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Effect of Finishing and Polishing Bulk-Fill Composites on Salivary and *Streptococcus mutans* Adhesion

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ABSTRACT

Bacterial adhesion over composite restorations may lead to secondary caries and periodontal inflammation. Post-curing finishing/polishing aim to reduce this exposure. This study investigated and compared the degree of single species *Streptococcus mutans (S. mutans)* and multi-species salivary biofilm adhesion on four restorative composites across three finishing/polishing systems. Standardized disc samples (2×10mm) were produced from each composite material. Ten discs of each material were subjected to three finishing and polishing systems. Half the samples (n=5) were incubated in human saliva and the other half were incubated in S. mutans for biofilm development for 48 and 24 h, respectively. Following dilution and bacterial growth, the mean number of colony forming units (CFU/mL×5; log10) was counted using a colony counter and analyzed using SPSS Statistics V22.0. Data were analyzed using three-way analysis of variance and the Tukey's post-hoc tests ($p<0.05$). There were no significant differences in biofilm adhesion in the saliva incubation group across the three polishing systems ($F=1.138$; $p=0.328$) or the four types of materials ($F=1.001$; $p=0.399$). There were significant differences in biofilm adhesion in the *S. mutans* group across the three polishing systems ($F=3.918$; $p=0.025$) and the four types of materials ($F=3.899$; $p=0.013$). Multiple comparisons revealed that biofilm adhesion was lowest in the Astropol® group. Filtek™ Bulk Fill had significantly lower biofilm adhesion than Filtek™ Z350 XT ($p=0.008$). Surface properties vary by composite materials and finishing and polishing techniques, which influences bacterial adhesion. The least bacterial adhesion was observed with Sof-LexTM finishing and polishing system and Filtek™ Bulk Fill composite material..

KEY WORDS: BULK-FILL COMPOSITES; RESIN COMPOSITE; SURFACE ROUGHNESS; BACTERIAL ADHESION; POLISHING SYSTEM; *STREPTOCOCCUS MUTANS*.

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INTRODUCTION

Resin-based composites (RBCs) can provide maximum tooth-like esthetics with a compatible tooth bonding structure and minimal cavity preparation (Chesterman et al., 2017). Due to these features, RBC dental materials are now widely used in daily clinical practice. However, RBCs also suffer from various limitations, including technique sensitivity and the multi-step etching and bonding procedure that requires incremental application. To address these concerns, manufacturers introduced bulk-fill RBCs. Clinicians can place bulk-fill RBCs in large increments, ranging from 4 to 10 mm, with less polymerization shrinkage and postoperative sensitivity, but improved aesthetics, durability, and working time. SDR and Filtek bulk fills were introduced as flowable composites with enhanced features, including radio-opacity, visible light cured, fluoride-containing, and applicable in relatively large increments of 4 mm (Chan et al., 2010, Benetti et al., 2015 Benetti et al., 2015; Van Ende et al., 2017. Chesterman et al., 2017).

Although there is a diverse number of available bulk-fill RBCs and their physical and chemical properties keep advancing, a variable degree of oral microbiota still adheres to RBCs. These microbiota can exert unwanted complications, including development of secondary caries and risks of periodontal disease, which restoration longevity. Among the complex bacterial colonies that are present in the oral flora, *Streptococcus mutans* (*S. mutans*) species play a major role in dental caries. Further, these cariogenic species are increasingly prevalent on composite restorations compared to natural teeth. Jaberi et al., (2014) evaluated 1,339 posterior teeth restored with amalgam or RBCs for secondary caries. RBC restorations showed a higher prevalence of secondary caries compared to amalgam restorations, (Loesche 1986, Thomas et al., 2008, Jaberi et al., 2014, Denson et al., 2018 Soliman et al., 2019).

RBC restorations also presented with a higher percentage of replacement due to secondary caries compared to amalgam restorations (Mjör and Jokstad 1993; Bernardo et al., 2007). Growing evidence indicates that the surface geometry of RBCs, namely the surface roughness, chemical composition, and clinical manipulation, influences attraction and colonization of microbial flora (Derchi et al., 2017). It is well established that material surface roughness is a key factor that makes composites vulnerable to bacterial adhesion and biofilm development (Montonaro et al., 2004). A rough, unfinished, and poorly polished composite surface is more likely to accumulate plaque (Eick et al., 2004). Finishing and polishing RBC materials can minimize plaque accumulation, subsequent marginal tissue inflammation, and recurrence of caries while improving wear behavior and the marginal integrity of posterior fillings (Ferreira et al., 2004, 2017).

Recent developments in finishing and polishing systems have allowed for smoother composite surfaces that may impact microbial adhesion levels (Ferreira et al., 2017). Pereira et al.,(2011) evaluated S. mutans biofilm adhesion on the surface of three RBCs subjected to different finishing and polishing techniques. There was a significant increase in bacterial adherence on all three composite surfaces, regardless of the polishing treatment performed.A limited number of studies have reported how the surface characteristics of bulk-fill RBC's influence biofilm formation and bacterial adhesion (Montonaro et al., 2004). Of these, none have investigated the effect of different finishing and polishing systems with variable steps on biofilm formation on bulk-fill RBCs. Therefore, the aim of this *in-vitro* study was to investigate and compare the degree of single species *S. mutans* and multi-species salivary biofilm adhesion on the surface of four common restorative composites across three finishing and polishing systems.

MATERIAL AND METHODS

This study was approved by the institutional review board of King Saud University (Reg. # E-18-3347). One conventional (FiltekTM Z350 XT (FZ)) and three bulk-fill (SDR® flow+ (SBF), FiltekTM (FBF), SonicFillTM 3 (SF)) commercially available RBCs with variable types of filler particles were utilized in the present study (Table 1). Discs were created from the four materials using a stainless-steel mold with a diameter of 10 mm and thickness of 2 mm. Briefly, the RBC material were placed in the mold and a clear matrix strip was pressed to produce a bubble-free, smooth surface disc. The disc was then light cured for 80 s by applying the tip of a hand-held light curing unit (Spectrum 800; Dentsply Inc., York, PA, USA) directly on the matrix strip. Thirty discs were created from each material, subdivided into three groups (n = 10), and subjected to Sof-LexTM (SL) finishing and polishing wheels, Astropol® (AS), or Enhance® PoGo ® (EP) finishing and polishing systems according to the manufacturer instructions (Table 1). The materials were then cleaned for excess fragments, washed with distilled water, and air dried. They were then sterilized by ultraviolet light at a wavelength of 253.7 nm for 1 min. The specimens were stored in sterile plastic containers with distilled water prior to the experimental phase.

Experimental phase Saliva group: Unstimulated human saliva was collected from one healthy participant who volunteered. Briefly, the participant was instructed to stop her oral hygiene practice one day prior to collection as well as not to eat or drink for at least 1 h prior to collection. Then, saliva was collected between 9:00 and 10:00 a.m. post-fasting after their mouth was washed with water and a 5 min resting period. A 50 ml sterile polypropylene tube was provided for the volunteer to expectorate unstimulated (drooled) saliva to achieve a volume of 5–7 ml. The saliva was directly placed on ice and transferred to the laboratory for processing. Five samples per RBC type and finishing and polishing subgroup were placed in 24-well polystyrene tissue culture plates (Thermo Fisher Scientific Inc., USA) and incubated in 1 ml of thawed sterilized human saliva for 48 h at 37C° in a CO2 chamber.

Table 1. Description of materials used in the study.

	Material Trade Name	Abbreviation Used	Manufacturer	Description (% weight)	Inorganic filler
Composite Materials	Filtek™ Z350 XT	FZ	3M ESPE	Resin: Bis-GMA, UDMA, TEGDMA, and bis-EMA. Filler system: Combination of non-agglomerated /non-aggregated 20 nm silica filler, non-agglomerated/ non-aggregated 4 to 11 nm zirconia filler, and aggregated zirconia/silica cluster filler (comprised of 20 nm silica and 4 to 11 nm zirconia particles).	78.5%
	SDR® flow+ Bulk Fill Flowable	SBF	Dentsply	Resin: Modified UDMA, TEGDMA, and EBPDMA. Filler system: Barium and Strontium AluminoFluoroboro Silicate	68%
	Filtek™ Bulk Fill	FBF	3M ESPE	Resin: Bis-GMA, Bis-EMA, UDMA, and Procrylat. Filler System: Combination of Ytterbium trifluoride and zirconia/silica particles	64.5%
	SonicFill™ 3	SF	Kerr	Resin: Bis-GMA, TEGDMA, and EBPDMA. Filler system: SiO2, glass, oxides, and chemicals	84%
Finishing and Polishing Materials	Sof-LexTM® Spiral Finishing and Polishing Wheels	SL	3M ESPE	2-step finishing and polishing system composed of thermoplastic elastomer impregnated with aluminum oxide particles	-
	Astropol®	AS	Ivoclar Vivadent	3-step finishing and polishing silicon rubber system: Astropol F: Silicon carbide particles and color pigments Astropol P: Silicon carbide particles and color pigments Astropol HP: Diamond particles, aluminum oxide, titanium oxide, and iron oxide	-
	Enhance® PoGo®	EP	Dentsply	1-Step Diamond Micro Polishers composed of pre-mounted, diamond impregnated polishers	-

Bis-GMA: bisphenol A-glycidyl methacrylate; UDMA: Urethane dimethacrylate; TEGDMA: Tri-ethylene-glycol-dimethacrylate; bis-EMA: bisphenol A glycol dimethacrylate; EBPDMA: ethoxylated Bis-GMA

S. mutans group: The other half of the samples (n = 5) for each group were placed in 24-well plates then covered with 1.5 ml of brain heart infusion agar (BHI agar, Difco, Detroit, MI, USA). A standard suspension of S. mutans was then prepared by seeding *S. mutans* onto BHI agar and samples were incubated for 24 h at 37C° in a CO2 chamber.

Bacterial adhesion: Following the protocol described by Pereira et al (2011), the samples were removed and washed with sterile physiological buffered solution (Gibco®, ThermoFisher Scientific, MA, USA) to remove loosely bound material. Then, the samples were placed in tubes with 1 ml of sterile physiological solution and mixed on a mixer (Super Mixer® II, LAB-LINE Instruments, IL, USA) for 30 s to disperse the biofilms. The obtained suspension was diluted 10, 100, 1,000, and 10,000 times and 0.1 ml aliquots were seeded in duplicate onto BHI agar and incubated for 48 h at 37C° in a CO2 chamber. Following incubation, the plates with bacterial colonies were counted in a colony counter (Reichert Quebec® Darkfield Colony Counter, Cambridge Instruments. NY. USA)

Data analysis: Data was analyzed using SPSS version 21.0 (IBM Inc., Chicago, IL, USA) statistical software. The mean saliva and *S. mutans* values (CFU/ml x 5) were converted to log10 to attain a normal distribution for analysis. Descriptive statistics (mean ± standard deviations) were used to describe the quantitative outcome variables in saliva and *S. mutans* groups. One-way analysis of variance was used to compare the mean saliva and *S. mutans* values across the three polishing systems (SL, AS, and EP) and four composite materials (FZ, SBF, FBF, and SF). Tukey's multiple comparisons tests were used to compare the mean values of different pairs of polishing systems and composite materials. A p-value of <0.05 was used as the cut-off for statistical significance.

Table 2. Comparison of biofilm adhesion levels following saliva or *S. mutans* incubation across the three polishing systems.

Type of polishing system	Mean (SD) biofilm adhesion (log10) following saliva incubation	F-value	p-value	Mean (SD) biofilm adhesion (log10) following *S. mutans* incubation	F-value	p-value
SL	5.99 (0.52)	1.138	0.328	7.26 (0.39)1	3.918	0.025*
AS	6.11 (1.07)			6.89 (0.64)2		
EP	6.37 (0.74)			7.23 (0.24)3		
Overall Mean	6.15 (0.77)			7.12 (0.42)		

*Statistically significant; (SL vs AS: p = 0.040; SL vs EP: p = 0.985; AS vs EP: p = 0.059) (by Tukey's test) SL: Sof-LexTM finishing and polishing wheels; AS: Astropol®; EP: Enhance® PoGo ®

Table 3. Comparison of biofilm adhesion following saliva or *S. mutans* incubation across the four RBCs.

Type of material	Mean (SD) biofilm adhesion (log10) following Saliva incubation	F-value	p-value	Mean (SD) biofilm adhesion (log10) following *S. mutans* incubation	F-value	p-value
FZ	6.46 (0.41)	1.001	0.399	7.44 (0.42)1	3.899	0.013*
SBF	6.10 (0.77)			7.10 (0.61)2		
FBF	5.99 (1.32)			6.90 (0.37)3		
SF	6.06 (0.38)			7.07 (0.33)4		
Over all mean	6.15 (0.72)			7.12 (0.43)		

*Statistically significant; (FZ vs SBF: p-value = 0.157; FZ vs FBF: p-value = 0.008; FZ vs SF: p-value = 0.118; SBF vs FBF: p-value = 0.626; SBF vs SF: p-value = 0.999; FBF vs SF: p-value=0.712) (by Tukey's test). FZ: FiltekTM Z350 XT; SBF: SDR® flow+; FBF: FiltekTM; SF: SonicFillTM 3

RESULTS AND DISCUSSION

Finishing & Polishing systems: The mean biofilm adhesion level following saliva incubation (6.15 ± 0.77) across the three polishing systems was less than following *S. mutans* incubation (7.12 ± 0.42). The type of polishing system used did not significantly alter biofilm adhesion in the saliva incubation group (F = 1.138; p = 0.328). In comparison, there was a significant difference in the *S. mutans* incubation group (F = 3.918; p = 0.025), with AS having significantly less biofilm adhesion than SF (p = 0.040) and EP (p = 0.059) (Table 2).

Composite materials: The mean biofilm adhesion level following saliva incubation (6.15 ± 0.72) across the four RBCs was less than following *S. mutans* incubation (7.12 ± 0.43). The type of RBC did not significantly alter biofilm adhesion in the saliva incubation group (F = 1.001; p = 0.399). In comparison, there was a significant difference in the *S. mutans* incubation group (F = 3.899; p = 0.013), with FBF having significantly less biofilm adhesion than FZ (p = 0.008) and no other significant group differences (Table 3).

The performance and long-term service of RBC restorations is not only dependent on their intrinsic properties, but also is greatly influenced by their extrinsic properties (Barbosa et al., 2005; Jung M 2007). The intrinsic properties are closely related to the success or failure of the restoration in terms of its internal composition and resistance to fracture. However, the external surface properties following finishing and polishing directly affect the surrounding microflora and influence bacterial adhesion (Ikeda et al., 2007). Biofilm formation that results from poorly finished and polished RBCs increases the chances of periodontal disease, secondary caries, and esthetic complications like discoloration (Attar 2007; Koh et al., 2008). Surface roughness is one element that makes RBC materials susceptible to bacterial attachment and biofilm formation (Mei et al., 2011). Previous studies have proposed that a surface roughness value of 200 nm is the upper limit for bacterial retention. No reductions were seen in measures of bacterial retention below this value, whereas biofilm accumulation increased with higher roughness values (Ikeda et al., 2007). RBC surface roughness is influenced by resin matrix, filler type, size, shape, and distribution of the fillers in the matrix, as well as the finishing and polishing techniques used (Türkün and Türkün 2004).

Stoddard and Johnson (1991) suggested that the material itself, filler size, content, type of abrasive used, number of strokes, amount of pressure applied, time spent on each abrasion, direction of the abrading surfaces, and geometry of the abrasive instruments impact the effectiveness of finishing and polishing systems. These factors determine whether a surface is properly polished, which decreases the risk of initial bacterial adherence and subsequent colonization (Gedik et al., 2005; Yap et al., 1998). Previous studies have reported that lower *S. mutans* biofilm adhesion rates were observed in FZ RBCs due to their smaller particles and fillers size and their wide distribution in the resin matrix. These factors reduce surface roughness after finishing and polishing, consequently decreasing S. mutans adherence (Montonaro et al., 2004). However, the present study found that the highest bacterial adhesion rate was observed on the conventional FZ RBC regardless of incubation type, with the SF and AS polishing groups in the presence or the absence of human saliva, and it was higher compared to the FZ specimens finished and polished using EP finishing system.

This finding is in opposition to previous studies observing finishing and polishing techniques. Antonson et al. (2011), concluded that the SF finishing system provided the smoothest surface when compared to AS and EP (Pereira et al., 2011). SF disks were also found to provide a smoother RBC surface than carbide bur finishing, followed by the Astrobrush. This may be due to SF disk's inability to displace filler particles in RBCs, thereby providing a homogenous abrasion of the fillers and resin matrix which promotes less bacterial adhesion (Gyo et al., 2008). The results of the current study showed that SL disks in the presence of human saliva recorded the lowest bacterial adhesion over systems and composite materials (p=0.040), which also coincided with material surface roughness (data not shown). However, in the absence of human saliva, the SL group recorded the highest bacterial adhesion on SBF and FBF materials among all groups. In contrast, in the absence of saliva, in the AS group, the SBF and FBF composite materials recorded the lowest bacterial adhesion among all groups.

However, a study by Abuelenain et al. (2017) observed a surface roughness value greater than 200 nm was observed in SBF and SF RBCs, suggesting roughened surface beyond this threshold will lead to more bacterial retention due to presence of micro-retentive surface alterations, which increases surface area for pellicle formation as previously reported by Øilo et al., (2015). In the presence of saliva, SF composite in the EP group recorded the lowest bacterial adhesion among the four types of composites in the same group. In contrast, in the absence of saliva, SF recorded the highest bacterial adhesion in the EP group. Although we did not perform specific characterization and quantification of the salivary sample utilized in the experiment, it has been reported that human saliva contains and serves as a growth media for S. mutans species (Newman 1974). The specimens in the current study that were incubated in human saliva containing S. mutans exhibited a significant increase in biofilm growth using the conventional FZ composite. This demonstrates the powerful ability of salivary components to modulate biofilm adhesion because oral bacteria adhere to receptors of the host origin in saliva pellicle (Øilo et al., 2015).

Bacterial adhesion is not only influenced by the physical and chemical composition of composites, but also by the material type, polishing medium, finishing and polishing technique performed, and the presence of saliva pellicle. A study done by Nasoohi N et al. (2017) regarding polishing medium reported that dry finishing

and polishing of microhybrid and nanohybrid RBCs increased the micro hardness and surface roughness (Abzal et al., 2016). By comparing the RBCs with other dental materials, one previous study showed that glass ionomers with a rough surface and increased inorganic components harbors more bacteria than RBCs. Therefore, the increase in bacterial adhesion to RBCs after finishing and polishing may be due to the change in the surface roughness (Stoddard and Johnson 1991).

A study by Derchi et al. (2017) revealed that indirect dental restorative composite resins were less prone to biofilm adhesion than direct composite resins (Derchi et al., 2017). Another study that investigated surface roughness and *S. mutans* adhesion in the presence and absence of saliva on composites and ceramics found that enamel was the roughest, Leucite/feldspathic ceramics were rougher than the feldspathic ceramic, and indirect composites were similar to direct composites. The highest level of bacterial adherence was found on enamel in the presence and absence of saliva, whereas the leucite/feldspathic ceramic demonstrated greater adherence than the feldspathic ceramic, and the composites were all statistically similar (Ikeda et al., 2007). The present study used *S. mutans* to promote biofilm adhesion because *S. mutans* bacterium is known to be the main etiological factor in the initiation and progression of dental caries. Moreover, the bulk of the microorganisms present in dental plaque are S. mutans (Montonaro L et al., 2004), and its adherence to enamel surface and restorative materials is a preliminary condition for biofilm formation. These formations can eventually promote secondary caries and periodontal diseases (Jung et al., 2007; Sissons et al., 1991).

A possible limitation of the current research is that it only observes a few of many techniques that are available to investigate RBC performance within an experimental oral environment. Further, there is a lack of consistency across some experimental protocols, which means that the current study may not be directly comparable to some published results. Further, short term investigations concerning bacterial adhesion may not provide information that is representative of long-term intraoral RBC use, which could be gained by clinical studies. Nonetheless, an attempt was made to standardize and reproduce the conditions present in the oral environment. Use of the artificial mouth continuous culture, or systems as suggested by Sissons et al., (1991) and Sissons (1997) is recommended for future studies related to oral biofilms. These models reproduce biofilm-growing conditions similar to the in vivo environment.

CONCLUSION

Multiple conclusions can be made within the limitations of this study. Firstly, RBC surface properties differ across materials and finishing and polishing techniques, which influence bacterial adhesion. Due to differences in the size, shape, number of filler particles, and the type of resin, one finishing and polishing system was incapable of creating the smoothest surface for all the RBCs tested. Pairing RBCs with the polishing system recommended by the manufacturer is suggested for clinical use. Overall, the lowest levels of bacterial adhesion were observed with the SL system and FBK RBC. Understanding the relationships among surface roughness, saliva, and biofilm formation in environments containing *S. mutans* is important to preventing secondary caries around composite restorations.

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