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The Influence of Cement Removal Techniques on *In Situ* Bacterial Adhesion and Biodegradation at the Marginal Interface of Ceramic Laminates

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Clinical Relevance

The presence of excess cement at the marginal interface of ceramic materials may increase surface roughness and facilitate bacterial adhesion, leading to clinical failure.

SUMMARY

Objectives: This *in situ* study aimed to analyze the influence of different resin cement removal techniques on bacterial adhesion and biodegradation at the marginal interface of ceramic laminates.

Methods and Materials: Eighty feldspathic ceramic (F) blocks were prepared and cemented onto bovine enamel slabs (7×2.5×2 mm). Excess cement was removed using a microbrush (MBR), a scalpel blade (SCP), or a Teflon spatula (TSP). For the biodegradation analysis, 40 disc-shaped

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resin cement specimens were prepared (7×1.5 mm) using a Teflon mold. The specimens were randomly allocated into two groups: (1) No finishing procedure (only Mylar strip), and (2) with finishing and polishing procedures using the Jiffy system (Ultradent, South Jordan, UT, USA) (n=20). The *in situ* phase consisted of using an intraoral palatal device by 20 volunteers for 7 days. Each device contained five cylindrical wells (8×3 mm), where three dental blocks and two cement specimens were included in the wells. Surface roughness (R_a) was measured using a contact profilometer. A micromorphological analysis was performed under a stereomicroscope and a scanning electron microscope. Bacterial adhesion was quantitated based on the number of colony-forming units (CFU/mL) and their biofilm development potential.

Results: The cement removal techniques directly affected surface roughness at the marginal interface (p<0.001), and the SCP technique produced higher mean roughness, regardless of the surface area analyzed. Surface polishing protected cement specimens from further biodegradation (p=0.148). There were no differences in CFU counts between the groups after the *in situ* phase (p=0.96). All specimens showed CFU with a strong ability to develop a biofilm.

Conclusions: The techniques used for cement removal increased the surface roughness of ceramic laminates, particularly SCP, but they did not affect bacterial adhesion at the marginal interface. Surface polishing of the resin cement is recommended to mitigate biodegradation.

INTRODUCTION

Ceramic laminates have been successfully used as dental restorations, particularly when a minimally invasive esthetic procedure in anterior teeth is required.[1,2] Bond stability between the cement, ceramic material, and dental tissues is an important factor determining the clinical success of all-ceramic restorations.[3,4]

The longevity of indirect restorations can be compromised by a marginal misfit, the presence of surface irregularities, and the excess of luting cement, which may favor the accumulation of microorganisms at the marginal interface.[2] Thus, the increased surface roughness may result in more significant biofilm development, causing periodontal issues associated with esthetic impairment. Besides, it may also negatively affect the cement bond strength between the tooth and ceramic material.[2,5]

Early bacterial accumulation largely depends on the physical and chemical nature of the surface.[6,7] Overall, a mean surface roughness (R_a) of <0.2 μm is desirable for dental materials. A lower surface roughness seems to reduce biofilm accumulation significantly.[8] In contrast, rougher surfaces have niches that may protect the microorganisms from the mechanical forces of toothbrushing, muscle activity, and salivary flow.[9]

Clinically, the resin-based cement film in ceramic restorations is located in an area with a higher concentration of organic acids.[10] These acids are metabolized by cariogenic bacteria, which can degrade methacrylate-based polymers, thereby affecting surface hardness and increasing surface roughness. This process is known as biodegradation.[11]

Several techniques have been described considering the importance of avoiding excess cement material around the interfacial region of ceramic restorations.[2,12] Most *in vitro* studies evaluated the use of sharp scalpel blades (SCPs), microbrushes (MBR), or brushes, cotton balls, and plastic instruments. The use of MBR provided a homogeneous and regular interfacial area, while a Teflon spatula (TSP) showed surface irregularities with higher bacterial concentration compared to the MBR technique.[12] The partial photoactivation for 5 seconds before cement removal reduced the surface roughness, especially when using a blade or an explorer. From a topographical point of view, a smoother surface was observed. Regarding bacterial adhesion, the polishing technique reduced the colony-forming unit (CFU/mL) count, particularly when a MBR was used compared to the other removal devices.[2]

A previous study showed the influence of different dental materials' surface roughness on bacterial adhesion *in vitro*.[13] However, no *in vitro* tests are capable of reproducing the complexity of the biodegradation process.[11] *In situ* models are recognized as an experimental design to examine biofilms properly.[11,14-18]

Thus, this *in situ* study aimed to analyze the influence of different cement removal techniques on bacterial adhesion and biodegradation at the marginal interface of ceramic laminates. The null hypotheses tested were that (1) the cement removal technique does not affect bacterial adhesion, and that (2) surface polishing of the resin-based cement has no influence on material biodegradation within the oral milieu.

METHODS AND MATERIALS

Figure 1 shows a schematic illustration of the experimental design. All tested materials and their specifications are listed in Table 1.

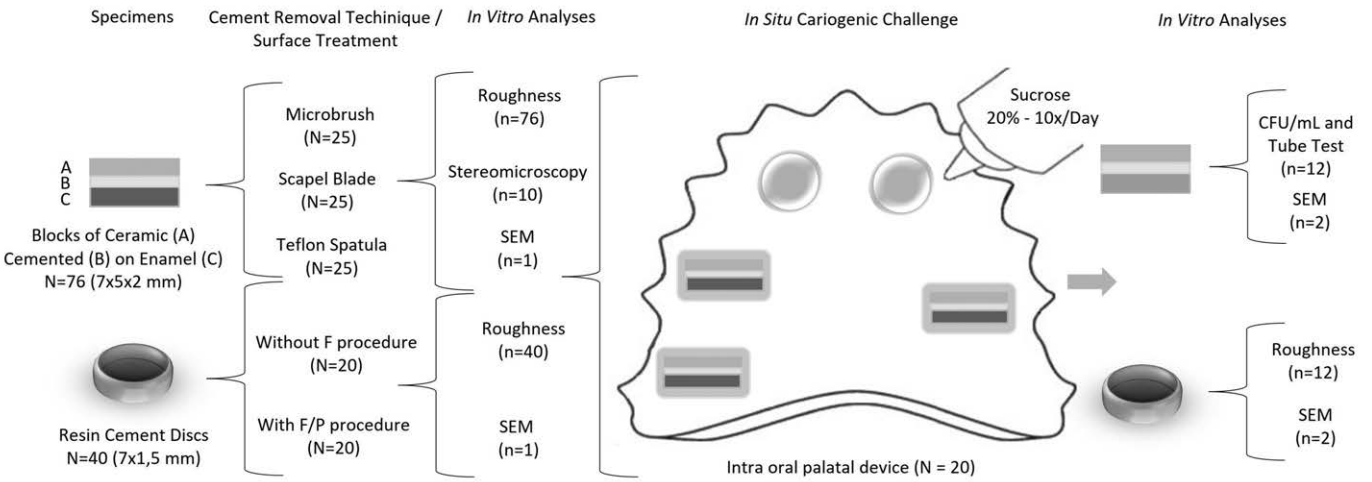

Figure 1. *Schematic illustration of the experimental design.*

For this *in situ* study, the sample size was calculated based on a previous study[15] in BioEstat 5.3 (Mamiraupa Sustainable Development Institute, Manaus, AM, Brazil), considering an α error of 0.05 and 0.8 statistical power. According to these parameters, a total of 17 volunteers were required to detect any significant differences. A final sample size of 20 volunteers was considered to compensate for possible outliers that could cause specimen loss.

Tooth Specimen Preparation

Eighty rectangular enamel slabs were obtained from extracted bovine incisors. The teeth were manually cleaned using periodontal curettes and a prophylaxis brush with pumice slurry and water. All cleaned teeth were stored in a 0.05% chloramine-T solution for disinfection.

The buccal surface of the tooth was ground with a silicon carbide paper (#600 and #1200) on a metallurgical polishing machine (METASERV 3000, Buehler, IL, USA) under constant water cooling. The tooth root was embedded into acrylic resin in a PVC mold (17×15 mm) to facilitate the handling. The tooth crown was longitudinally sectioned with a diamond saw (Isomet Diamond Wafering Blades - Buehler) in a low-speed precision cutting machine (Cutmaster Erios, São Paulo, SP, Brazil). The final dimensions of the enamel slab were obtained using diamond discs (7016, American Burs, Palhoças, SC, Brazil) mounted in a handpiece. The dentin was cut to obtain a block

Table 1: *Tested Materials, Composition, and Specifications*

Material (Color)	Composition	Manufacturer Batch Number
Duceram Kiss Bonding Porcelain –(A3)	Silicon Oxide, Aluminum Oxide, Potassium Peroxide, Sodium Oxide, Lithium Oxide, Barium Oxide, Boron Oxide, Calcium Peroxide, Titanium Oxide, Cerium Oxide, Tin Oxide, Phosphorus Oxide, Antimonious Oxide, Fluorine and Zirconium Oxide and pigments that are added in basic powders with variation between 1% and 10%	Dentsply Sirona Company (Hanau-Wolfgang, Germany) 118008
Tetric N Bond Universal	Methacrylates, ethanol, water, highly dispersed silicon dioxide, initiators, and stabilizers	Ivoclar Vivadent (Ontario, Canada) X25012
Variolink Esthetic LC (Light)	Monomers: BisGMA, UDMA, TEGDMA, HEMA, and GDMA (30 wt%) Inorganic Filler: ytterbium trifluoride and spheroid mixed oxide. Initiators, stabilizers, pigments and additional ingredients Filler loading (Wt%/Vol%)/size: (30%/38%)/0.04-0.2 µm	Ivoclar Vivadent (Ontario, Canada) Y05760

Abbreviations: Bis-GMA, bisphenol A glycidyl dimethacrylate; UDMA, urethane dimethacrylate; TEGDMA, triethylene glycol dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; and GDMA, glycidyl dimethacrylate.

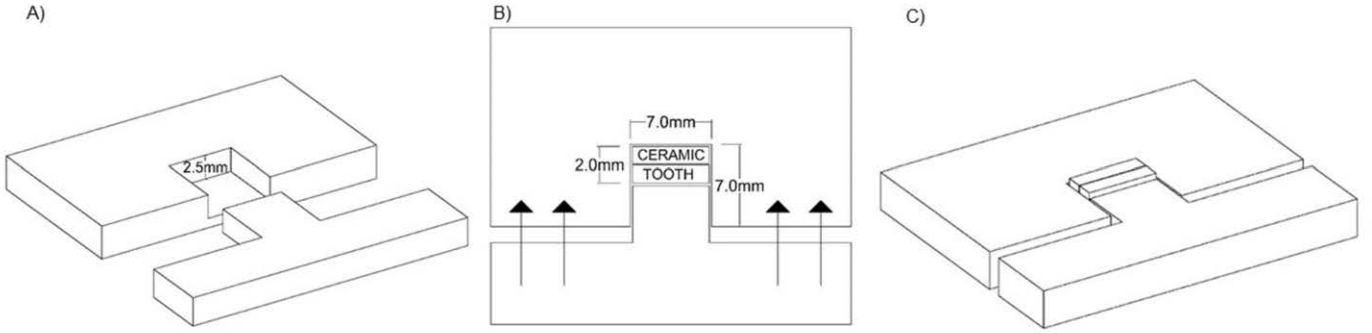

Figure 2. *Schematic illustration of the custom-made metal apparatus used for specimen fixture during cementation and cement removal technique. A) Side view of the metal apparatus; B) Front-view of the metal apparatus with the ceramic and teeth block. Black arrows indicate the direction of the parts; C) side view of the cemented block attached to the metal apparatus.*

with 7×2.5×2 mm using a digital caliper with 0.01-mm precision (Digimess, São Paulo, SP, Brazil). The slabs remained stored in distilled water at room temperature until the cementation procedure.

Ceramic Specimen Preparation

Eighty F blocks (Duceram Kiss Bonding Porcelain, Dentsply Sirona Company, Hanau-Wolfgang, Germany) were prepared according to the manufacturer's instructions. A rectangular stainless-steel split mold (25×2×2 mm) was filled in excess with the mixture, and the moisture was gently dried with absorbent paper. The ceramic blocks were submitted to a sintering cycle in an appropriate furnace (Multimat NTX Press, Dentsply). The blocks were sectioned using a handpiece with a diamond disc under constant water cooling to obtain the final dimension (7×2.5×2 mm) with the digital caliper. In addition to the cementation surface, a layer of glaze (InSync Glaze System, Chemichl AG Landstrasse, Vaduz, Liechtenstein) was applied onto each ceramic surface. The specimens were submitted to a second cycle in the furnace.

Ceramic Cementation and Cement Removal Techniques

Enamel surfaces were cleaned with pumice, and excess water was removed using an air-jet until dry. The cementation surface was etched with 5% hydrofluoric acid for 2 minutes (Condac Porcelana 5%, FGM Joinville, SC, Brazil), rinsed, and air-dried. The enamel surface was then actively etched with 37% phosphoric acid (Condac 37, FGM) for 30 seconds, rinsed, and air-dried. A layer of a silane coupling agent (Prosil, FGM) was applied onto the entire surface and left in contact for 2 minutes to promote water/alcohol evaporation.

A custom-made device was used to fix the specimens (Figure 2). Each enamel block was positioned and treated with 37% phosphoric acid for 30 seconds, rinsed for 15 seconds, and dried with air jets. An adhesive system (Tetric N Bond Universal, Ivoclar Vivadent, Ontario, Canada) was applied according to the manufacturer's instructions. A light-cured resin-based cement (Variolink LC Esthetic, Ivoclar Vivadent) was applied, followed by adapting the ceramic specimens.

A horizontal load was applied to fix the device, and maintain the correct position of the enamel and the ceramic blocks during excess cement removal (Table 2). Each specimen was cured at the marginal interface area using an LED light-curing unit (Radii-Cal, Dental Products, SDI, Baywater, Victoria, Australia) for 40 seconds with 1200 mW/cm² irradiance. After curing, the marginal interface of 10 specimens from each group was examined under a stereomicroscope (SteREO Discovery.V12, Carl Zeiss Microscopy GmbH, Jena, Germany) with 82× magnification.

Biodegradation of Resin Cement

The influence of polishing procedures on the biodegradation of resin cement materials was further examined. Briefly, 40 disc-shaped specimens (7×1.5 mm) were prepared. A Teflon mold was filled to excess with the resin cement, and a Mylar matrix strip under a microscope glass slab was placed on the top surface. Slight finger pressure was applied against the glass to minimize voids. Each cement specimen was cured at the central area, and the excess was removed using a sharp blade and silicon carbide papers (#600 and #1200).

The specimens were randomly allocated into two groups (n=20) according to the cement surface treatment: (1) no finishing procedure (light cured, Mylar strip) and (2) with finishing-polishing procedure by a single operator using Jiffy rubber points (Ultradent, South Jordan, UT, USA). Yellow and the white flame-shape points were used for 20 seconds each and then replaced after every five cycles.

Table 2: *Excess Cement Removal Technique*

Group	Removal Technique
Microbrush[A] (MBR)	A fine MBR was used in the cementation line in one direction before photoactivation (n=25)[D]
Scalpel blade[B] (SCP)	The excess of cement was displaced with a SCP after the first 5 seconds of curing and then continued the final photoactivation (n=25)
Teflon spatula[C] (TSP)	The excess of cement on the marginal interface was removed using a TSP before the photoactivation (n=25)

A: KG, Sorensen, Cotia, Brazil
B: Advantive (Sterilance, Sterilance Medical Inc. Suzhou, China)
C: Esthetic Plus, TDV, Pomerode, Santa Catarina, Brazil
D: Monowave LCU (Radii-Cal, SDI, Austrália)

Measurement of Surface Roughness

The R_a of the specimens was measured (μm) using a contact profilometer (SURFTEST SJ 310, Mitutoyo Corp, Kanagawa, Japan). For cemented blocks, the surface roughness was measured before the *in situ* phase. Ten successive in-line measurements were taken, with the needle in two different points of each predefined location: (1) ceramic surface; (2) ceramic surface, closer to the cement line; (3) cement line; (4) tooth, closer to the cement line, and (5) tooth surface (T). All measurements were performed in the specimen's long axis at a constant speed of 0.5 mm/s, with 0.7 load and 0.25 mm cut off.

For the biodegradation analysis, disc-shaped resin cement specimens were measured before and after the *in situ* phase. Three successive in-line traces were used to determine the mean surface roughness (R_a) from different angles. A trace length of 6.0 mm was used for both cemented blocks and cement disc specimens. A calibration step was performed periodically to monitor the device's performance.

Volunteer Selection

Twenty volunteers aged from 21 to 35 years, who were undergraduate and graduate dental students, participated in this study. The following inclusion criteria were considered: good systemic and oral health; no caries activity or any signs of gingivitis; and no use of antibiotics up to 2 months before the experimental phase or administration of any drugs that could affect salivary flow. Volunteers with poor oral hygiene, diagnosed with diabetes or chronic mouth breathing, with motor difficulties, palatal torus, denture use, or those wearing orthodontic appliances were not included in this study. A dentist carried out visual and oral examinations. All volunteers signed an informed consent form to authorize their participation. Before the experiment, the specimens were sterilized in a gamma radiation camera (25 kGy) for a period of 15 hours.

In Situ Experimental Phase

An acrylic custom-made palatal device was made for each volunteer. Each device contained five disc-shaped cavities (8×3 mm), to which three dental blocks and two cement specimens were fixed with wax (Figure 2). A plastic mesh was fixed over each cavity, maintaining a 1-mm space from the specimen surface to allow biofilm accumulation and to protect the specimens from mechanical disturbance.

During the 7-day experimental period, volunteers were instructed to brush their teeth with a regular fluoridated dentifrice three times per day (Colgate Maximum Cavity Protection—Palmolive Company, New York, NY, USA). There were no dietary restrictions during the experimental phase. The instructions were presented orally and written. Particular recommendations were given towards removing the device before eating or ingesting any food or beverages. In any case, the instruction was to keep the intraoral device constantly moistened in the plastic case provided by the authors.

The cariogenic challenge consisted of an extraoral application of one drop of a 20% sucrose solution onto each specimen 10 times per day at predetermined time intervals (8 am, 9:30 am, 11:00 am, 12:30 pm, 2:00 pm, 3:30 pm, 5:00 pm, 6:30 pm, 8:00 pm and 9:30 pm). The device was removed from the mouth, and excess saliva was cleaned with a gauze. Subsequently, a drop of sucrose was applied to the specimen. A 5 minute waiting period was established before the palatal device was repositioned in the mouth to enable sucrose diffusion into the biofilm.

After the experimental period, the devices were collected for further analysis. Cemented blocks were carefully removed from the devices and inserted into swab tubes (Absorve, Cral Artigos para Laboratório Ltda, San José, Cotia-SP, Brazil) containing 2 ml of Mueller Hinton broth. The disc-shaped specimens

were placed in tubes with sterile saline solution, washed in an ultrasonic bath for 30 minutes, and measured for their surface roughness.

Scanning Electron Microscopy (SEM)

One specimen from each group was selected for Scanning Electronic Microscopy (EVO LS 15, Carl Zeiss) analysis before and after the *in situ* phase. The specimens not submitted to *in situ* tests were dehydrated, dried (40°C/12 hours), and gold-sputtered (Q150T ES, Quorum Technologies Ltd, Laughton, UK) before SEM analysis. The specimens submitted to *in situ* tests were removed from the intraoral device and washed with 3 mL of sterile saline solution to remove nonadherent material from the surface.

Each specimen was placed in Eppendorf tubes containing a solution of glutaraldehyde (2.5%)/ paraformaldehyde (4%) in 0.1 M phosphate buffer (pH 7.2) for 2 hours at 4°C. The specimens were washed with the same solution and postfixed for 1 hour with osmium tetroxide in 0.1 M phosphate buffer (pH 7.2). Once again, they were washed and dehydrated with increasing concentrations of ethanol (30, 50, 70, 90, and 3× 100% for 30 minutes), dried using the critical point method, gold-sputtered, and observed under an SEM operated at 10kV with a working distance of 10 mm.

Colony-Forming Units Count (CFU/mL)

The cemented blocks from four volunteers were analyzed for CFU counting and biofilm formation. The specimens were removed from the swab, placed into tubes containing 2 mL of Mueller Hinton broth, and then sonicated for 30 seconds in a 50-60 W power ultrasonic homogenizer (Unique Ultrasonic Cleaner, USC-3300, São Paulo, SP, Brazil). A 1:1000 dilution was performed, and duplicate aliquots were seeded onto Muller Hinton agar. The plates were incubated at 37°C for 48 hours, and those containing 30-300 colonies were counted for CFU/mL.

Biofilm Formation

After CFU counting, bacterial colonies were also examined for their ability to develop a biofilm. Colonies were isolated from the specimens, and five colonies of each species were added to a Falcon tube containing 3 mL of saline solution. The tubes were vortexed, and the absorbance of the cell suspension was read at 600 nm (with a variation of 0.145-0.155). Then, 140 μL of Mueller Hinton culture medium, 20 μL of sterile distilled water, and 40 μL of the adjusted inoculum were added into a 96-well plate.

A standard bacterial colony was added as a biofilm starter (*Klebsiella pneumoniae*)—positive control. The absorbance was read at 600 nm at baseline (0 hour) and after 24 hours of incubation at 37°C. The supernatant was removed, and the plate was washed three times with sterile saline solution (0.85%) and then dried in an oven at 60°C for 60 minutes. Next, 200 μL of a violet crystal (0.4%) was added to the wells, and the plate was kept at room temperature for 15 minutes, followed by three washes under running water. Finally, 200 μL of ethanol (PA) was added to the wells, and the plate was kept for an additional 30 minutes at room temperature. The wells' absorbance was read at 570 nm, and the optical density was calculated and interpreted as follows: nonadherent, weakly adherent, moderately adherent, and strongly adherent, according to the methodology proposed by Stepanović and others (2000).[19]

Statistical Analysis

The data were analyzed descriptively and inferentially in SPSS version 21.0 (IBM Corporation). Shapiro–Wilk test was used to check for the normality of data distribution. Kruskal–Wallis test determined the difference between the groups, and the Mann–Whitney test was applied when significant differences were observed. In all tests, the significance level was set at $\alpha = 0.05$.

RESULTS

Analysis of Surface Roughness

Twenty volunteers were selected for this study, but only 18 completed the experimental phase. Two volunteers did not complete the established protocol and were excluded from the analysis. Surface roughness (R_a) measurements of the ceramic material after cementation are described in Table 3. Significant differences were observed between the techniques regarding the cement line ($p<0.001$), the area between the cement and the tooth surface ($p=0.002$), and the tooth surface ($p=0.003$). The mean roughness between the ceramic–cement area was nearly significant ($p=0.054$). The SCP removal technique produced the highest mean roughness, regardless of the surface area. Figure 3 shows the characteristics of the surfaces of different specimens, according to stereomicroscopy and SEM analysis.

Table 4 shows the contribution of surface finishing and polishing to the biodegradation of the resinous cement. The specimens without finishing procedure showed a significantly lower initial mean roughness (0.07 μm), which may be due to the Mylar strip's smoothness. However, after the *in situ* phase, this group showed a significant increase in surface roughness ($p<0.001$). For the specimens submitted to finishing and polishing procedures, no statistical difference was observed between evaluation periods ($p=0.148$).

Table 3: *Mean (SD) Roughness on the Surface and Interface in µm Following the Three Cement Removal Technique*[a]

Group	MBR	SCP	TSP	*p**
Ceramic surface	0.68 (0.38)	0.53 (0.25)	0.54 (0.32)	0.145
Ceramic/Cement	0.60 (0.33)	0.99 (0.61)	0.69 (0.37)	0.054
Cement line	0.86 (0.39) A	1.39 (0.42) B	0.97 (0.44) A	<0.001
Cement/Teeth	0.67 (0.29) A	1.30 (0.66) B	0.74 (0.54) A	0.002
Teeth surface	0.60 (0.28) A	0.49 (0.30) B	0.37 (0.18) C	0.003

Abbreviations: MBR, microbrushes; SCP, scalpel blades; TSP, Teflon spatula.

[a] *Different letters indicate statistical significance between groups through Mann–Whitney test. Uppercase letters indicate differences in each removal technique within the surface.*

**Kruskal–Wallis.*

Surface Micromorphology

Figure 3 shows images of the ceramic surface after block cementation for each cement removal technique. Excess cement can be seen at the cementation line in Figures 3A and 3B in specimens submitted to the MBR technique, with the presence of some irregularities and flaws (*red arrows*) in this area. The excess cement remaining after the use of SCP (3C and 3D) covered most of the feldspathic ceramic and tooth surface (*blue arrows*). The TSP removal technique (3E and 3F) seemed to have produced a smoother surface (*green arrow*), with fewer irregularities at the cementation line.

SEM images of the cement specimen submitted to *in situ* biodegradation are shown in Figure 4. The unpolished cement specimen (4A) showed rougher surface areas before the *in situ* phase and, therefore, exhibited a higher adhesion of bacterial colonies (4C). The polished specimen (4a) showed a smoother surface and promoted less bacterial adhesion after the *in situ* phase (4b and 4c).

CFU/mL Counting

The mean (±SD) CFU/mL (Log_{10}) is shown in Table 5. There was no statistical difference between the groups after the *in situ* phase (p=0.96).

Analysis of Biofilm Formation

The CFU counts of the specimens from four volunteers were determined, and volunteer number 2 showed the highest amount of isolated bacterial species (n=8). Table 6 shows the number of isolated bacterial species, biofilm formation analysis, and the Gram staining procedure for each strain.

When excess cement was removed using a MBR, two bacterial species were recovered from the specimens, as per the violet crystal technique. Both species were found to be Gram-negative and had a strong and moderate ability to form a biofilm.

When excess cement was removed using a TSP or a SCP, three bacterial species were recovered. Two species in the TSP group showed a strong potential

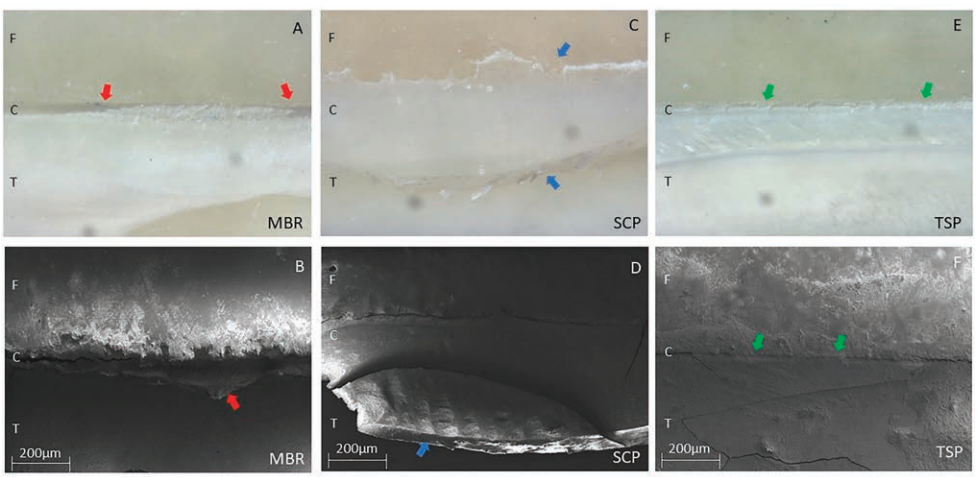

Figure 3. *Images A, C, E) in stereomicroscopy (82×); B, D, F) in SEM (60×) for the cement removal technique. (A/B) red arrow indicates the presence of irregularities and flaws at the cement line after cement removal with MBR; Blue arrow shows the excess of cement left after the use of SCP covering the feldspathic ceramic (F) and the tooth surface (T) (D/D). A more defined interface (green arrows) was observed after cement removal with TSP (Figures E and F).*

Table 4: *Mean (SD) Surface Roughness of Resin Cement Specimens in µm, Before and After Biodegradation*[a]

Group	Before	After	*p*[b]
Without finishing procedure	0.07 (0.02) Aa	0.36 (0.12) Ab	<0.001
With finishing/polishing procedure	0.19 (0.09) Ba	0.43 (0.22) Ba	0.148
p[c]	<0.001	<0.001	

[a] *Different letters indicate differences between groups.*
[b] *Wilcoxon signed-rank test was used to compare roughness in different moments of observation (lowercase letters in lines).*
[c] *According to the Mann–Whitney test (uppercase letters in columns).*

to form biofilm and were found to be Gram-negative, whereas one species showed a weak ability to form a biofilm. As for the SCP group, two species showed a moderate potential for biofilm formation, and another one showed a strong ability to do so. One of the species with moderate potential for biofilm formation was found to be gram-positive.

DISCUSSION

In our study, the cement removal technique did not significantly affect bacterial adhesion to the ceramic material, which confirms our first hypothesis. The results showed that bacterial adhesion was not associated with the excess cement removal technique. A previous study showed that surface roughness of up to 0.2 µm would accumulate less biofilm.[8] However, a recent systematic review[9] showed that a reduction in surface roughness (less than 0.2 µm) had no further impact on supra- or subgingival bacterial adhesion or biofilm composition compared to R_a above 0.2 µm, which is in agreement with others findings.[20-25]

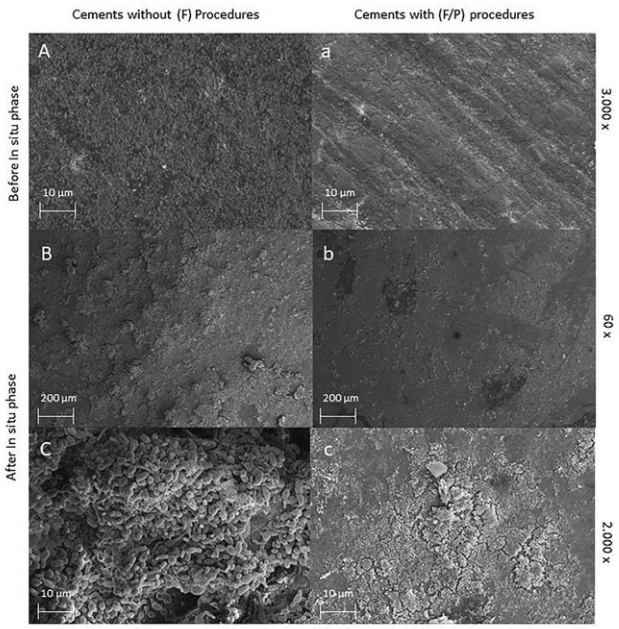

Figure 4. *SEM images of cement samples without finishing procedures (A,B,C) and cements sample with finishing-polishing procedures (a,b,c) in different magnifications.*

The bacterial adhesion was determined by analyzing the CFU/mL count. Several parameters may influence the bacterial adhesion, such as factors related to the microenvironment, surface characteristics, and the bacteria itself.[26] Among the factors related to the surface, surface roughness is one of them. However, in the present study, despite differences in surface roughness between groups, no differences were observed in the formation of CFU/mL. The surface roughness of each surface (tooth, resin cement, and ceramic) showed R_a means higher than 0.2 µm (Table 3), regardless of the removal device used.

In vitro studies previously demonstrated a significant association between the cement removal technique and bacterial adhesion onto the restorative material.[2,12] According to Anami and others,[12] the TSP technique showed the highest R_z value (arithmetic mean between the five highest peaks and five deepest valleys within a specific length), in addition to greater bacterial adhesion and biofilm volume. Pereira and others[2] found that the MBR removal technique was associated with lower CFU counts.

The clinical longevity of restorations is influenced by resin cement physical and mechanical properties and its ability to adhere to the dental structures. The outcomes of an *in vitro* study are generally more limited, because some factors are controlled, such as the type of bacterial inoculation, temperature, pH, and nutritional status.[9] Instead, *in situ* study designs are more versatile and can be used for various analytical purposes, such as assessing erosive or cariogenic potential.[27] Clinical and biological aspects such as temperature changes, salivary composition, and pH can contribute to the degradation phenomenon.[3] On the other hand, these factors may also be considered a limitation of *in situ* studies, as the oral milieu and the microbiome itself are specific to each volunteer.

For *in situ* studies, the cariogenic challenge's acceleration is commonly undertaken using 20% sucrose solutions 4×,[27] 8×,[28] or even 10× daily.[15] The time

Table 5: *Mean (SD) of Colony-forming Units (CFU/mL) log_{10} After* In Situ *Phase (n=4)*

Group	CFU/mL log_{10}	*p**
MBR	5.29 (0.19)	0.96
SCP	5.24 (0.18)	
TSP	5.26 (0.18)	

Abbreviations: MBR, microbrushes; SCP, scalpel blades; TSP, Teflon spatula.
**Kruskal–Wallis*

established for the duration of an *in situ* experiment is also highly variable. *In vitro* studies assessing bacterial adherence and colonization may have a duration of 24[12]-48 hours.[2] However, biofilm formation and maturation depends on the cohesion and coaggregation of different species and gene expression.[29] After 7 days, a climax community is established, having a dynamic balance with minor variations in species composition and proportion.[30] While extended *in situ* observation periods have been considered in the literature,[16] participant adherence to the protocol established in our study for more extensive periods may prove challenging to achieve and may be considered a limitation of our study.

The interaction of *Streptococcus mutans* with the surface of resinous materials promotes biodegradation. Organic acids produced by bacterial metabolism change the oral environment's pH (from 7.3 to 4.0), which may affect the surface of resinous materials.[31] An *in vitro* study[29] examined bacterial adhesion on the surface of resin composites using a 4 hour protocol. The authors found that early colonization of bacterial species is considered an essential factor for biofilm formation and maturation. Also, topographic characteristics and material composition affect only early bacterial adhesion but not biofilm maturation.[32-34]

The tube test is the most frequently used method to measure biofilm formation. Biofilm cultures may be formed on a culture tube and stained with a cationic dye or grown in a microtiter plate. The optical density of stained biofilm is assessed using a spectrophotometer.[19] The classification used herein to determine bacterial biofilm formation was based on a study of Christensen and others.[35] Here, all isolated species were adherent, and classified as moderate and strong biofilm-forming microorganisms, except for one species recovered from the TSP group, which showed a weak ability to form a biofilm.

The second tested hypothesis was that the cement polishing technique does not affect biodegradation in the oral environment. This hypothesis was rejected, as statistically significant differences were observed between baseline and final roughness measurements when no surface polishing was performed. Such a difference was not observed in the specimens submitted to finishing and polishing procedures. This phenomenon is frequently observed when metabolic acids from cariogenic bacteria cause surface damage, such as corrosion and increased roughness of restorative materials, but no *in vitro* test can reproduce the complex process of biodegradation.[36,37] Lactic acid is the most critical product metabolized by cariogenic bacteria, such as *S. mutans*, in the presence of sucrose.[38] However, the pH conditions in an *in vitro* environment may differ from those observed in oral conditions.

Table 6: *Analysis of Bacterial Biofilm Formation and Gram Test of the Isolated Bacteria in Each Sample Analyzed*

	Removal Technique	Biofilm Formation	Gram Staining
1	MBR	Strong (+++)	-
2		Moderate (++)	-
3	SCP	Moderate (++)	+
4		Moderate (++)	-
5		Strong (+++)	-
6	TSP	Strong (+++)	-
7		Weak (+)	-
8		Strong (+++)	-

Abbreviations: MBR, microbrushes; SCP, scalpel blades; TSP, Teflon spatula.

Although no differences in roughness measurements were observed before and after the polished specimens' cariogenic challenge, this does not imply that there was less bacterial adhesion. Other factors, such as the material's surface free energy, may also directly affect biofilm formation,[7,20] which could be confirmed in the micrographs shown in Figure 4. At the same magnification (2000×), more significant colonization of microbial species was observed than the specimens submitted to finishing and polishing procedures.

A positive correlation between increased surface roughness and bacterial adhesion was observed,[12,22,39,40] to the extent that it can even exceed other properties' influence, such as surface free energy.[32] Although the recommended (low) mean roughness measurement was obtained at baseline (<0.2 μm), polished cement specimens showed an increase in surface roughness over time due to the biodegradation of the polymeric matrix.[8]

The chemical composition of resinous materials is important for bacterial colonization. Monomer

polymerization is not fully complete, and approximately 5%-10% of unpolymerized content can be eluted. Some components present on the surface can favor or impair bacterial adhesion. The literature shows that the monomers ethylene glycol dimethacrylate (EGDMA) and triethylene glycol dimethacrylate (TEGDMA) are more easily released. These monomers can be used as carbon sources by anaerobic bacteria and are also known to increase cariogenic bacteria's viability.[30]

The Variolink resin cement contains bisphenol A glycidyl dimethacrylate (*Bis*-GMA), urethane dimethacrylate (UDMA), TEGDMA, 2-hydroxyethyl methacrylate (HEMA), and glycidyl dimethacrylate (GDMA) (30% wt) in its organic matrix composition. TEGDMA is a molecule that absorbs more water than *Bis*-GMA, leading to this material's higher solubility. In contrast, TEGDMA can modulate bacterial growth[41] and reduce surface degradation caused by acid exposure.[42]

The polishing procedure aims to improve the esthetic characteristics and durability of resinous materials by decreasing surface porosity and improving mechanical properties.[43] Furthermore, the organic matrix is removed, and exposure of inorganic particles avoids early degradation.[11]

Clinicians may choose to use more than one device for excess cement removal. However, the present study did not evaluate this synergistic effect. The combination of cement removal methods could lead to smoother surfaces, although time consuming. If the combination of methods is chosen, clinicians must be aware of maintaining the ceramic laminates in position, avoiding pressing and loosening the laminate to the prepared tooth, therefore, avoiding more outflow of the resin cement. Independent of solo or combined use, from our results, final polishing has shown a significant impact on the surface roughness of the resin cement. Further *in situ* studies are encouraged to determine the behavior of different resinous cements and preheated resin composites as luting agents for indirect restorations.

CONCLUSIONS

To conclude, our findings suggest that the three techniques used for cement removal increased the surface roughness of ceramic laminates, particularly with the scalpel blade (SCP). Still, they did not affect bacterial adhesion at the marginal interface. Finishing and polishing procedures at the cement interface should be periodically performed to minimize the biodegradation of the resinous interface.

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Regulatory Statement

This study was previously approved by the Institutional Research Ethics Committee, under protocol number 3.201.874. The approval code issued for this study is 08553219.3.0000.5207.

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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