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Bacterial Colonization in the Marginal Region of Ceramic Restorations: Effects of Different Cement Removal Methods and Polishing

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Clinical Relevance

The polishing of the ceramic-dentin marginal region rather than excess cement removal techniques reduces significantly the surface roughness. This procedure has an effect in bacterial adhesion, especially when the excess cement is removed with a microbrush.

SUMMARY

This study evaluated the effects of excess cement removal techniques, with or without subsequent polishing, on biofilm formation and micromorphology in the marginal region of the tooth/restoration. From bovine teeth, 96 dentin blocks (4 × 8 × 2 mm) were produced, molded, and reproduced in type IV gypsum, on which 96 pressed ceramic blocks (Vita PM9, Vita Zahnfabrik; 4 × 8 × 2 mm) were produced via the lost wax technique. The dentin blocks and their respective ceramic blocks were cemented with a self-adhesive resin cement (RelyX U200, 3M ESPE), and cement excess was removed from the margin using four different

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techniques, followed or not by polishing with silicone rubber tips: MBr, removal with microbrush and photoactivation; MBr-Pol, MBr + polishing; Br, removal with brush and photoactivation; Br-Pol, Br + polishing; Photo-Expl, 5 seconds of initial photoactivation, removal with explorer, and final curing; Photo-Expl-Pol, Photo-Expl + polishing; Photo-SB, 5 seconds of initial photoactivation, removal with scalpel, and final curing; and Photo-SB-Pol, Photo-SB + polishing. After 24 hours, the roughness in the marginal region was analyzed using a profilometer (three measurements on each sample). Micromorphological analyses of the region were performed by stereomicroscope and scanning electron microscopy (SEM). Then the samples were contaminated with sucrose broth standardized suspension with *Streptococcus mutans*, *Staphylococcus aureus*, and *Candida albicans* and incubated for a period of 48 hours. The samples were quantitatively analyzed for bacterial adherence in the marginal region by confocal laser scanning microscopy and counting of colony-forming units (CFUs/mL) and qualitatively analyzed using SEM. Roughness data (Ra) were submitted to two-way analysis of variance, Tukey test at a confidence level of 95%, and Student *t*-tests. CFU, biomass, and biothickness data were analyzed by Kruskal-Wallis, Mann-Whitney, and Dunn tests. The removing technique statistically influenced Ra (MBr, $p=0.0019$; Br, $p=0.002$; Photo-Expl, $p=0.0262$; Photo-SB, $p=0.0196$) when comparing the polished and unpolished groups. The MBr and MBr-Pol technique differed significantly for CFU/mL values ($p=0.010$). There was no significant difference in the amounts of biomass and biothickness comparing polished and unpolished groups and when all groups were compared ($p>0.05$). Different morphological patterns were observed (more regular surface for polished groups). We conclude that margin polishing after cementation of feldspar/pressed ceramic restorations is decisive for achieving smoother surfaces, as the excess cement around the edges can increase the surface roughness in these areas, influencing bacterial adhesion.

INTRODUCTION

In recent years, adhesively cemented ceramic restorations, such as inlays/onlays and veneers, have been used as the main approach for minimally invasive esthetic restorations in anterior and posterior teeth. However, its clinical success is related, among other factors, to the quality of tooth restoration.[1] Factors such as marginal misfit,[2] surface irregularities,[3] and cement excess[3,4] may favor the accumulation of microorganisms,[3] compromising restoration clinical longevity.

The cement retained on the tooth surface can promote plaque accumulation, leading to gingivitis and radicular surface demineralization.[4] In the case of the implant/prosthetic restoration interface, excess cement has a clinical impact, resulting in peri-implantation bone loss and bleeding on probing and resulting in implant loss.[5-11]

Excess cement on the tooth-restoration interface promotes bacteria adhesion due to the presence of surface irregularities.[3] Biofilm establishment in that area promotes periodontal tissues and peri-implant inflammation, compromising esthetics.[12,13] At the same time, biofilm can also promote a deleterious effect on the bond strength between the tooth and the restoration.[14-17]

Due to esthetic contingencies, preparation margins are commonly positioned at the intrasulcular level hindering access to proper excess cement removal. Consequently, a more favorable environment promoting the deleterious effects of biofilm can be seen.[18-25]

Considering the surface roughness effect on microbial adhesion and retention,[19,23,26-32] various excess cement removal protocols for tooth restoration[3,4,33] and implant-restoration interfaces[5-11] have been investigated. Anami and others[3] found that the morphology of the tooth-ceramic interface, according to different protocols of excess cement removal, influenced the *in vitro* adhesion of *Streptococcus mutans*.

However, polishing-associated evidence of different effects of cement removal methods on the morphology of ceramic restoration margins, roughness, and bacterial colonization is scarce. As is known, the polishing of ceramic and polymeric surfaces is performed for surface smoothing by light surface abrasion, which might impact bacterial adherence.

Considering the aforementioned premises, the research questions for this study were the following: Do different resin-cement excess removal techniques affect marginal region morphology and *in vitro* biofilm formation? Does marginal polishing have an effect on surface roughness and *in vitro* biofilm formation after excess cement removal?

Thus, the objective of this study was to assess whether the resin-cement excess removal methods in the marginal area between tooth and ceramic restorations, with or without polishing of the margin region, have an effect on surface roughness and *in vitro* biofilm formation.

METHODS AND MATERIALS

Obtaining Tooth Samples

Ninety-six dentin blocks were obtained from freshly extracted bovine incisors. The teeth were cleaned with periodontal curettes, Robinson brushes, and pumice. Crowns were removed under constant cooling using diamond discs (EDENTA, Au, Switzerland) mounted in a handpiece. The buccal surface was ground with silicon carbide paper (#100) adapted to a polishing device (METASERV 3000, Buehler, Lake Bluff, IL, USA) until an 8 × 4-mm area of dentin had been exposed. Then the dentin was cut into a block of 2-mm thickness. The smear layer was standardized by grinding with silicone carbide paper (#600) under constant cooling.[34,35] The final dimensions of 8 × 4 × 2 mm were verified with a digital caliper (Mitutoyo, São Paulo, Brazil). The blocks remained immersed in distilled water in an oven (Orion 502, Fanem, São Paulo, Brazil) at 37°C until the time of the cementation procedure.

Obtaining Ceramic Samples

In order to enable proper positioning of the ceramic blocks on the dentin surface during cementation, two 1-mm-deep cavities (guides) were made with a spherical diamond bur (EDENTA) on a 4 × 8-mm surface of each dentin block. These guides were made with an adapted device to standardize depth and the insertion axis.

The marked dentin surface of each specimen was molded with impression material (Express, 3M ESPE, St Paul, MN, USA) and a model in plaster type IV (Durone, Dentsply, Petrópolis, Brazil) was obtained on which a wax-up was performed for each specimen with dimensions similar to its respective dentin block. Prior to waxing, VITA In-Ceram interspace varnish (Vita Zahnfabrik, Bad Säckingen, Germany) was applied on dye surfaces, except in marginal areas, to facilitate fitting between blocks.

Ceramic blocks were produced by the lost-wax casting technique, followed by ceramic material injection. For this, the waxing was included in investment, and the ceramic (PM9, Vita Zahnfabrik) was pressure injected (Vita Vacumat 6000 MP, Vita Zanhfabrik) following the manufacturer's instruction. Ceramic samples were removed from coating with the aid of sandblasting.

Table 1: *Experimental Design, Considering Two Study Factors (Cement Removal Technique and Polishing: Presence/Absence) (n=12)*

Groups (Codes)	Study Factors	
	Cement Removal Technique	Polishing[a]
MBr	Microbrush:[b]	—
MBr-Pol	Excess removal with microbrush and photoactivation	With*
Br	Brush:[c]	—
Br-Pol	Excess removal with brush and photoactivation	With*
Photo-Expl	Photoactivation and explorer:	—
Photo-Expl-Pol	Photoactivation for 5 s, excess removal with explorer	With*
Photo-SB	Photoactivation and scalpel blade:	—
Photo-SB-Pol	Photoactivation for 5 s, excess removal with scalpel blade #12	With*

[a] *Polishing with silicon rubber tips (Shofu, San Marcos, CA, USA).*
[b] *Microbrush (KG Sorensen, Cotia, Brazil).*
[c] *Brush (Kota, Cotia, Brazil).*

The corresponding ceramic and dentin blocks were simultaneously polished with silicon carbide paper (#400, #600, #800, #1200, #1500) through a polishing machine (Metaserv 3000, Buehler) under constant cooling. The cementation surfaces of both blocks were not polished. All ceramic blocks were submitted to ultrasonic cleaning in isopropyl alcohol for five minutes (Vitasonic, Vita Zanhfabrik).

Ceramic and tooth blocks were analyzed by a stereomicroscope (10×/50×, Discovery V20, Zeiss, Göttingen, Germany) to ensure the absence of bubbles. Ninety-six sets of ceramic/dentin samples were counted and randomly distributed into eight groups (Table 1) using a specific Internet-based tool (www.randomizer.org), considering two study factors (n=12): excess cement removal technique (in four levels) and polishing (in two levels). The experimental unit of this study was the ceramic/dentin interface.

Cementation of Samples

Dentin blocks were cleaned with pumice and water, and excess water was removed with air jets without drying of the dentin. Ceramic blocks were cleansed with isopropyl alcohol ultrasonic bath for 480 seconds; surfaces were etched with 10% hydrofluoric acid for 60 seconds, washed, and air jet dried. Silane agent (Ceramic Primer, 3M/ESPE) was applied and air jet dried for five seconds.

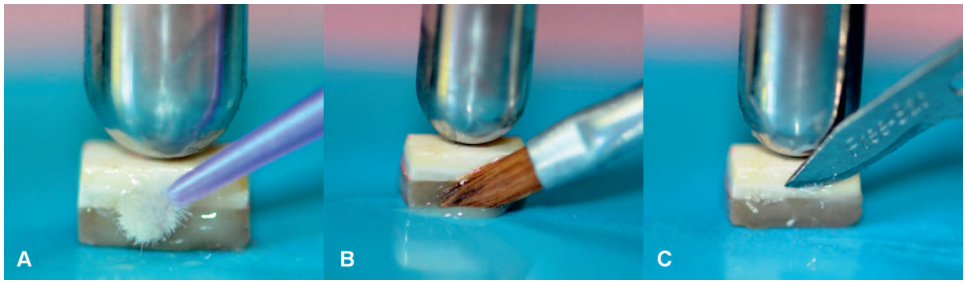


Figure 1. *Photos show cement removal using microbrush (A), brush (B), and blade (C) from the marginal region.*

Self-adhesive resin cement (RelyX U200, 3M ESPE) was used according to the manufacturer's recommendations. The cement was dispensed and applied with the aid of a plastic spatula on the ceramic surface, and it was positioned on its respective dentin block. A 750-g vertical load was used to correctly position the blocks during curing. The excess of resin-cement was removed according to the techniques described in Table 1 (Figure 1).

Microbrushes and brushes were passed through the marginal region in one direction to remove the remaining excess cement (one microbrush/brush for each specimen). The brush was perpendicularly positioned to the cementation line to prevent the input of bristles onto the cementation line and the formation of grooves in the region. The explorer and scalpel blade were used in one lever movement to displace excess cement. Polishing was carried out in dry conditions.

Each side of the set was photoactivated for 40 seconds, and after five minutes at room temperature, the cemented blocks were stored in distilled water at 37°C for 24 hours.

Surface Roughness

Quantitative analysis of surface roughness was performed with a contact profilometer (SJ 400, Mitutoyo, Tokyo, Japan). The samples were positioned with the interfacial area, perpendicular to the tip, which performed three measurements (about 1-mm distance between measurements) following a 3-mm path on each sample. Mean values of roughness arithmetic average (Ra, in μm) were obtained for each sample.

Sterilization

All samples were sterilized by gamma radiation. For this, they were individually wrapped in hot sealed surgical-type paper. The Embrarad Company (Cotia, Brazil) is authorized by ANVISA (Brazilian Health Surveillance Agency, São Paulo, Brazil) and ISO 9001 certified and performed the sterilization process with a gamma radiation camera (cobalt 60) at 20 kGy for six hours.

Morphological Analysis by Scanning Electron Microscopy

One sample of each group was evaluated by scanning electron microscopy (SEM) under 300× magnification (Inspect S50, FEI Company, Brno, Czech Republic) for marginal quality characterization. The samples were fixed in the sampler with a double-sided carbon adhesive tape (SPI, West Chester, PA, USA). The top surface was coated with gold-palladium alloy (Polaron SC 7620 Sputter Coater, Quorum Technologies, Newhaven, UK) (time: 130 s; 10-15 mA current; 130 mTorr vacuum; plating rate: 3.5 nm/min; Pd-Au layer: about 80 A). The SEM was operated at 20 kV.

Biofilm Analysis

Biofilms were formed according to the following methodology: standardized suspensions were prepared using standard strains of *Candida albicans* (ATCC 18804), *Staphylococcus aureus* (ATCC 6538), and *S. mutans* (ATCC 35688). The *C. albicans* strains were subcultured onto Sabouraud dextrose agar (Difco, Detroit, MI, USA). The strains of *S. aureus* and *S. mutans* were picked in brain heart infusion agar (BHI) (Brain Heart Infusion, Difco). The microorganisms were incubated in a bacteriological incubator at 37°C for 24 hours. All tests with *S. mutans* strains were incubated in a bacteriological oven under microaerophilic conditions (5% CO_2). After the incubation period, microorganism colonies were suspended in sterile saline solution (0.9% NaCl) and set in a spectrophotometer (B 582, Micronal, São Paulo, Brazil) for obtaining a standardized suspension containing 10^6 cells/mL. The parameters of optical density and wavelength were, respectively, 0.284 and 530 nm for *C. albicans*, 0.374 and 490 nm for *S. aureus*, and 0.620 and 398 nm for *S. mutans*.

Table 2: *Mean, Standard Deviation, Confidence Interval, Tukey Test Groups, and Student* t*-Test Results for Ra Data (μm) for the Experimental Groups*[a]

Cement Removal Technique	Ra (μm)		
	Polishing		*p*-Value[d]
	Absence[b]	Presence (Pol)[c]	
MBr	4.5 ± 2.2 (2.90-6.16) a	1.8 ± 0.6 (1.37-2.26) A	0.0019
Br	4.6 ± 1.7 (3.38-5.92) a	1.7 ± 0.7 (1.27-2.31) A	0.0002
Photo-Expl	3.9 ± 1.2 (3.12-4.86) ab	2.5 ± 1.3 (1.63-3.56) A	0.0262
Photo-SB	2.7 ± 1.0 (2.06-3.50) bc	1.8 ± 0.4 (1.55-2.21) A	0.0196

[a] *One-way analysis of variance and Tukey tests.*
[b] *Different lowercase letters indicate statistically significant differences ($p<0.05$) in the absence of polishing.*
[c] *Different capital letters indicate statistically significant differences ($p<0.05$) in the presence of polishing.*
[d] *Student* t*-test; $p<0.05$ indicates statistically different means.*

For biofilm formation, the broth proposed by Gybbons and Nygaard[36] was used, which is composed of 20 g trypticase, 2 g NaCl, 3 g K_2HPO_4, 2 g KH_2PO_4, 1 g K_2CO_3, 120 mg $MgSO_4$, 15 mg $MnSO_4$, and 50 g $C_6H_8O_7$ g, dissolved in 1000 mL of distilled water. The broth was sterilized by autoclaving at 121°C for 15 minutes. The sterilized specimens were placed in the first row of 24-well plates (Costar Corning, Corning, NY, USA) with the aid of a sterile tweezers, and 0.1 mL of each microbial suspension was inoculated in each well of the plate for the formation of the multispecies (heterotypic) biofilm. The plates were incubated in a bacteriological incubator maintained at 37°C for 48 hours.

CFU Counting (CFU/mL)—In order to remove the nonadherent microbial cells, 2 mL of broth were substituted by 2 mL of sterile saline solution in each well, and the plate was stirred for five minutes with an orbital shaker (Solab, Piracicaba, Brazil).

The CFU count was performed on 10 samples of each group. For this, specimens were individually placed in falcon tubes containing 10 mL of sterile saline solution and then homogenized for 30 seconds using a 50 W power ultrasonic homogenizer (Sonoplus HD 2200, Electronic Bandelin, Berlin, Germany). The obtained microbial suspension had 10^{-1} dilution factor, and new decimal dilutions were performed (10^{-2}, 10^{-3}, 10^{-4}, 10^{-6}) in sterile saline solution; 0.1-mL aliquots of each dilution were placed into duplicate Petri dishes with selective media for each microorganism as follows: 1) *C. albicans* in Sabouraud dextrose agar with 50 mg/L of chloramphenicol (Union Chemicals, São Paulo, Brazil), 2) *S. aureus* in NaCl BHI agar (Difco), and 3) *S. mutans* in Mitis Salivarius agar (Difco) plus 0.2 IU/mL of bacitracin (Chemical Union, São Paulo, Brazil) and 15% sucrose. The plates were incubated at 37°C for 48 hours, and the plates containing from 30 to 300 colonies were counted for the CFU number.

Since samples were contaminated by a biofilm composed of three microorganisms (*S. mutans*, *C. albicans*, and *S. aureus*), for each sample, the definitive CFU/mL value was obtained by summing each individual CFU/mL value. Moreover, due to data variation, CFU/mL values were converted into $\log_{10}$.

Biofilm Analysis Using SEM—Sixteen samples were fixed using the following protocol: immersion in glutaraldehyde solution at 2.5% (for 1 hour) and subsequent dehydration through a series of passages

Table 3: *Mean, Standard Deviation, Confidence Interval, Dunn Test Groups, and Mann-Whitney Test Results of CFU/mL $\log_{10}$ Values for the Experimental Groups*

Cement Removal Technique	CFU/mL $\log_{10}$		
	Polishing		*p*-Value[c]
	Absence[a]	Presence (Pol)[b]	
MBr	9.35 ± 0.19 (9.21-9.49) ab	9.16 ± 0.09 (9.09-9.24) A	0.010
Br	9.21 ± 0.17 (9.08-9.34) b	9.25 ± 0.11 (9.16-9.33) A	0.424
Photo-Expl	9.33 ± 0.12 (9.23-9.42) ab	9.30 ± 0.16 (9.18-9.41) AB	0.725
Photo-SB	9.57 ± 0.37 (9.31-9.84) a	9.46 ± 0.09 (9.39-9.53) B	0.896

[a] *Comparison between unpolished groups using Kruskal-Wallis and Dunn tests: different lowercase letters indicate statistically significant differences.*
[b] *Comparison between polished groups using the Kruskal-Wallis and Dunn tests: different capital letters indicate statistically significant differences.*
[c] *Comparison between absence and presence of polishing of each cement removal technique using Mann-Whitney test: $p<0.05$ indicates statistical difference.*

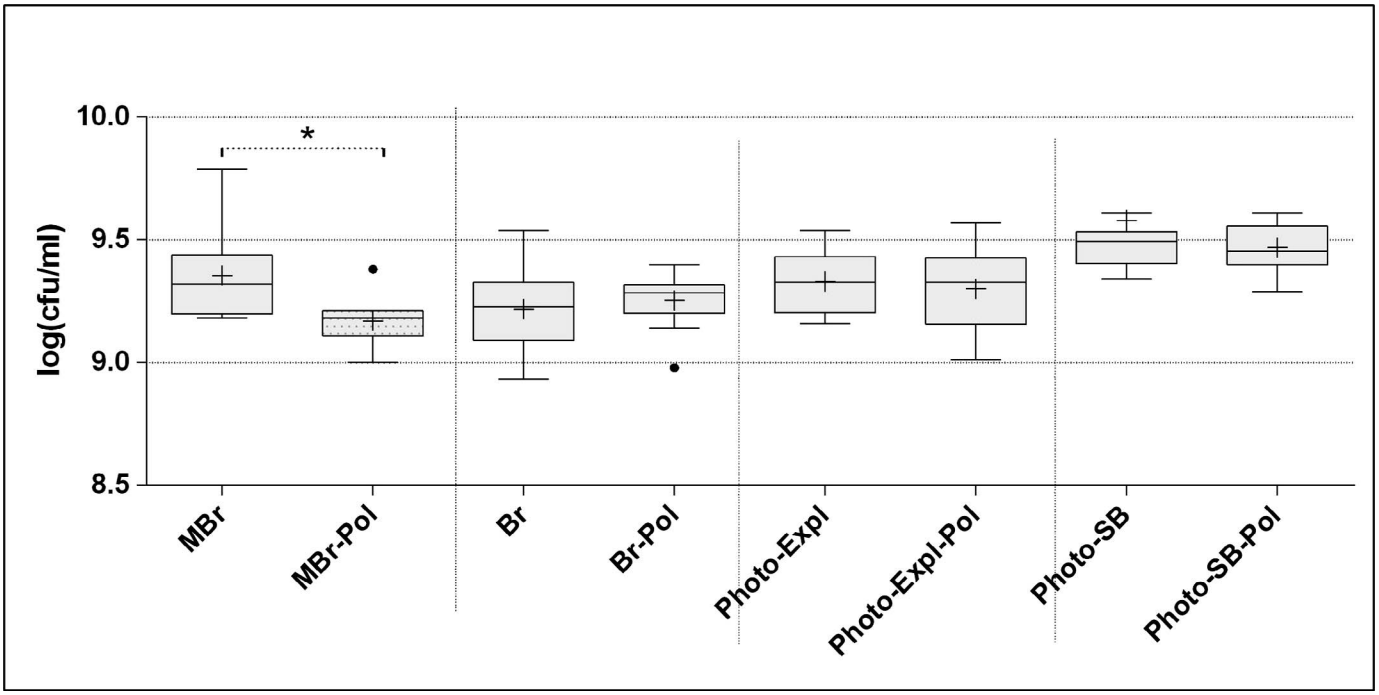


Figure 2. *Box-whisker plots of the CFU/mL log_{10} for the different experimental groups. The means are represented by +, and the medians are represented by the central line. The boxes represent the interquartile ranges. Groups that are statistically significant are denoted by * (Mann-Whitney test).*

with an ethanol concentration increase (50%, 70%, 80%, 90%, and 95% for 20 minutes and 100% for 1 hour).

Samples were gold coated, and SEM (4000×) was operated as previously described. A descriptive analysis of the material formed on the samples was performed.

Biofilm Analysis by Laser Scanning Confocal Microscopy—Ten samples from each experimental group were removed from the culture medium with heterotypic biofilm and then placed on a glass slide with the aid of a sterile tweezers. The Live/Dead Light BAC Bacterial Viability and Counting Kit (Molecular Probes, Eugene, OR, USA) was used for bacterial population viability monitoring, as described by Anami and others.[3]

The contaminated surface of each specimen was analyzed by laser scanning confocal microscopy (LSCM) (LSM 510 META, Zeiss). COMSTAT software was used to characterize the biofilm in terms of mean thickness (μm) and biovolume ($\mu m^3/\mu m^2$).

Data Analysis

After confirming normality assumptions, the roughness data were analyzed by two-way analysis of variance and Tukey and Student *t*-tests with a significance level of 95%. CFU/mL log_{10}, mean thickness, and biovolume were analyzed by nonparametric tests (Kruskal-Wallis, Dunn, and Mann-

Table 4: *Mean, Standard Deviation, Confidence Interval, and Mann-Whitney Test Results of Biovolume Values ($\mu m^3/\mu m^2$) for the Experimental Groups*

Cement Removal Technique	Biovolume		
	Polishing		*p*-Value[c]
	Absence[a]	Presence (Pol)[b]	
MBr	0.027 ± 0.030 (0.01-0.052)	0.021 ± 0.035 (−0.006-0.048)	*p*=0.3176
Br	0.006 ± 0.010 (−0.06-0.019)	0.023 ± 0.034 (−0.005-0.050)	*p*=0.4103
Photo-Expl	0.047 ± 0.095 (−0.05-0.14)	0.015 ± 0.027 (−0.007-0.037)	*p*=0.9038
Photo-SB	0.036 ± 0.063 (−0.00-0.76)	0.020 ± 0.024 (0.001-0.038)	*p*=0.7859

[a,b,c] *No statistically significant difference was detected.*

Table 5: *Mean, Standard Deviation, Confidence Interval, and Mann-Whitney Test Results of Biofilm Thickness Values (μm) for the Experimental Groups*

Cement Removal Technique	Biofilm Thickness		
	Polishing		*p*-Value[c]
	Absence[a]	Presence (Pol)[b]	
MBr	0.060 ± 0.080 (−0.001-0.120)	0.042 ± 0.074 (−0.011-0.094)	*p*=0.2179
Pi	0.003 ± 0.004 (−0.003-0.008)	0.050 ± 0.093 (−0.028-0.127)	*p*=0.6848
Photo-Expl	0.005 ± 0.005 (−0.000-0.009)	0.052 ± 0.092 (−0.025-0.128)	*p*=0.5869
Photo-SB	0.103 ± 0.166 (−0.015-0.221)	0.096 ± 0.171 (−0.035-0.227)	*p*=0.9047

[a,b,c] *No statistically significant difference was detected.*

Whitney) with significance level of 95% (Statistix 8.0, Analytical Software Inc., Tallahassee, FL, USA).

RESULTS

Surface Roughness

The mean and standard deviation data of surface roughness in the experimental groups are shown in Table 2.

Regardless of the cement removal technique, polishing reduced the surface roughness significantly (Table 2). In the absence of polishing, the photoactivation prior to cement removal reduced surface roughness values, particularly when the blade was used to remove the cement. No difference was observed between the cement removal techniques when the polishing was performed (Table 2).

CFU/mL $\log_{10}$

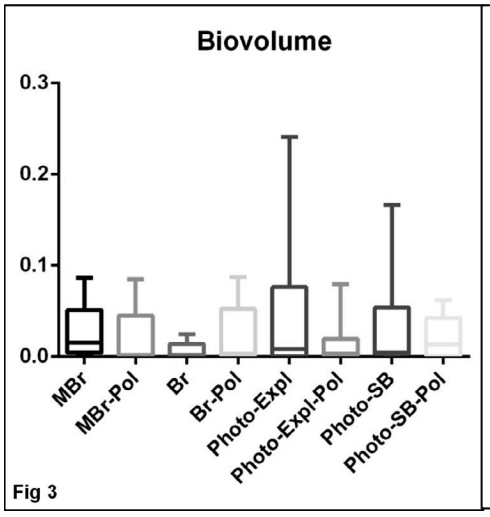
The mean and standard deviation data of CFU/mL $\log_{10}$ in experimental groups are shown in Table 3. Medians and interquartile ranges for CFU/mL $\log_{10}$ are shown in Figure 2.

In the absence of polishing, a statistically significant difference was observed only between Br and Photo-SB groups. When polishing was carried out, cement removal by microbrush or brush resulted in lower biofilm accumulation compared to the blade removal technique. The polishing reduced the counts of CFU/mL for the MBr group (MBr vs MBr-Pol) significantly but had no influence on the other excess cement removal techniques (Table 3).

Biovolume and Biofilm Thickness

The mean and standard deviation data of biovolume and biofilm thickness in the experimental groups are shown in Tables 4 and 5, respectively. Medians and interquartile ranges of biovolume and biofilm thickness data are shown in Figures 3 and 4, respectively.

Biovolume and biofilm thickness were not influenced by the applied cement removal technique



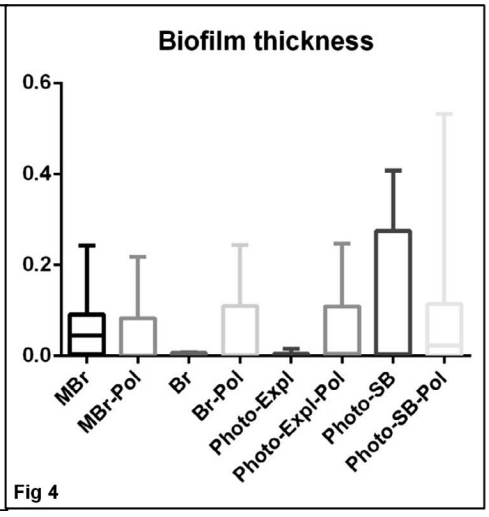


Figure 3. *Graph of median and interquartile ranges for biovolume values for experimental groups.*
Figure 4. *Graph of median and interquartile ranges for biofilm thickness values for experimental groups.*

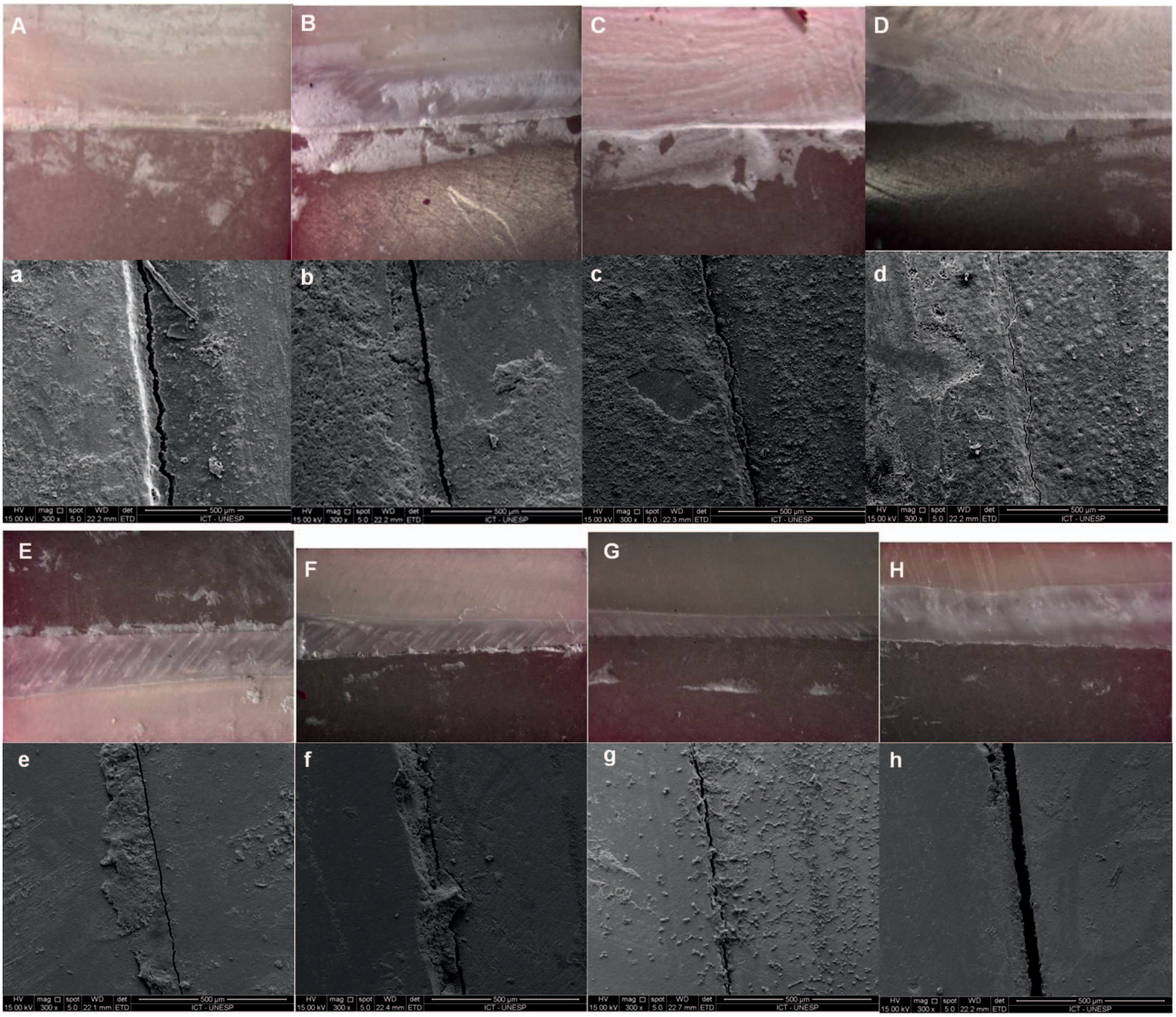


Figure 5. *Stereomicroscopic (A-H) (20×) and SEM (a-h) images (500×) of the marginal region for excess cement removal methods: MBr (A, a), MBr-Pol (B, b), Br (C, c), Br-Pol (D, d), Photo-Expl (E, e), Photo-Expl-Pol (F, f), Photo-SB (G, g), Photo-SB-Pol (H, h).*

(p=0.389 and p=0.249, respectively) or by the polishing (p=0.950 and p=0.723, respectively).

Micromorphological Analysis

The surface characteristics of the region/ceramic interface after the application of different excess cement removal techniques are presented in Figure 5.

The cement had overlaid the tooth and ceramic surface in the marginal region in all groups. In polished samples, the remaining cement appeared to be smoother and with a less rough appearance, especially in the MBr-Pol and Br-Pol groups compared with the unpolished MBr and Br groups (Figure 5Aa,Bb,Cc,Dd). In the margin, resulting from the Photo-Expl and Photo-SB techniques, small irregularities concentrated near the marginal line region were observed (Figure 5Ee,Gg). Grooves are still visible in the enamel (Figure 5H).

Biofilm Analysis by SEM

Figure 6 shows representative micrographs of the heterotypic biofilm on the samples. After 48 hours of *in vitro* biofilm formation, significant adhesion and the presence of colonies of *S. mutans*, *C. albicans*, and *S. aureus* were noted regarding the experimen-

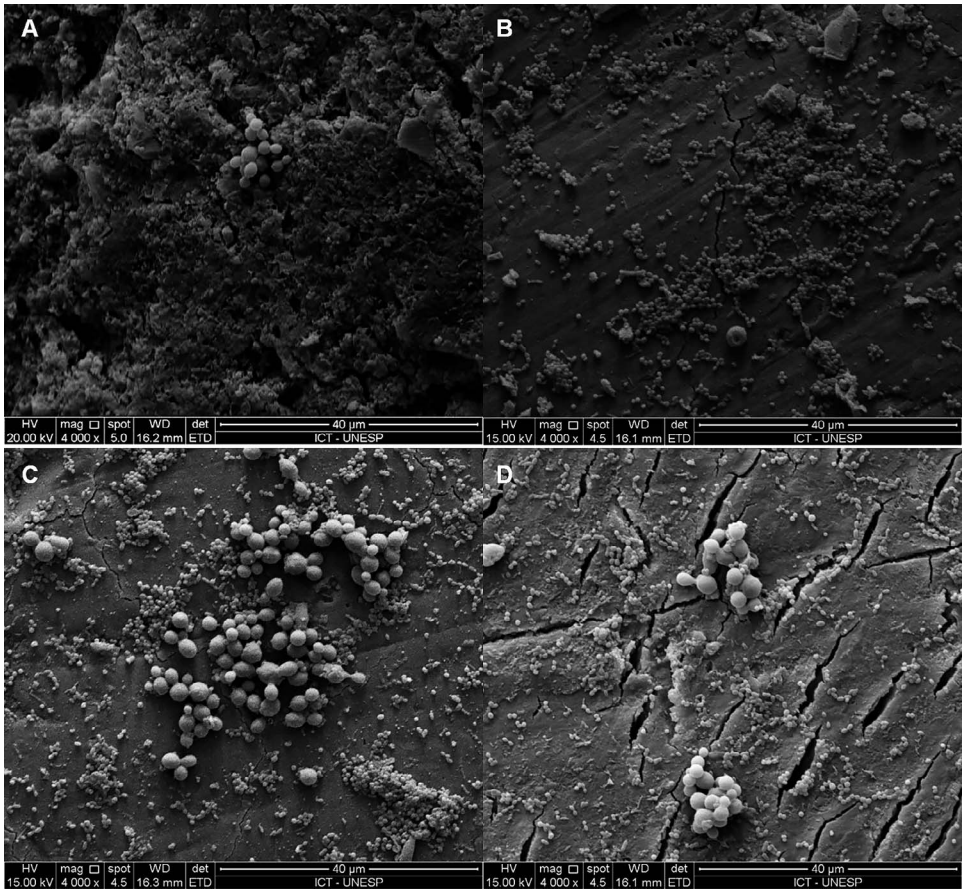


Figure 6. *Representative micrographs (4000×) of the biofilm formed on samples in the Pi-Pol (A), Pi- Pol (B), Photo-Expl-Pol (B), and Photo-Bi-Pol (C) groups.*

tal groups. The time of biofilm formation (48 hours) was not enough to recover the surface of specimens with microorganism colonies.

Biofilm Analysis by LSCM

Figure 7 shows representative LSCM images of the formed biofilm on samples. The three types of microorganisms were found (*S. mutans*, *S. aureus*, and *C. albicans*). Green points in the images correspond to "live" microorganisms and red points to "dead" microorganisms. In Figure 7A,B, it is possible to observe a balance between live and dead microorganisms. Figure 7C,D shows mainly dead microorganisms. These models of biofilm presentation were observed in all experimental groups.

DISCUSSION

This study showed that polishing ceramic and tooth marginal areas provides a significantly smoother surface regardless of the applied cement removal technique. Thus, polishing the margins after cementation is more important than the selected cement removal technique.

Considering the microbiological analysis, polishing significantly reduced the CFU/mL counts for the MBr group (MBr vs MBr-Pol) but had no influence on other excess cement removal techniques. In the LSCM analyses of biofilm thickness and biovolume, no difference was observed between groups despite polishing.

Taking into account the experiments without polishing, photoactivation prior to cement removal reduced surface roughness, particularly when the blade was used over the microbrush or brush. This result probably occurred because the prior five seconds of photoactivation provided partial cement polymerization and possibly facilitated explorer and blade excess removal before final photoactivation. This was evidenced by the stereomicroscope micrographs (Figure 5), through which it was observed that prior photoactivation, associated with explorer or blade to cement removal, resulted in a smoother surface, from a topographical point of view, in all tooth/cement/ceramic regions.

Considering the polishing experiments, the stereomicroscopic micrographs demonstrated that the

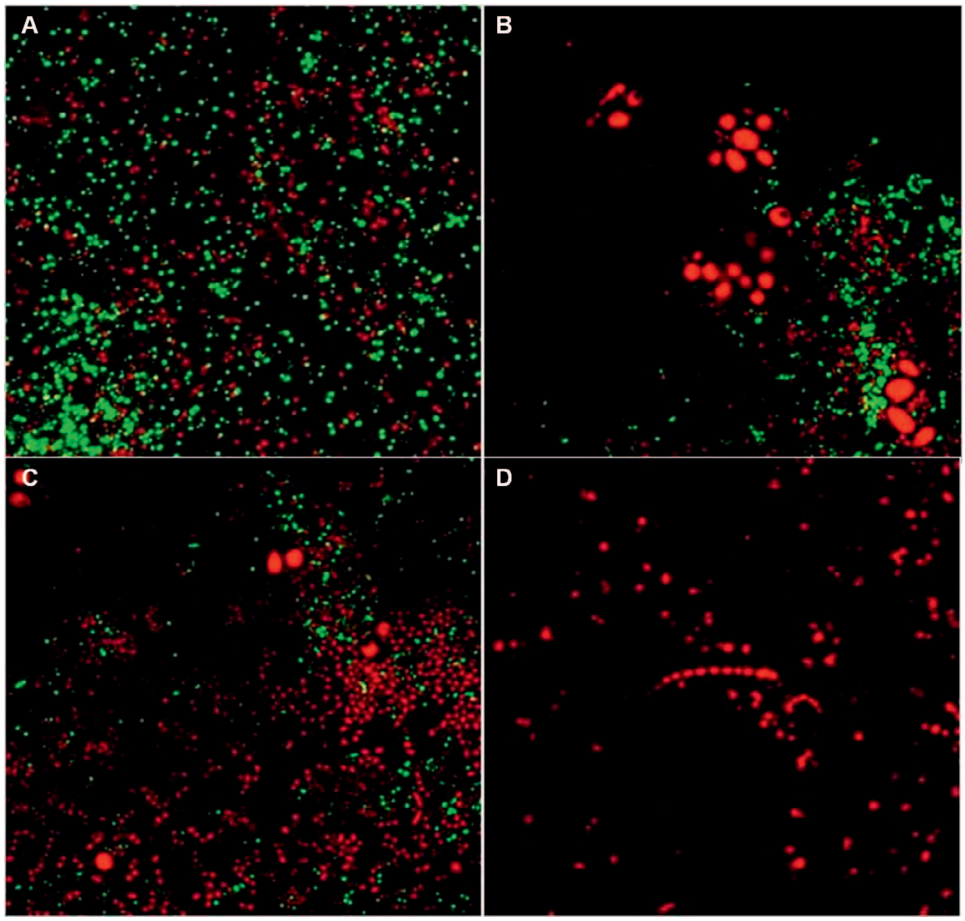


Figure 7. *Representative micrographs of the biofilm formed on the samples in Br-Pol (A) and Photo-Expl-Pol (B-D) groups; the presence of the three types of microorganisms can be verified.*

residual cement was present on the tooth and the ceramic for the microbrush or brush techniques. This did not occur when prior photoactivation was carried out. However, the subsequent polishing provided similar roughness between the techniques despite the presence of residual cement. Contrasting the results of this study, Anami and others[3] demonstrated that polishing, subsequent to cement removal, promoted an increase in surface roughness. The divergent findings may be related to the type of abrasive material for polishing, which can abrade the surface differently, making it rougher or smoother. We used the proper polishing silicone tips indicated for margin restoration.

In the microbial culture analysis, statistical differences were observed between the testing groups (Table 3). In the absence of polishing, the Br group presented a statistically lower number of the CFU/mL $\log_{10}$ than the SB group. However, the Br group showed higher surface roughness values than the SB group. This finding shows that other factors besides surface roughness affect bacterial adhesion.

Importantly, polishing significantly reduced roughness values but did not affect the reduction in microbial adhesion. This can be explained by the fact that biofilm formation time (48 hours) is a rather long time for the roughness effect on microbial adhesion to take place and because of other factors that have an influence on biofilm formation, apart from roughness, such as the material's surface free energy.[37] Undoubtedly, surface roughness has an effect on initial microorganism adherence. Irregularities increase the available adhesion area and protect bacteria from regulatory and control mechanisms of the oral microbiota.[37] Accordingly, biofilm may exhibit more rapid maturation in these areas.[37-40] In addition, evidence that microorganisms remain in surface irregularities despite tooth brushing were presented.[41] Thus, considering frequent subgingival positioning of the tooth-cement-ceramic margin and that microorganism retention in surface irregulari-

ties is greater, smoother margins may have a clinical impact favoring restoration longevity.

The LSCM images allied with the Constat and Matlab programs can be useful in the quantitative analysis[19,31,42] of biofilm formed on the material and provides, among other things, biovolume and biofilm thickness. For biovolume and biofilm thickness values, it was observed that cement removal techniques were statistically similar in both polishing conditions (absence and presence). When comparing the influence of polishing in each technique, no significant difference was observed. Using LSCM, Brentel and others[19] observed differences among the groups and positive correlations between surface roughness/biovolume and roughness/biofilm thickness.

From the SEM and LSCM qualitative analyses,[3,18,19,43] the presence of the three microorganisms (*S. mutans*, *S. aureus*, and *C. albicans*) showed similar behavior of adhesion for the different techniques.

In conclusion, a well-polished surface is important for biofilm formation and definitive esthetic results. Thus, after the excess cement removal technique, polishing has a great importance when considering the evaluated materials and techniques. Clinical trials and/or *in situ* studies could be conducted to assess the clinical behavior of various cement removal techniques and polishing conditions in the marginal region.

CONCLUSIONS

- Marginal surface polishing has a significant influence on surface roughness for all of the cement removal techniques.
- The microbrush polishing technique significantly reduced the CFU/mL $\log_{10}$ values compared with the unpolished condition, although it has not been relevant to other techniques.

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Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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REFERENCES

1. Liebenberg WH (1999) Interproximal integrity of posterior partial-coverage ceramic restorations: Sequence of delivery and finishing *Quintessence International* **30(12)** 807-818.
2. Cenci MS, Pereira-Cenci T, Cury JA, & Ten Cate JM (2009) Relationship between gap size and dentine secondary caries formation assessed in a microcosm biofilm model *Caries Research* **43(2)** 97-102.
3. Anami LC, Pereira CA, Guerra E, Souza ROA, Jorge AO, & Bottino MA (2012) Morphology and bacterial colonization of tooth/ceramic restoration interface after different cement excess removal techniques *Journal of Dentistry* **40(9)** 742-749.
4. Mansour YF, Pintado MR, & Mitchell CA (2006) Optimizing resin cement removal around esthetic crown margins *Acta Odontologica Scandinavica* **64(4)** 231-236.
5. Korsch M, Robra BP, & Walther W (2015) Cement-associated signs of inflammation: Retrospective analysis of the effect of excess cement on peri-implant tissue *International Journal of Prosthodontics* **28(1)** 11-8.
6. Korsch M, Marten SM, Dötsch A, Jáuregui R, Pieper DH, & Obst U (2015) Effect of dental cements on peri-implant microbial community: Comparison of the microbial communities inhabiting the peri-implant tissue when using different luting cements *Clinical Oral Implants Research* 1-6 In press.
7. Korsch M, Robra BP, & Walther W (2015) Predictors of excess cement and tissue response to fixed implant-supported dentures after cementation *Clinical Implant Dental Related Research* **17(Supplement 1)** e45-e53.
8. Korsch M, & Walther W (2015) Peri-implantitis associated with type of cement: A retrospective analysis of different types of cement and their clinical correlation to the peri-implant tissue *Clinical Implant Dental Related Research* **17(Supplement 2)** e434-443.
9. Korsch M, Obst U, & Walther W (2014) Cement-associated peri-implantitis: A retrospective clinical observational study of fixed implant-supported restorations using a methacrylate cement *Clinical Oral Implants Research* **25(7)** 797-802.
10. Korsch M, Walther W, Marten SM, & Obst U (2014) Microbial analysis of biofilms on cement surfaces: An investigation in cement-associated peri-implantitis *Journal Applied Biomaterials & Functional Materials* **12(2)** 70-80.
11. Wilson TG Jr (2009) The positive relationship between excess cement and peri-implant disease: A prospective clinical endoscopic study *Journal of Periodontology* **80(9)** 1388-1392.
12. Christensen RP, & Ploeger BJ (2010) A clinical comparison of zirconia, metal and alumina fixed-prosthesis frameworks veneered with layered or pressed ceramic: A three-year report *Journal American Dental Association* **141(11)** 1317-1329.

13. Lange RT, & Pfeiffer P (2009) Clinical evaluation of ceramic inlays compared to composite restorations *Operative Dentistry* **34(3)** 263-272.

14. Mutluay MM, Zhang K, Ryou H, Yahyazadehfar M, Majd H, Xu HH, & Arola D (2013) On the fatigue behavior of resin-dentin bonds after degradation by biofilm *Journal of Mechanical Behavior of Biomedical Materials* **18(February)** 219-231.

15. Schmidlin PR, Siebenmann J, Kocher P, Seemann R, Attin T, & Bindl A (2008) Effects of de- and remineralization of dentin on bond strengths yielded by one-, three-, and four-step adhesives *Journal of Adhesive Dentistry* **10(2)** 119-126.

16. Silva EM, Almeida GS, Poskus LT, & Guimaraes JG (2012) Influence of organic acids present in the oral biofilm on the microtensile bond strength of adhesive systems to human dentin *Journal of Biomedical Materials Research: Part B Applied Biomaterials* **100(3)** 735-741.

17. Zhang K, Cheng L, Imazato S, Antonucci JM, Lin NJ, Lin-Gibson S, Bai Y, & Xu HH (2013) Effects of dual antibacterial agents MDPB and nano-silver in primer on microcosm biofilm, cytotoxicity and dentine bond properties *Journal of Dentistry* **41(5)** 464-474.

18. Azevedo SM, Kantorski KZ, Valandro LF, Bottino MA, & Pavanelli CA (2012) Effect of brushing with conventional versus whitening dentifrices on surface roughness and biofilm formation of dental ceramics *General Dentistry* **60(3)** 123-130.

19. Brentel AS, Kantorski KZ, Valandro LF, Fucio SB, Puppin-Rontani RM, & Bottino MA (2011) Confocal laser microscopic analysis of biofilm on newer feldspar ceramic *Operative Dentistry* **36(1)** 43-51.

20. Carlen A, Nikdel K, Wennerberg A, Holmberg K, & Olsson J (2001) Surface characteristics and in vitro biofilm formation on glass ionomer and composite resin *Biomaterials* **22(5)** 481-487.

21. Frojd V, Chavez de Paz L, Andersson M, Wennerberg A, Davies JR, & Svensater G (2011) In situ analysis of multispecies biofilm formation on customized titanium surfaces *Molecular Oral Microbiology* **26(4)** 241-252.

22. Hickel R, Roulet JF, Bayne S, Heintze SD, Mjor IA, Peters M, Rousson V, Randall R, Schmalz G, Tyas M, & Vanherle G (2007) Recommendations for conducting controlled clinical studies of dental restorative materials. Science Committee Project 2/98—FDI World Dental Federation study design (part I) and criteria for evaluation (part II) of direct and indirect restorations including onlays and partial crowns *Journal of Adhesive Dentistry* **9(1)** 121-147.

23. Kantorski KZ, Scotti R, Valandro LF, Bottino MA, Koga-Ito CY, & Jorge AO (2009) Surface roughness and bacterial adherence to resin composites and ceramics *Oral Health Preventive Dentistry* **7(1)** 29-32.

24. Kawai K, Urano M, & Ebisu S (2000) Effect of surface roughness of porcelain on adhesion of bacteria and their synthesizing glucans *Journal of Prosthetic Dentistry* **83(6)** 664-667.

25. Lima FG, Romano AR, Correa MB, & Demarco FF (2009) Influence of microleakage, surface roughness and biofilm control on secondary caries formation around composite resin restorations: An in situ evaluation *Journal of Applied Oral Science* **17(1)** 61-65.

26. Aykent F, Yondem I, Ozyesil AG, Gunal SK, Avunduk MC, & Ozkan S (2010) Effect of different finishing techniques for restorative materials on surface roughness and bacterial adhesion *Journal of Prosthetic Dentistry* **103(4)** 221-227.

27. Al-Marzok MI, & Al-Azzawi HJ (2009) The effect of the surface roughness of porcelain on the adhesion of oral *Streptococcus mutans Journal of Contemporary Dental Practice* **10(6)** 017-024.

28. Kantorski KZ, Scotti R, Valandro LF, Bottino MA, Koga-Ito CY, & Jorge AO (2008) Adherence of *Streptococcus mutans* to uncoated and saliva-coated glass-ceramics and composites *General Dentistry* **56(7)** 740-747.

29. Scotti R, Zanini Kantorski K, Scotti N, Monaco C, Valandro LF, & Bottino MA (2006) Early biofilm colonization on polished- and glazed-zirconium ceramic surface: Preliminary results *Minerva Stomatology* **55(9)** 493-502.

30. Tanner J, Robinson C, Soderling E, & Vallittu P (2005) Early plaque formation on fibre-reinforced composites in vivo *Clinical Oral Investigations* **9(3)** 154-160.

31. Auschill TM, Arweiler NB, Brecx M, Reich E, Sculean A, & Netuschil L (2002) The effect of dental restorative materials on dental biofilm *European Journal of Oral Science* **110(1)** 48-53.

32. Rimondini L, Fare S, Brambilla E, Felloni A, Consonni C, Brossa F, & Carrassi A (1997) The effect of surface roughness on early in vivo plaque colonization on titanium *Journal of Periodontology* **68(6)** 556-562.

33. Akgüngör G, Aydin M, Sen D, & Issever H (2008) Clinical efficacy of the Vector system in excess cement removal during fixed prosthodontic treatment *Quintessence International* **39(4)** 139-146.

34. Burrow MF, Nopnakeepong U, & Phrukkanon S (2002) A comparison of microtensile bond strengths of several dentin bonding systems to primary and permanent dentin *Dental Materials* **18(3)** 239-245.

35. Ogata M, Harada N, Yamaguchi S, Nakajima M, & Tagami J (2002) Effect of self-etching primer vs phosphoric acid etchant on bonding to bur-prepared dentin *Operative Dentistry* **27(5)** 447-54.

36. Gybbons RJ, & Nygaard M (1968) Synthesis of insoluble dextran and its significance in the formation of gelatinous deposits by plaque-forming streptococci *Archives of Oral Biology* **13(10)** 1249-1262.

37. Quirynen M, Marechal M, Busscher HJ, Weerkamp AH, Darius PL, & van Steenberghe D (1990) The influence of surface free energy and surface roughness on early plaque formation: An in vivo study in man *Journal of Clinical Periodontology* **17(3)** 138-144.

38. Quirynen M, van der Mei HC, Bollen CM, Schotte A, Marechal M, Doornbusch GI, Naert I, Busscher HJ, & van

Steenberghe D (1993) An in vivo study of the influence of the surface roughness of implants on the microbiology of supra- and subgingival plaque *Journal of Dental Research* **72(9)** 1304-1309.

39. Gatewood RR, Cobb CM, & Killoy WJ (1993) Microbial colonization on natural tooth structure compared with smooth and plasma-sprayed dental implant surfaces *Clinical Oral Implants Research* **4(2)** 53-64.
40. Quirynen M, & Bollen CML (1995) The influence of surface roughness and surface-free energy on supra- and subgingival plaque formation in man. A review of the literature *Journal of Clinical Periodontology* **22(1)** 1-14.
41. Scotti R, Kantorski KZ, Monaco C, Valandro LF, Ciocca L, & Bottino MA (2007) SEM evaluation of in situ early bacterial colonization on a Y-TZP ceramic: A pilot study *International Journal of Prosthodontics* **20(4)** 419-422.
42. Staudt C, Horn H, Hempel DC, & Neu TR (2004) Volumetric measurements of bacterial cells and extracellular polymeric substance glycoconjugates in biofilms *Biotechnology and Bioengineering* **88(5)** 585-592.
43. Pereira SM, Kantorski KZ, Brentel AS, Valandro LF, & Bottino MA (2008) SEM analysis of the in situ early bacterial colonization on two novel feldspathic ceramics submitted to different types of glazing *Journal of Contemporary Dental Practice* **9(2)** 49-56.