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Evaluation of Surface Roughness and *Streptococcus mutans* Adhesion to Bulk-Fill Resin Composites Polished with Different Systems

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Abstract

Purpose: Bacterial adhesion represents the initial step in biofilm formation, dental caries and decay. This study aimed to evaluate and compare surface roughness and bacterial adhesion to bulk fill resin composites polished with different systems. **Methods:** Filtek Z350 XT (Incremental-fill resin composite), Filtek Bulk-fill Posterior (Bulk-fill resin composite), and Tetric N Ceram (Bulk-fill resin composite) were used as resin composites. The polishing systems used in this study were Sof-Lex multi-step, PoGo one step, and Mylar strip. Scanning electron microscope (SEM) was used to examine the surface roughness and adhesion of *Streptococcus mutans* ATCC 25175 standard strain to bulk-fill resin composites. **Results:** The type of restorative materials did not affect the surface roughness or bacterial adhesion ($p > 0.05$) but the polishing systems were significant ($p < 0.05$) influencing factors. Furthermore, Pearson correlation revealed a statistically significant ($p < 0.001$) association ($R = 0.943$) between surface roughness and bacterial adhesion to the tested surfaces. **Conclusion:** Regardless of the restorative material, Mylar polishing system revealed the smoothest surface and the lowest adhesion of *S. mutans* as compared to Pogo one step and Sof-Lex multi-step polishing systems.

Keywords

Bacterial Adhesion, Surface Roughness, *Streptococcus mutans*,

Polishing Systems

1. Introduction

Oral biofilm is formed of miscellaneous microbes found on the tooth surface, and enclosed in a matrix of polymers of bacterial and salivary origin. The main causes for restoration replacement are dental surface biodegradation, secondary caries, and periodontal inflammation associated with oral biofilm formation. The initial and critical step of plaque formation includes adhesion of bacteria known as early colonizers such as oral streptococci [1] [2]. These bacteria bind to various proteins including alpha-amylase, proline-rich proteins, and glycoprotein [3]. Oral biofilm formation was influenced by many factors such as surface roughness and surface free energy [4] [5]. The adhesion and initial colonizing of bacteria along cracks and pits in enamel were shown by microscopic examination of early plaque formation, indicating the influence of surface structure on bacterial adhesion [2]. The increasing demands for tooth-colored restorations and seek for amalgam replacements have strong association with enlarged requests for direct tooth-colored restorative materials. Within the past few years this esthetic look of tooth-colored restorations is of large significance to each the dentist and patient [6].

One of the predictable disadvantages of dental composites is its polymerization shrinkage. In the event that occurs while the resin composite materials are inside the cavity bonded to its walls, stresses may develop inside resin composite. Consequently, debolding, postoperative sensitivity, marginal staining, recurrent caries, and 8 cuspal deflections may develop and induce smaller scale breaks as well as cusp cracks [7]. Incremental composites application technique is anticipated to decrease the C-factor, enabling a specific amount of flow to reduce the shrinkage stress partially [8]; on the other hand, it has number of impediments, for example, entrapment of voids between the increments, bond failure between the increments and the long time required to cure each increment separately [9] [10].

Novel restorative materials have been introduced to solve many of the problems associated with the incremental method of employing resin that are used as bulk fill composites. Bulk-fill resin-based composites carry the advantages of improving polymerization depth, and diminishing polymerization shrinkage stresses and cuspal deflection rates, as well as shortening the time of incremental layering techniques [11]. Appropriate finishing and polishing of dental restorations is required for oral health protection. A rough composite resin surface may decrease the shine and esthetic appearance. Moreover, it raises the number of sites on the restoration surface prone to bacterial biofilm accumulation, which results in increasing risk of both caries and periodontal inflammation [12] [13]. Generally, the finished/polished or surface sealant-coated composite resins have low susceptibility to adhere to oral microorganisms [14].

Surface roughness has effects on recoloring and bacterial attachment to the restoration. Accordingly, many strategies for finishing and polishing of tooth-colored restoratives have been developed. Recently, specialists have implemented different trials to accomplish a high surface quality by applying one-step polishing systems [15]. It has been demonstrated that, the outcomes of one-step strategy are better or possibly practically identical to multi-step procedures and may be item related [16] [17]. Concurrently in view of the most recent technology, it is hard to acquire all around well-polished restorations even when utilizing appropriate restorative materials and the best polishing system may act as a predisposing factor for biofilm formation [18].

Salivary pellicles quickly coat every uncovered surface in the mouth. Pellicle arrangement is trailed by the grip of facultative anaerobic pioneer bacteria [19], for example, *Streptococcus gordonii*, *Streptococcus oralis*, and *Streptococcus sanguine* [20]. Early colonizing microbes play a crucial role in the consequent adhesion of cariogenic bacteria, such as *Streptococcus mutans*. Substratum surface roughness (Ra) and surface free energy are supposed to be as the primary variables influencing dental plaque formation [21]. Bacterial plaque formation and secondary caries are caused by buildup of bacteria on the marginal areas of enamel and restorative material [4]. The main reason of replacement is caries formation around dental restorations which require efforts to decrease or avert plaque formation on restorative materials [22]. Multiple *in vitro* and *in vivo* models have examined both the adhesion of a variety of microorganisms to dental restorations and the mechanisms involved in [23] [24].

The outcome of diverse finishing/polishing systems on surface roughness and bacterial adhesion of composite resins has been reported in the literature [25] [26]. Nevertheless, little data about the bacterial adhesion to bulk-fill resin composites are available in the literature. In this context, the current study aimed to compare and evaluate surface roughness and adhesion of *Streptococcus mutans* to bulk-fill resin composites with different polishing systems.

2. Materials and Methods

2.1. Materials

Filtek Z350 XT (Incremental-fill resin composite), Filtek Bulk-fill Posterior (Bulk-fill resin composite), and Tetric N Ceram (Bulk-fill resin composite) were used as resin composites in this study (**Table 1**).

The finishing/polishing systems (F/P) used in this study were Sof-Lex Pop-on Discs Multi-step, PoGo One step, and Mylar strip. The composition and manufacturers of different polishing systems are summarized in **Table 2**. *Streptococcus mutans* ATCC 25175 (*S. mutans* ATCC 25175) standard strain is used in all bacterial adhesion experiments.

2.2. Methods

2.2.1. Specimen Preparation

A total of 90 standardized specimens, 30 specimens of each restorative material,

Table 1. Resin composites used in this study.

Resin composite	Manufacturer	Composition	Filler load
Filtek Z350 XT (Incremental-fill resin composite)	3M ESPE, St. Paul, MN, USA	**Matrix** Bis-GMA, UDMA, TEGDMA, PEGDMA, Bis-EMA **Filler** 20 nm silica filler, 4 to 11 nm zirconia filler, zirconia/silica cluster filler (0.6 to 10 μm)	63.3 vol% 78.5 wt%
Filtek Bulk-fill Posterior (Bulk-fill resin composite)	3M ESPE, St. Paul, MN, USA	**Matrix** Aromatic UDMA, UDMA, ERGP-DMA, Diurethane-DMA and 1,2-dodecane-DMA **Filler** Non-agglomerated/non aggregated 20 nm filler, non agglomerated/non aggregated 4_11 zirconia filler, aggregated zirconia/silica cluster filler and a ytterbium trifloride filler	58.4 vol% 76.5 wt%
Tetic N Ceram (Bulk-fill resin composite)	Ivoclar Vivadent, AG, Schaan, Liechtenstein	**Matrix** Dimethacrylates **Filler** Barrium glass, ytterbium trifluoride, mixed oxides, polymer fillers	54 vol% 75 - 77 wt%

Table 2. Polishing systems used in this study.

Polishing systems	Composition	Manufacturer
Sof-Lex Pop-On Discs Multi-step	Medium aluminum oxide disc (40 μm) Fine aluminum oxide disc (24 μm) Ultra-fine aluminum oxide disc (8 μm)	3M Dental products, St Paul, MN, USA
PoGo One step	Diamond coated micro-polisher	Dentsuply/Caulk, Milford DE, USA
Mylar Matrix only	polyethylene terephthalate matrix	SS White, Philadelphia, PA, USA

were fabricated using a cylindrical plastic mold (10 mm diameter × 2 mm depth). The specimens were prepared by standardized method by applying the mold above a glass slap covered with Mylar strip (SS White, Philadelphia, PA, USA), followed by injecting the restorative material into the mold, covering it again with Mylar strip, and placing a glass slide on top. The material was then pressed between both glass slides to extrude excess material and to reduce voids at the surface. Specimens were cured for 40 seconds with a LED light curing unit through the glass slide and Mylar strip on the top of the specimens once being

pressed. The light intensity was measured at 800 mW/cm^2. Additional 20 seconds curing on both sides of the specimens was done after removing the stripes and glasses. The resulting specimen's extraneous flanges were removed. All specimens were stored in distilled water at 37˚C for 24 h in the incubator until usage [27].

2.2.2. Surface Roughness

To evaluate surface roughness, the tested materials are categorized into the following groups; Group 1: These specimens were kept without F/P after removal of Mylar strip to act as control group (30 specimens; ten of each restorative material). After that, the outermost surface of the remaining 60 specimens were surfaced with the super-fine grit finishing diamond bur (25 μm, No. 837 KREF.314.014, Brasseler) attached to high speed hand piece (W&H, RC-90RM, Austria) for 30 seconds at 200,000 rpm. Group 2: Thirty specimens (ten of each restorative material the specimens) were polished with flat broad surface of the Pogo diamond micro polisher disc for 40 seconds, one-step system according to manufacturer's instructions. Group 3: Thirty specimens (ten of each restorative material, the specimens) were polished with three step Sof-Lex aluminum oxide disc system according to manufacturer's instructions. After each polishing step, all the specimens were thoroughly rinsed with water for 10 seconds to remove debris and air-dried for 5 seconds. After completing polishing procedures, specimens were rinsed, cleaned in an ultrasonic cleaner for 3 min, and air dried [28].

2.2.3. Surface Roughness Measurement

All the 90 specimens were assessed for surface roughness by using FEI Quanta 200 FEG ESEM (FEI Co., Hillsboro, OR, USA) combined with image analysis to provide both qualitative and quantitative assessments of surface roughness [28].

2.2.4. Bacterial Adhesion Assay

Samples used for testing surface roughness were used for assessing bacterial adhesion with the same grouping. Sterilization of each specimen after packing in dry plastic bag in an autoclave at 121˚C before tested with bacteria. Standard strain of *S. mutans* ATCC 25175 was used for the *in vitro* adhesion assay. The standard strain was cultured on blood agar and incubated at 37˚C for 24 h. The colony count was adjusted to 1×10^6 CFU/mL from 0.5 McFarland (1.5×10^8 CFU/mL) equivalence turbidity standard (Thermo Scientific™ Remel, Waltham, MA, USA). In sterile 12-Well Corning microplates (Corning, NY, USA), 2 ml of Muller Hinton (MH) broth culture (1×10^6 CFU/mL) of *S. mutans* ATCC 25175 were aseptically transferred to each well. The disk materials were then aseptically transferred to the 12-Well Corning microplates (one/well) using sterile forceps and the plates were incubated at 37˚C for 4 h. After incubation, the disk materials were carefully washed thrice with sterile isotonic saline solution (0.9% w/v

NaCl) to remove non-adhering cells. Each disk material was then transferred to a sterile tube containing 1 ml of saline solution and vortexed for 5 min to ensure detachment of bacteria adherent to the discs surfaces. After vortexing, the cell suspensions were tenfold serially diluted in sterile saline and aliquots (10 µl) were surface cultured on blood agar plates, followed by incubation at 37˚C for 24 h for determination of viable cell count as colony forming units per milliliter (CFU/mL) [29].

2.2.5. Scanning Electron Microscopy (SEM)

The samples of disk materials were washed with phosphate buffered saline (PBS) and fixed in solution of 4% v/v paraformaldehyde with 1% glutaraldehyde in PBS for 1 h and rinsed with PBS three times for 2 min each. Finally, samples were washed with deionized water thrice for 2 min each and dehydrated through an ethanol series (50%, 70%, 80%, 95%, and 100%) for 15 min each, desiccated, sputter-coated, and visualized by a SEM (JSM-5310LV JEOL, Tokyo, Japan). Photographs of representative areas of the polished surfaces were captured at 3000× magnifications [30].

2.3. Statistical Analysis

The results were statistically analyzed by IBM SPSS Statistics version 15.0 (SPSS Inc., Chicago, IL, USA). Data were presented as means ± standard deviations (±SD) for each group. Analyses of data variables were performed using ANOVA followed by Tukey's high significant difference (HSD) test at p-value < 0.05. Pearson correlation coefficient (PCC), at two-tailed, was used to evaluate the potential association between surface roughness and bacterial adhesion to the tested surfaces.

3. Results

3.1. Surface Roughness

Two-way ANOVA showed that both the restorative materials and the finishing/polishing systems have significant effects ($p < 0.05$) on the surface roughness (**Table 3**).

The results (**Table 4**) revealed that the polishing systems significantly ($p < 0.05$) influenced the surface roughness of different restorative materials. In this context, the Mylar strip showed the smoothest surface followed by PoGo, and the roughest surface was recorded for Sof-lex. On the contrary, different resin composites (FiltekZ350 XT, FiltekZ350 XT, and Tetric N Ceram) had no statistically significant ($p > 0.05$) influence on the surface roughness and this can be attributed to the existence of polishing systems.

The two dimensional microphotographs of resin composites surfaces (FiltekZ350 XT, FiltekZ350 XT, and Tetric N Ceram) polished with different systems (Mylar strip, Pogo one step, and Sof-lex multi-step) are shown in **Figure 1**.

Table 3. Two way ANOVA test results of surface roughness means (nm) among the tested restorative materials and different finishing/polishing systems.

Source	Type III Sum of Squares	df	Mean Square	F	Significance
Corrected model	35187.1*	8	4398.3	156.7	0.000
Intercept	3323803.0	1	3323803.0	118481.9	0.000
restoration	619.4	2	309.7	11.0	0.000
finishing	33770.3	2	16885.1	601.8	0.000
restoration finishing	797.3	4	199.3	7.1	0.000
Error	2272.3	81	28.0		
Total	3361262.5	90			
Corrected Total	37459.4	89			

*R^2 = 0.939 (Adjusted R^2 = 0.933).

Table 4. Post hoc Tukey's test results of surface roughness of the tested restorative materials with different finishing/polishing systems.

Polishing system	Surface roughness of restorative material (nm)		
	Filtek Z350 XT	Filtek Bulk Fill	Tetric N Ceram
Mylar strip	161.07 ± 6.82[a]	162.08 ± 6.82[a]	164.19 ± 6.82[a]
Pogo one step	188.33 ± 6.03[b]	190.41 ± 6.03[b]	191.42 ± 6.03[b]
Sof-lex multi-step	215.87 ± 3.876[c]	217.89 ± 3.87[c]	217.88 ± 3.87[c]

Each value represents mean (±SD) and values with different superscript letters a, b, c indicate statistically significant difference in surface roughness.

3.2. Bacterial Adhesion

The results of two-way ANOVA test revealed that types of polishing systems significantly ($p < 0.05$) influence the bacterial adhesion (Table 5).

The results demonstrated that adhesion *S. mutans* to Filtek Z350 XT varied significantly ($p < 0.05$) between different finishing/polishing systems. The highest bacterial adhesion was observed with Sof-lex multi-step and the lowest one was observed with Mylarstrip. On the other hand, different types of restorative materials (FiltekZ350 XT, FiltekZ350 XT, and Tetric N Ceram) showed non-significant ($p > 0.05$) effect on bacterial adhesion (Table 6).

Adhesion of *S. mutans* ATCC 25175, as captured by SEM, to resin composites surfaces (FiltekZ350 XT, FiltekZ350 XT, and Tetric N Ceram) polished with different systems (Mylar strip, Pogo one step, and Sof-lex multi-step) are shown in Figure 2. Pearson correlation test revealed a statistically significant ($p < 0.001$) and strong association (PCC = 0.943) between surface roughness and bacterial adhesion to the tested surfaces.

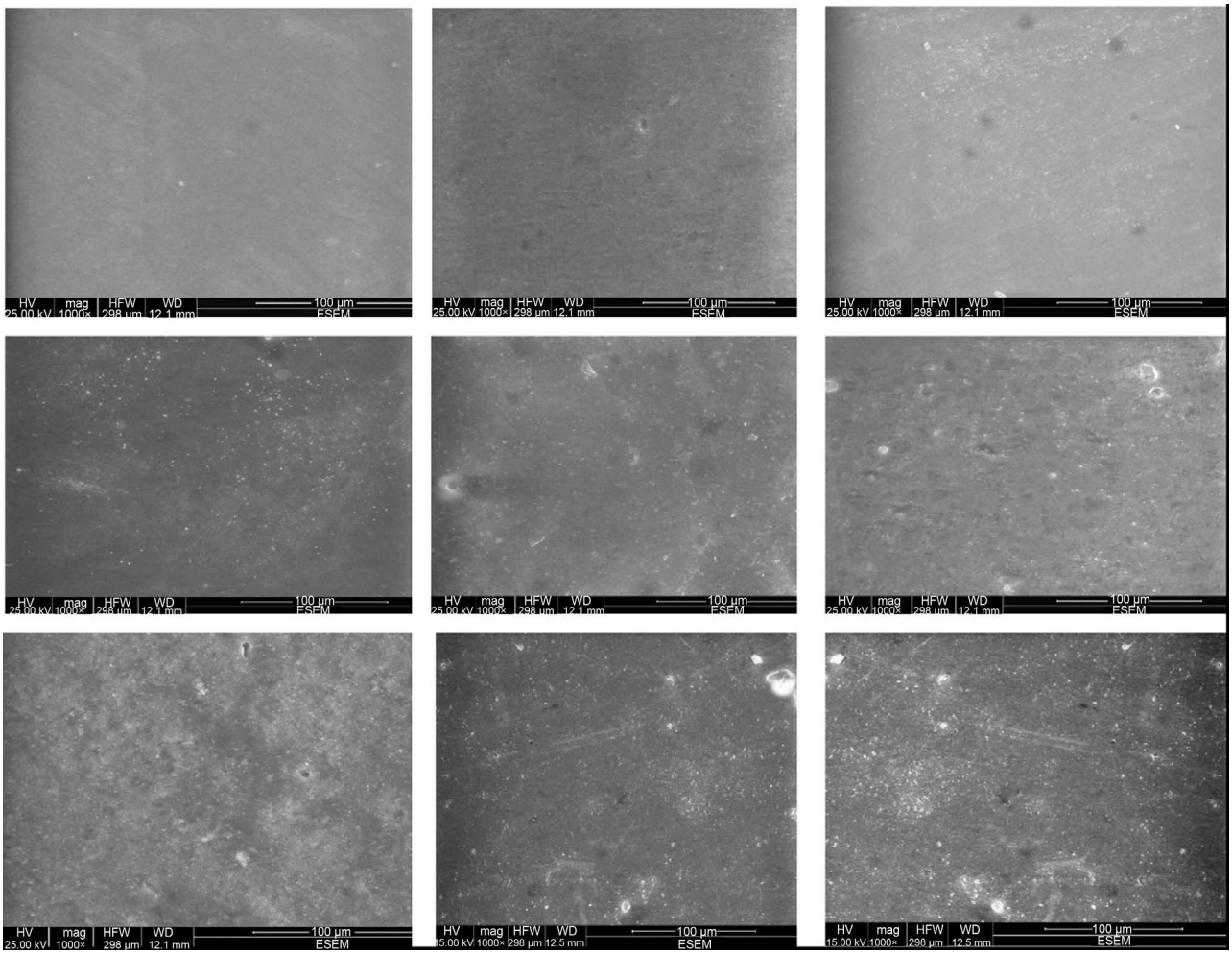

Figure 1. Two dimensional photomicrograph of resin composites surfaces polished with different polishing systems: (A) FiltekZ350 XT with Mylar strip, (B) Filtek Bulk-fill with Mylar strip, (C) Tetric N Ceram with Mylar strip, (D) FiltekZ350 XT with Pogo one step, (E) Filtek Bulk-fill with Pogo one step, (F) Tetric N Ceram with Pogo one step, (G) FiltekZ350 XT with Sof-lex multi-step, (H) Filtek Bulk-fill with Sof-lex multi-step, (I) Tetric N Ceram with Sof-lex multi-step.

4. Discussion

Bulk fill restorative materials have been developed to enable dentists to efficiently reduce placement time and effort. Little information is available about the performance of this new bulk fill materials. Successful esthetic restoration should mimic the surface smoothness and gloss of human enamel. The effectiveness of finishing and polishing procedures on esthetic restorative materials is a crucial factor in restorative treatment. Accordingly, smoother surfaces are generally obtained by curing the materials against Mylar matrix strips. Unfortunately, this procedure is often clinically insufficient because post-curing finishing procedures have to be performed to remove excess material, obtain the correct anatomical form, and polish the surfaces [18]. Surface roughness in this study was evaluated by ESEM which provides both qualitative & quantitative data of the surface [28]. In accordance with the published data [31], the results of this study revealed that the surface roughness of the tried restorative materials

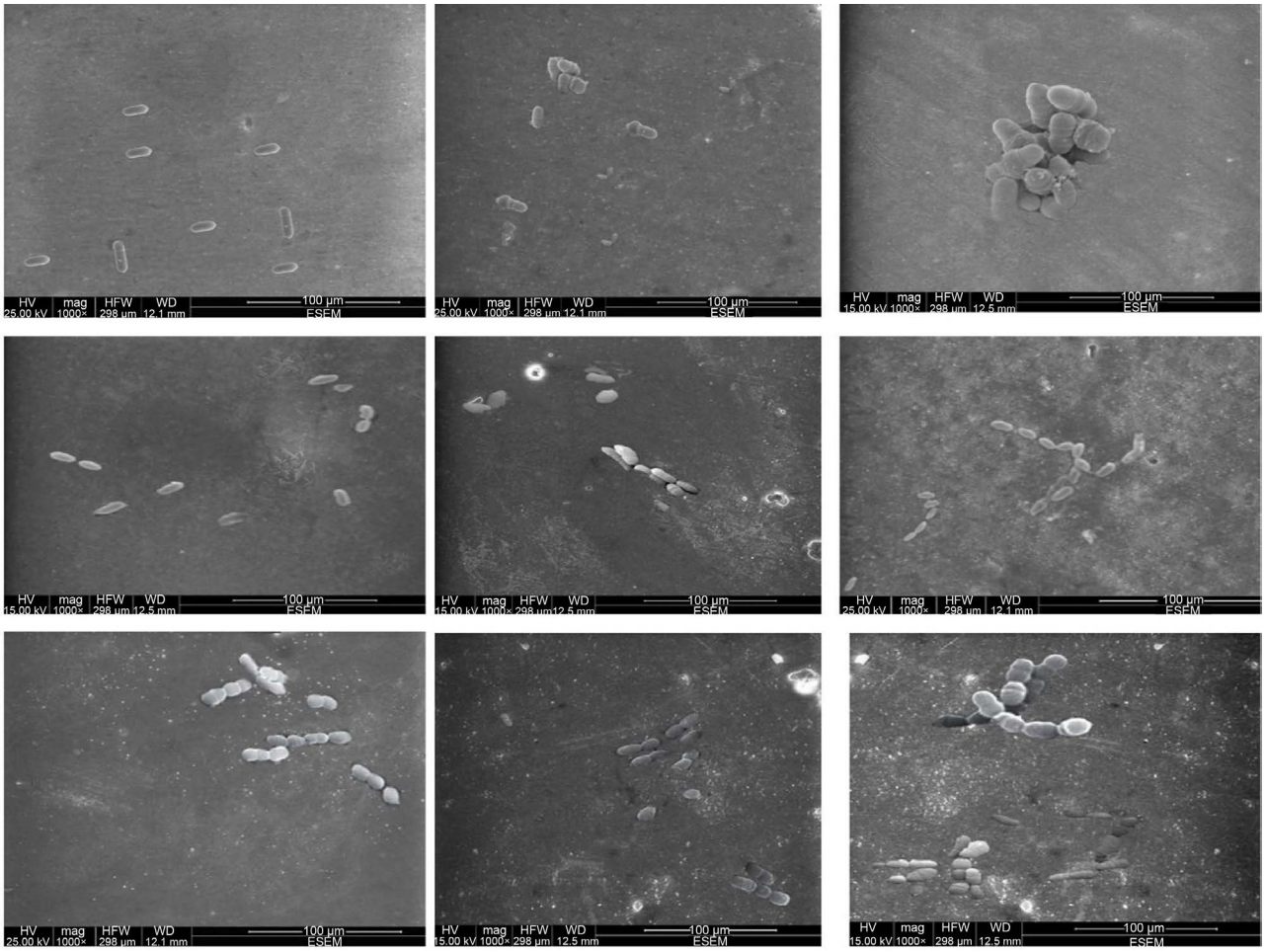

Figure 2. Two dimensional SEM micrographs of *Streptococcus mutans* ATCC 25,175 adhesion to the surfaces of resin composites polished with different polishing systems: (A) FiltekZ350 XT with Mylar strip, (B) FiltekZ350 XT with Pogo one step, (C) FiltekZ350 XT with Sof-lex multi-step, (D) Filtek Bulk-fill with Mylar strip, (E) Filtek Bulk-fill with Pogo one step, (F) Filtek Bulk-fill with Sof-lex multi-step, (G) Tetric N Ceram with Mylar strip, (H) Tetric N Ceram with Pogo one step, (I) Tetric N Ceram with Sof-lex multi-step.

have effect on the polishing systems with statistical significance.

In the present study, the lowest roughness values were recorded with the Mylar strip samples. The smoothest surface next to Mylar strip was obtained with one-step Pogo polishing system followed by multi-step Sof-lex polishing system. These findings are in agreement with that of Costa *et al.* [32], who reported that one-step system provides the highest gloss values. Conversely, the outcome of this study contradicts with that of Nasoohi *et al.* [33], who reported that finishing and polishing techniques need sequential usage of instrumentation with progressively smaller grained abrasives to finallyatta in the anticipated glossy surface. In this study, Sof-Lex multi-step finishing system revealed rougher surfaces than PoGo one-step finishing system with all tested composites.

The results of the present study revealed that the surface roughness of all tested restorative materials were significantly influenced by the type of finishing/polishing system and Mylar strip produced the smoothest surface and that

Table 5. Two way ANOVA test results of bacterial adhesion means among tested restorative materials and different finishing/polishing systems.

Source	Type III Sum of Squares	df	Mean Square	F	Significance
Corrected model	106012.8*	8	13251.6	255.6	0.000
Intercept	3537870.4	1	3537870.4	68249.8	0.000
restoration	785.0	2	392.5	7.5	0.001
finishing	103731.8	2	51865.9	1000.5	0.000
restoration finishing	1496.0	4	374.0	7.2	0.000
Error	4198.8	81	51.8		
Total	3648082.0	90			
Corrected total	110211.6	89			

*R^2 = 0.939 (Adjusted R^2 = 0.933).

Table 6. Post hoc Tukey's test results of *Streptococcus mutans* ATCC 25,175 adhesion to the tested restorative materials with different finishing/polishing systems.

Polishing system	Bacterial adhesion to restorative material (CFU ×10³/mL)		
	Filtek Z350 XT	**Filtek Bulk Fill**	**Tetric N Ceram**
Mylar strip	156.40 ± 6.50[a]	158.40 ± 6.50[a]	159.40 ± 6.50[a]
Pogo one step	199.40 ± 5.31[b]	201.40 ± 5.31[b]	202.40 ± 5.31[b]
Sof-lex multi-step	226.50 ± 4.00[c]	228.50 ± 4.00[c]	229.50 ± 4.00[c]

Each value represents mean (±SD) and values with different superscript letters a, b, c indicate statistically significant difference in bacterial adhesion.

may be due to its highly lustrous feature, which cannot be created with other polishing system, and this findings are consistent with some previous studies [34] [35]. Both Filtek Z350XT and Filtek Bulk-fill have nearly the same surface roughness which may be attributed to the similarity of the filler particle size (pure nano-filled) as compared to Tetric N Ceram which has higher nano-hybrid size as described previously [36] [37].

Cariopathogenic biofilms on tooth surfaces or artificial dental substrata are primarily formed by initial adhesion of specific oral bacteria to such surfaces. Within the complex process of biofilm development, *S. mutans* is primarily responsible for the initiation of tooth decay as well as for the progression of an established lesion[38].The selection of *S. mutans* for adhesion assay in this study was based on the fact that *S. mutans* is considered as a major etiological agent of dental caries [39] [40]. In this study, the adhered cells were removed for subsequent quantification after 4 h. This time of exposure was chosen because initial steps of biofilm development in the oral cavity normally occur within 4 h [41] [42].

The results of the present study indicated that bacterial adhesion differs significantly between various polishing systems, where Mylarstrip produced the lowest bacterial adhesion, followed by PoGo, and the highest value recorded with Sof-lex. This finding maybe attributed to the variable polishing capacity

and the surface roughness of these systems which significantly influence the bacterial adhesion to the substrata as describe previously [43] [44] [45]. Bacterial adhesion is governed by non-specific interactions (physico-chemical interactions) and specific (ligand-receptor like interactions). Non-Specific interactions involve van der Waals, acid-base, and electrostatic interactions. The integration of such interactions plays a fundamental role in the initial bacterial adhesion as well as in biofilm formation [46].

In the current study, Pearson correlation test indicated a strongly positive correlation (PCC = 0.943) between surface roughness and *S. mutans* adhesion to the tested surfaces. In accordance with our results, some investigators have mentioned potential correlations between surface roughness and bacterial adhesion [47] [48]. Similarly, it has been reported that *S. aureus* adhesion was strongly correlated to the surface roughness [49]. Furthermore, qualitative and quantitative adhesion analyses on different surfaces demonstrated significant aggregation of bacterial cells on untreated surfaces than on electro polished smooth surfaces [50]. Conversely, Eick *et al.* [51] disagreed with this relationship and reported that nocorrelation was observed between surface roughness and the number of colony forming units (CFU) of *S. mutans* in their study.

5. Conclusion

The current study revealed a strong association between surface roughness and *S. mutans* adhesion to the tested surfaces. Irrespective of the restorative material, Mylar polishing system presented the smoothest surface and the least bacterial adhesion as compared to Sof-Lex multi-step and Pogo one step polishing systems. Consequently, Mylar polishing system would be more recommended for clinical application.

Conflicts of Interest

The authors declare no conflict of interest.

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