-bioluminescence generated by the bacterial cells in the saline (100 µL) was quantified using the BacTiter-Glo™ Microbial Cell Viability Assay Kit (Promega, Madison, WI, USA). Both CFU counting and ATP-bioluminescence measurements were technically repeated 2 or 3 times.

Statistical analysis was performed using the software (IBM SPSS Statistics v21.0; SPSS Inc., Chicago, IL, USA). In each group, all data were subjected to normality and homogeneity of variance using the Shapiro-Wilk test. The t-test was performed, and the significance level was set at 5%.

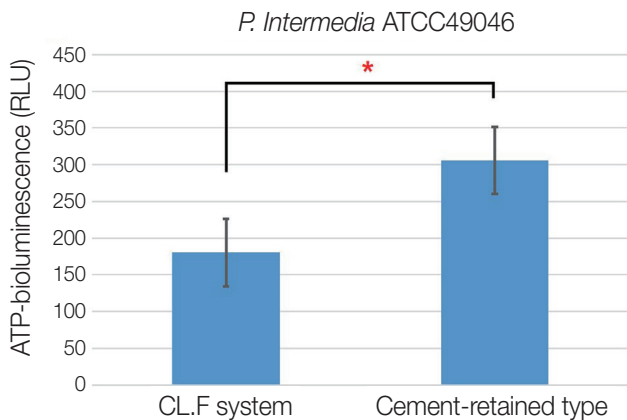
**RESULTS**

Physiological saline (100 µL) containing *P. intermedia* cells that penetrated the crown-abutment interface was serially diluted and spread on the brucella blood agar. After 72 h, the number of bacterial CFUs in the CL.F system was approximately 33% lower than that in the cement-retained type (Table 1, *P* < .05). ATP-bioluminescence, which reflects bacterial viability, was also measured and expressed in relative light unit (RLU). The RLU values of the physiological saline (100 µL) containing the bacterial cells were lower by approximately 41% in the CL.F system than in the cement-retained type (Fig. 5, *P* < .05).

**Table 1.** Colony forming unit (CFUs) of *P. intermedia* cells penetrating at the crown-abutment interface

Retention type	Mean (× 10 <sup>4</sup> )	SD (× 10 <sup>4</sup> )
CL.F system	767.22	0.93
Cement-retained type	1146.52*	35.40*

\* Statistically significant difference (*P* < .05)



**Fig. 5.** ATP-bioluminescence assessed by evaluating *P. intermedia* cells penetrating into the crown-abutment interface. \**P* < .05

**DISCUSSION**

The unique nature of the implant-bone anchorage, which allows limited movement of around 10 µm,<sup>8</sup> led to the hypothesis that passively fitting superstructures are a prerequisite for long-lasting osseointegration of dental implants.

In the cement-retained type, the cement layer can compensate the existing inaccuracies, thereby achieving a passive fit. However, excessive cement in the submucosal peri-implant region is difficult to access and remove. The rough surface of the excessive cement also increases plaque deposition, which is a major etiologic factor for peri-implant diseases.<sup>9</sup> The cement-retained type implant prosthesis has been frequently associated with biological complications, such as suppuration and fistula formation.<sup>10</sup> Moreover, the development of peri-implantitis with bone loss exceeding 2 mm is more frequently observed in the cement-retained type compared with the screw-retained type.<sup>11</sup>

To solve this problem, several cementation techniques have been introduced. The abutment replica technique is a representative cementation technique proposed to reduce cement remnants, where a resin abutment replica is used in the initial extraoral cementation. The extruded excess cement is cleaned outside the mouth, then, the crown is placed into the intraoral abutment to complete setting.<sup>12</sup> However, abutment replica technique tends to make relatively wide voids, which can lead to increased bacterial penetration.<sup>13</sup> Hence, in the present study, the traditional cementation technique was modified by using a microbrush to apply an extremely thin cement layer on the intaglio surface of the crown to minimize the extruded cement and to prevent creating a wide void. In addition, the application of a layer of Vaseline over the external marginal contour of the crown was effective in completely removing the excess cement.

Although either permanent or provisional cement can be used for cement-retained type implant restorations,<sup>14,15</sup> clinicians are highly encouraged to use the least retentive provisional cements because of no risk of decay on the abutments and high retrievability of the restorations.<sup>15-18</sup> The efficacy of provisional cement can be further supported by the fact that there is no clear evidence that the difference in retentiveness between provisional and permanent cement is clinically relevant to the frequency of crown dislodgement.<sup>19,20</sup> Among the provisional cements, the use of methacrylate-based cements, including the Premier Implant Cement has resulted in increased biofilm formation<sup>21</sup> and the development of suppuration and peri-implantitis.<sup>22</sup> Meanwhile, ZOE is contained in probably most common provisional cements, which are used in provisional fixed prostheses and cement-retained type implant restorations. ZOE also has been proven to have antibacterial properties and is cost-effective.<sup>23,24</sup> Based on these data, ZOE is considered the most appropriate cement that can be used to set cement-retained type prosthesis in this study.

Microleakage, which is defined as the passage of ions, molecules, fluid, or bacteria between a cavity wall and the

restorative material that was applied, is one of the most important problems that must be addressed in restorative dentistry. The use of restorative materials that do not perfectly adhere on enamel and dentin causes microleakage and eventually lead to hypersensitivity of the restored teeth, discoloration at the margins of the cavities and restorations, recurrent caries, and plaque deposition.<sup>25,26</sup> In implant restorations, microleakage is associated with bacterial penetration and plaque deposition, which are important causes of peri-implantitis. To evaluate microleakage and marginal gap between the crown and abutment, researchers have commonly assessed dye infiltration using a microscope,<sup>27,28</sup> or have scanned and analyzed with a three-dimensional analysis software.<sup>29,30</sup> However, dye infiltration is a diffusion phenomenon, and hence, the results cannot be obtained immediately. In addition, dye infiltration can be evaluated semi-quantitatively and only in two-dimensional sections.<sup>31</sup> Furthermore, dye penetration between the crown and abutment would not directly reflect bacterial penetration and their viability, which are clinically correlated to the development of peri-implantitis. In this study, we quantitatively measured bacterial penetration by both CFUs counting and ATP-bioluminescence that reflect bacterial viability,<sup>32</sup> thereby evaluating the marginal fitness of the specimens. The experimental procedure performed four times using same protocol, because there is a possibility of contamination during too long ten specimens procedure due to high sensitivity to contamination, and to prevent that the overall results were determined by an error, for example, contamination of the medium.

As shown in Table 1 and Fig. 5, CFUs and ATP-bioluminescence in the CL.F system were lower by approximately 33% and 41%, respectively, than those in the cement-retained type. These results clearly indicate that the CL.F system exhibited less microleakage and higher resistance to bacterial penetration than the cement-retained type. The fitness of the crown increased by relining the resin on the intaglio surface of the zirconia crown and by relatively expanding the relined composite resin. In clinical practice, the CL.F system would be more beneficial in preventing peri-implantitis as it is essentially free of residual excessive cement that increases plaque deposition. Moreover, with its passive fit, the CL.F system is biologically and mechanically advantageous for maintenance.

The degradation of the provisional cement and resulting microleakage inevitably lead to bacterial penetration in the cement-retained type prosthesis. However, the degradation process is slow and is accelerated after the occurrence of microfractures.<sup>33</sup> In this study, within a relatively short period of time (48 h), ZOE has not sufficiently degraded to allow bacteria to penetrate deep into the crown-abutment interface, therefore degradation of provisional cement could be negligible. Rather, comparing with the CL.F system, the characteristics of the cement-retained type prosthesis that favored bacterial adhesion, particularly at the marginal area, may have influenced the number of bacteria penetrating into the abutment-crown interface. Interestingly, in four

repeated bacterial penetration experiments, more than 10<sup>4</sup> CFUs of bacteria were collected from both types of specimens. We speculated that most bacteria were attached to the crown-abutment interface, particularly near the margin. However, we could not rule out the possibility that the bacteria attached to the outer surface of the specimens remained, even after the washing process that was performed before separating the crown and the abutment, and they could be partly included in the CFU counting.

The present study had some limitations that it is a short-term and *in vitro* evaluation. However, we founded that the CL.F system shows precise marginal fit and higher resistance to bacterial penetration than the cement-retained type. Further studies are needed about long-term durability of a precise fit by cyclic loading and thermocycling test, and *in vivo* studies are also needed.

## CONCLUSION

Within limitation of this study, implant prosthesis of the CL.F system is less susceptible to bacterial adhesion and penetration at the abutment-crown interface than the cement-retained type implant prosthesis, due to precise fit. Bacterial cell viability at the abutment-crown interface, which was evaluated by ATP-bioluminescence, is also lower in the CL.F system than in the cement-retained type.

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## REFERENCES

1. Anadioti E, Aquilino SA, Gratton DG, Holloway JA, Denry I, Thomas GW, Qian F. 3D and 2D marginal fit of pressed and CAD/CAM lithium disilicate crowns made from digital and conventional impressions. *J Prosthodont* 2014;23:610-7.
2. Salvi GE, Bragger U. Mechanical and technical risks in implant therapy. *Int J Oral Maxillofac Implants* 2009;24:69-85.
3. Shadid R, Sadaqa N. A comparison between screw- and cement-retained implant prostheses. A literature review. *J Oral Implantol* 2012;38:298-307.
4. Linkevicius T, Vindasiute E, Puisys A, Peciuliene V. The influence of margin location on the amount of undetected cement excess after delivery of cement-retained implant restorations. *Clin Oral Implants Res* 2011;22:1379-84.
5. Linkevicius T, Puisys A, Vindasiute E, Linkeviciene L, Apse P. Does residual cement around implant-supported restorations cause peri-implant disease? A retrospective case analysis. *Clin Oral Implants Res* 2013;24:1179-84.
6. Linkevicius T, Vindasiute E, Puisys A, Linkeviciene L, Maslova N, Puriene A. The influence of the cementation margin position on the amount of undetected cement. A prospective clinical study. *Clin Oral Implants Res* 2013;24:71-6.

7. Lee H, Park S, Kwon KR, Noh G. Effects of cementless fixation of implant prosthesis: a finite element study. *J Adv Prosthodont* 2019;11:341-9.
8. Assif D, Marshak B, Schmidt A. Accuracy of implant impression techniques. *Int J Oral Maxillofac Implants* 1996;11:216-22.
9. Jepsen S, Berglundh T, Genco R, Aass AM, Demirel K, Derks J, Figuero E, Giovannoli JL, Goldstein M, Lambert F, Ortiz-Vigon A, Polyzois I, Salvi GE, Schwarz F, Serino G, Tomasi C, Zitzmann NU. Primary prevention of peri-implantitis: managing peri-implant mucositis. *J Clin Periodontol* 2015;42:S152-7.
10. Wittneben JG, Millen C, Brägger U. Clinical performance of screw- versus cement-retained fixed implant-supported reconstructions—a systematic review. *Int J Oral Maxillofac Implants* 2014;29:84-98.
11. Säiler I, Mühlemann S, Zwahlen M, Hämmerle CH, Schneider D. Cemented and screw-retained implant reconstructions: a systematic review of the survival and complication rates. *Clin Oral Implants Res* 2012;23:163-201.
12. Lee JH, Park IS, Sohn DS. A digital approach to fabricating an abutment replica to control cement volume in a cement-retained implant prosthesis. *J Prosthet Dent* 2016;116:25-8.
13. Canullo L, Cocchetto R, Marinotti F, Oltra DP, Diago MP, Loi I. Clinical evaluation of an improved cementation technique for implant-supported restorations: a randomized controlled trial. *Clin Oral Implants Res* 2016;27:1492-9.
14. Gómez-Polo M, Ortega R, Gómez-Polo C, Celemin A, Del Rio Highsmith J. Factors affecting the decision to use cemented or screw-retained fixed implant-supported prostheses: A critical review. *Int J Prosthodont* 2018;31:43-54.
15. Lemos CA, de Souza Batista VE, Almeida DA, Santiago Júnior JF, Verri FR, Pellizzer EP. Evaluation of cement-retained versus screw-retained implant-supported restorations for marginal bone loss: a systematic review and meta-analysis. *J Prosthet Dent* 2016;115:419-27.
16. Carnaggio TV, Conrad R, Engelmeier RL, Gerngross P, Paravina R, Perezous L, Powers JM. Retention of CAD/CAM all-ceramic crowns on prefabricated implant abutments: an in vitro comparative study of luting agents and abutment surface area. *J Prosthodont* 2012;21:523-8.
17. Mehl C, Harder S, Wolfart M, Kern M, Wolfart S. Retrievability of implant-retained crowns following cementation. *Clin Oral Implants Res* 2008;19:1304-11.
18. Schierano G, Manzella C, Menicucci G, Parrotta A, Zanetti EM, Audenino AL. In vitro standardization of two different removal devices in cemented implant prosthesis. *Clin Oral Implants Res* 2016;27:1026-30.
19. Nejatidanesh F, Savabi O, Ebrahimi M, Savabi G. Retentiveness of implant-supported metal copings using different luting agents. *Dent Res J (Isfahan)*. 2012;9:13-8.
20. Garg P, Gupta G, Prithviraj DR, Pujari M. Retentiveness of various luting agents used with implant-supported prostheses: a preliminary in vitro study. *Int J Prosthodont* 2013;26:82-4.
21. Busscher H, Rinastiti M, Siswomihardjo W, van der Mei H. Biofilm formation on dental restorative and implant materials. *J Dent Res* 2010;89:657-65.
22. Korsch M, Marten SM, Dötsch A, Jáuregui R, Pieper DH, Obst U. Effect of dental cements on peri-implant microbial community: comparison of the microbial communities inhabiting the peri-implant tissue when using different luting cements. *Clin Oral Implants Res* 2016;27:e161-6.
23. Markowitz K, Moynihan M, Liu M, Kim S. Biologic properties of eugenol and zinc oxide-eugenol. A clinically oriented review. *Oral Surg Oral Med Oral Pathol* 1992;73:729-37.
24. Trowbridge HO. Intradental sensory units: physiological and clinical aspects. *J Endod* 1985;11:489-98.
25. Taylor MJ, Lynch E. Microleakage. *J Dent* 1992;20:3-10.
26. Boruziniat A, Gharaee S, Sarraf Shirazi A, Majidinia S, Vatanpour M. Evaluation of the efficacy of flowable composite as lining material on microleakage of composite resin restorations: a systematic review and meta-analysis. *Quintessence Int* 2016;47:93-101.
27. Yavuz I, Aydın H, Research M. New direction for measurement of microleakage in cariology research. *J Int Dent Med Res* 2010;3:19-24.
28. T'jan AH, Dunn JR, Grant BE. Marginal leakage of cast gold crowns luted with an adhesive resin cement. *J Prosthet Dent* 1992;67:11-5.
29. Park JY, Bae SY, Lee JJ, Kim JH, Kim HY, Kim WC. Evaluation of the marginal and internal gaps of three different dental prostheses: comparison of the silicone replica technique and three-dimensional superimposition analysis. *J Adv Prosthodont* 2017;9:159-69.
30. Holst S, Karl M, Wichmann M, Matta RE. A new triple-scan protocol for 3D fit assessment of dental restorations. *Quintessence Int* 2011;42:651-7.
31. Hepdeniz OK, Temel UB, Ugurlu M, Koskan O. The effect of surface sealants with different filler content on microleakage of Class V resin composite restorations. *Eur J Dent* 2016; 10:163-9.
32. Takahashi N, Ishihara K, Kimizuka R, Okuda K, Kato T. The effects of tetracycline, minocycline, doxycycline and ofloxacin on *Prevotella intermedia* biofilm. *Oral Microbiol Immunol* 2006;21:366-71.
33. White SN, Ingles S, Kipnis V. Influence of marginal opening on microleakage of cemented artificial crowns. *J Prosthet Dent* 1994;71:257-64.