

## Effect of Polishing Wheels on Composite Resin Surface Properties and Bacterial Adhesion *In Vitro*

Guy Melamed, Victoria Glinkin and Nir Sterer\*

Department of Prosthodontics, Goldschleger School of Dental Medicine, Sackler Faculty of Medicine, Tel-Aviv University, Ramat-Aviv, Israel

\*Corresponding Author: Nir Sterer, Department of Prosthodontics, Goldschleger School of Dental Medicine, Sackler Faculty of Medicine, Tel-Aviv University, Ramat-Aviv, Israel.

Received: July 21, 2020; Published: August 29, 2020

### Abstract

**Introduction:** Bacterial adhesion to composite resin and biofilm accumulation may cause gingivitis and secondary caries formation resulting in restoration failure.

**Aim:** The aims of the present study was to compare the effect of different finishing and polishing means on composite resin surface properties and bacterial adhesion.

**Materials and Methods:** Composite resin samples were assigned to four different finishing and polishing test groups: (i) no finishing (mylar strip control), (ii) finishing burs only, (iii) Finishing burs and polishing discs, and (iv) Finishing burs and polishing wheels. Following treatment samples were evaluated for surface roughness using SEM and non-contact profilometer, surface hydrophobicity using contact angle goniometer, salivary proteins adsorption using Bradford assay and bacterial adhesion using crystal violet assay and fluorescence microscopy.

**Results:** Polishing wheels use resulted in a significant increase in bacterial adhesion concomitant with a significant decrease in surface hydrophobicity and an increase in salivary proteins adsorption as compared with polishing discs. Whereas, no significant difference was observed in surface roughness between the two.

**Conclusion:** Results show that the use of polishing wheels may increase bacterial adhesion and that this might be associated with reduced surface hydrophobicity and increased salivary proteins adsorption rather than increased surface roughness.

**Keywords:** Bacterial Adhesion; Composite Resin; Polishing; Surface Properties

### Introduction

Finishing and polishing techniques strongly effect the aesthetic appearance and surface properties of composite resin restorations [1]. Polishing gives the restoration 'enamel-like' appearance by providing their surface with shine and luster as well as decreasing the risk for discoloration and bacteria and debris retention [2-4].

Bacterial adhesion to composite resin restoration is the first step in biofilm formation [5]. Microbial biofilm may increase the risk of gingival inflammation and secondary caries formation resulting in restoration failure [6,7]. Bacteria adherence to dental and restorative surfaces is mediated by the adsorption of salivary proteins onto these surfaces. These adsorbed proteins form a layer known as the ac-

quired pellicle and constitute the connecting layer between the surface and adhering bacteria [8]. The first adhering oral bacteria or early colonizers adhere to specific salivary proteins and allow other bacteria including oral pathogens to attach, thus forming the mature oral biofilm [9].

Whereas some studies showed significant correlations between surface roughness and bacterial adhesion [10,11], others reported only partial correlations between the two [12,13]. Other studies linked bacterial adhesion with different surface properties such as salivary proteins adsorption [8].

### Aim of the Study

The aim of the present study was to test the effect of polishing wheels on composite resin surface properties (hydrophobicity, roughness and protein adsorption) and bacterial adhesion.

### Materials and Methods

#### Tested materials

Nano-filled composite resin (Filtek Ultimate®, 3M-ESPE) discs were prepared using a fabricated Teflon mold (9 mm diameter and 2 mm thickness) and covered with a Mylar strip. Discs were light cured for 20 seconds using LED curing light (Elipar S10, 3M-ESPE) with a curing light intensity of 1200 mW/cm<sup>2</sup>. Discs were divided into 4 treatment groups as follows: (i) no finishing (Hawe transparent strip) as control, (ii) finishing burs only (199-018 XF, MDT), (iii) Finishing burs followed by polishing discs system (Sof-Lex® extra thin, 3M-ESPE) and (iv) Finishing burs followed by polishing wheels (Sof-Lex® spiral, 3M-ESPE).

Discs preparation, finishing and polishing were carried out by a single operator. Finishing and polishing were applied to one side of the discs, using light pressure in a single direction. Burs were applied for 10 seconds using water cooled high speed hand-piece and replaced every 6 samples. Finishing discs and wheels were applied for 10 seconds using air cooled low speed hand-piece and replaced for each new sample. Following finishing and polishing discs were rinsed with water and allowed to dry for 24 hours before they were tested for surface properties and bacterial adhesion as described below.

#### Surface morphology and roughness

Samples were mounted on aluminum stabs and sputter coated with gold. Surface morphology was examined using scanning electron microscopy (SEM, JSM-IT100, Jeol) in 2,000x magnification.

Surface roughness (Ra) was measured using a non-contact profilometer (µsurf explorer, Nanofocus) in 1,000x magnification.

#### Surface hydrophobicity

Contact angle test was performed to evaluate the hydrophobic properties of the tested discs. Contact angle was measured using a goniometer (model 100, Ramé-Hart) by placing a drop of deionized water (10 µL) on the leveled disc, measuring the contact angle on both sides of the drop and recording the mean result.

#### Salivary proteins adsorption

A drop (40 µL) of filtered saliva (0.22 mm, stericup, Millipore) was placed on each of the polished or control discs for 3 minutes at room temperature. Discs were washed three times with saline to remove any non-adhered salivary proteins. Discs were placed on the bottom of a 48 wells plate and the wells were added with 250 µL of Bradford reagent [14]. The reagent was allowed to react for 10 minutes and transferred to a 96 well plate that was read at 600 nm.

### Bacterial adhesion

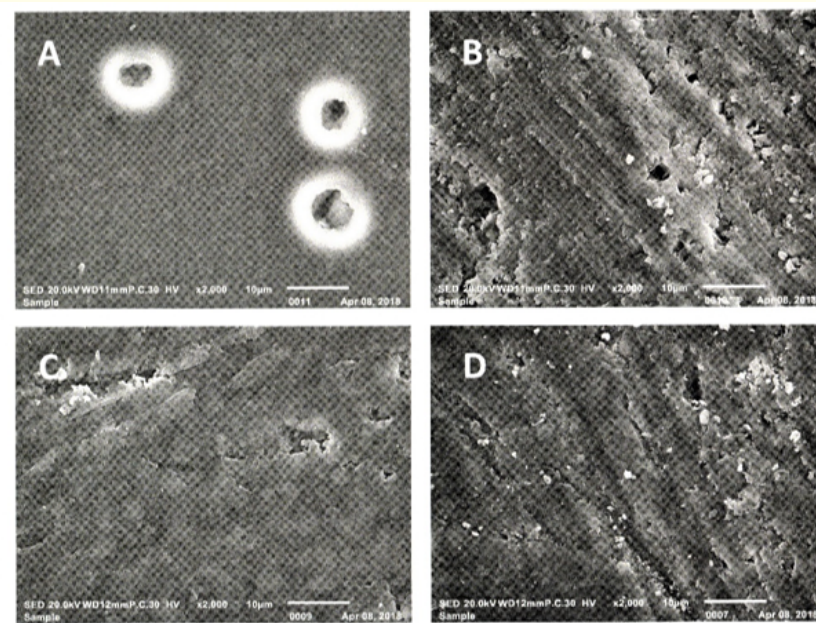
Bacterial adhesion was tested using the early colonizer bacterium *Streptococcus salivarius* (NS1). Discs were treated with filtered saliva as stated above. Following salivary proteins adsorption the discs were placed at the bottom of a 48 wells plate that were filled with 500  $\mu$ L of test bacterium suspension (10D) and incubated for 4 hours at 37°C. Following incubation the discs were washed three times with saline to remove any non-adhered bacteria. Bacterial adhesion was evaluated by fluorescence microscopy and quantified using crystal violet assay. Fluorescent microscopy was done by staining the adhered bacteria with a Rose Bengal solution (0.1% w/v) covering the discs with a cover slip and using immersion oil magnification (1,000x). Crystal violet (100  $\mu$ L) drop was placed on the discs for 1 minute and the discs were washed three times with saline to remove any non-cell-bound stain. The stain was extracted using a distaining solution that was transferred to a 96 well plate and read at 600 nm.

### Statistical analysis

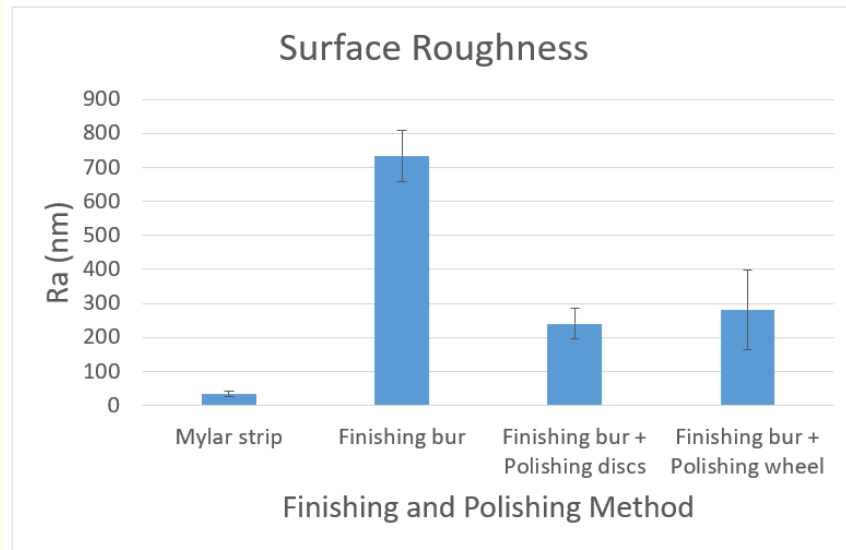
To compare the effect of the different polishing measures on surface properties and bacterial adhesion ANOVA was applied with post-hoc pairwise comparisons according to Dunnet and Scheffe. Tests applied were two-tailed and  $p \leq 0.05$  was considered statistically significant. Experiments were conducted in six replicates.

### Results

Results of the composite resin surface morphology and roughness following the various treatments are presented in figure 1 and 2. Results show that the surface roughness (Ra) of the composite resin was slightly lower in the samples polished using polishing discs as compared with the polishing wheels. However, these differences were not statistically significant.

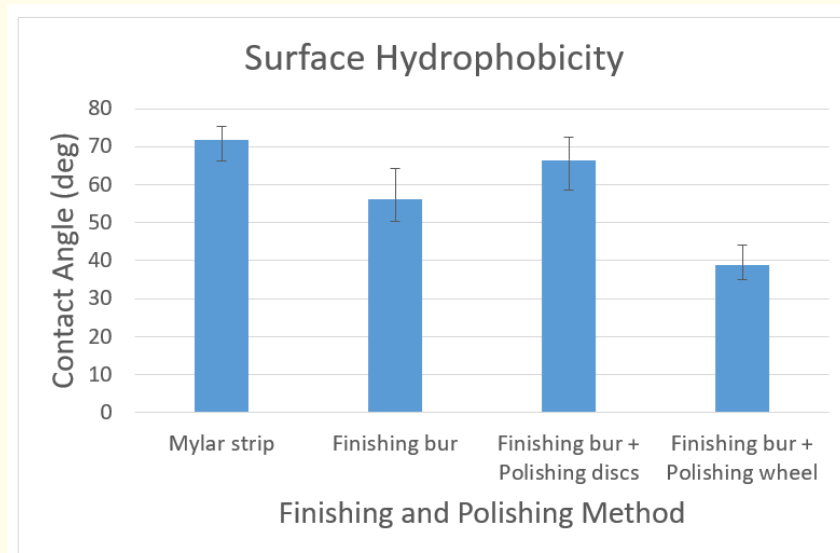


**Figure 1:** SEM images of the various treated surfaces: (A) Mylar strip control, (B) Finishing burs only, (C) Finishing burs and polishing discs set, (D) Finishing burs and polishing wheels set.



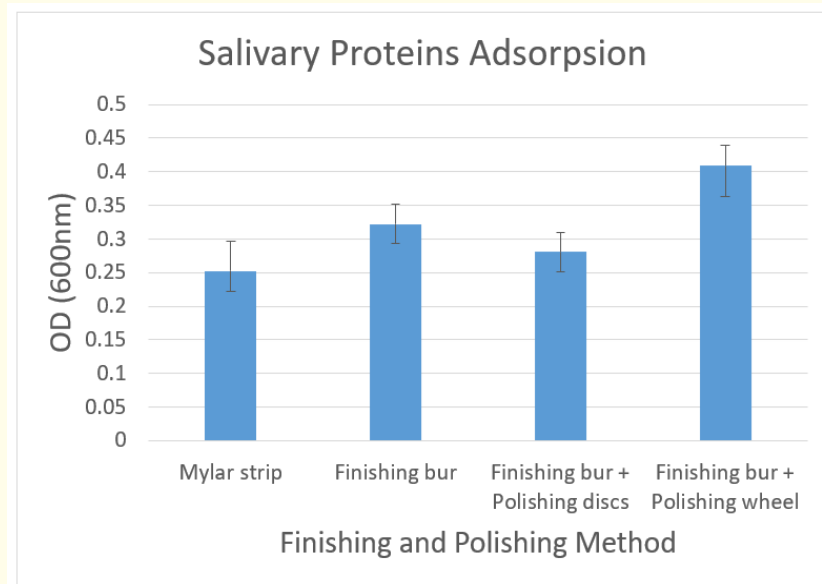
**Figure 2:** Mean results ( $\pm$  SD) of surface roughness (Ra) for the various treated surfaces measured using a non-contact profilometer.

Results of the composite resin surface hydrophobicity following the various treatments are presented in figure 3. Results show that the surface hydrophobicity (contact angle) of the composite resin was significantly lower in the samples polished using polishing wheels as compared with the polishing discs ( $p < 0.001$ ) and the mylar strip and finishing burs controls.



**Figure 3:** Mean results ( $\pm$  SD) of surface hydrophobicity for the various treated surfaces measured using a contact angle goniometer.

Results of the salivary proteins adsorption to composite resin surface following the various treatments are presented in figure 4. Results show that salivary proteins adsorption onto the composite resin surface was significantly higher in the samples polished using polishing wheels as compared with the polishing discs ( $p < 0.001$ ) as well as the mylar strip and finishing burs controls.



**Figure 4:** Mean results ( $\pm$  SD) of salivary proteins adsorption onto the various treated surfaces measured using Bradford assay.

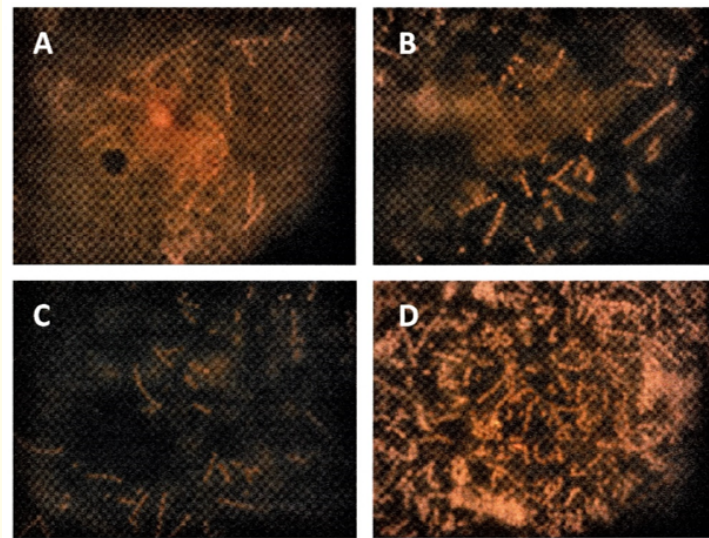
Results of the bacterial adhesion to composite resin surface following the various treatments are presented in figure 5 and 6. Results show that bacterial adhesion to the composite resin surface was significantly increased in the samples polished using polishing wheels as compared with the polishing discs ( $p < 0.001$ ) as well as the mylar strip and finishing burs controls.

## Discussion

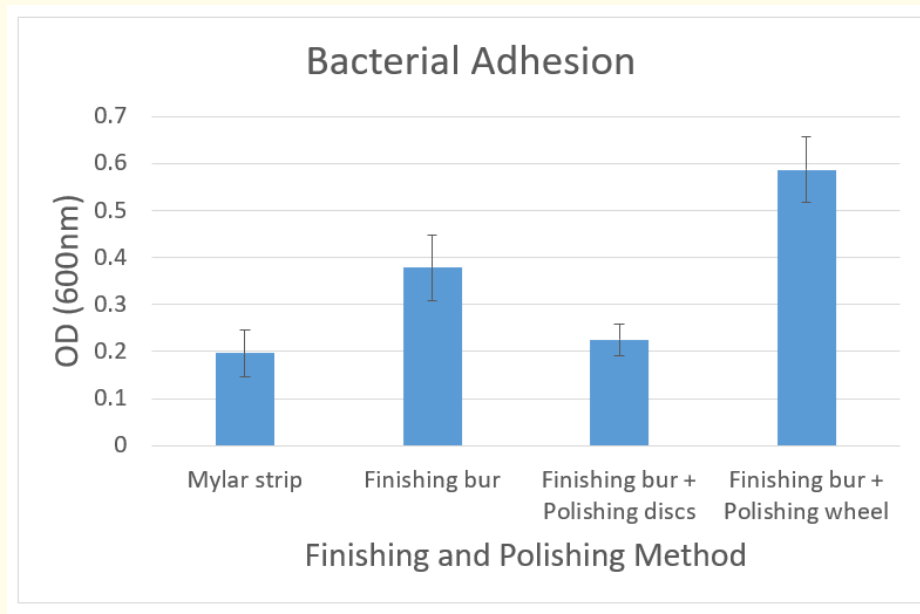
Finishing and polishing procedures have a strong effect on the outcome of a composite resin restoration both aesthetically and biologically. Composite resin restorations are prone to biofilm accumulation and the risk of secondary caries formation [15]. Surface properties that favors bacterial adhesion such as increased surface roughness was shown to increase the risk for gingival inflammation and staining [2,16].

Results of the present *in vitro* study showed that the use of polishing wheels significantly increased bacterial adhesion to the treated composite resin surface as compared with polishing discs. However, unlike some previous studies in the present study bacterial adhesion was not associated with a significant increase in surface roughness but rather with an increase in salivary proteins adsorption. Increased salivary proteins adsorption promotes the formation of the acquired pellicle on dental and restorative surfaces. These proteins enables the adhesion of early colonizing bacteria such as oral streptococci, which in turn initiates the formation of the dental biofilm [9].

Previously we suggested the incorporation of antibacterial nanoparticles in the composite resin matrix as a safety measure against secondary caries formation [17]. However, killing or inactivating the adhered bacteria will not solve this problem since residual non-vital



**Figure 5:** Fluorescence microscopy images of *Streptococcus salivarius* adhered to the various treated surfaces: (A) Mylar strip control, (B) Finishing burs only, (C) Finishing burs and polishing discs set, (D) Finishing burs and polishing wheels set.



**Figure 6:** Mean results ( $\pm$ SD) of bacterial adhesion to the various treated surfaces measured using crystal violet assay.

biofilm structure has been shown to facilitate and promote secondary bacterial adhesion [18]. Therefore, it was suggested that the reduction of bacterial adhesion might be a better strategy [19].

Previous studies reported that initial salivary proteins adsorption and acquired pellicle formation was a rapid, reproducible and highly selective process [20] and that the pattern of adhered proteins varied between different surfaces [21]. It was further claimed that the adsorption of specific salivary proteins onto dental materials surfaces was imperative to the adhesion of early colonizing bacteria [8].

### **Conclusion**

Salivary proteins adsorption is mediated through electrostatic and hydrophobic reactions. Taken together results of the present study suggest that the use of polishing wheels may increase salivary proteins adsorption and bacterial adhesion by significantly reducing surface hydrophobicity. The mechanism through which this mode of treatment affects the composite resin surface's hydrophobicity is yet unclear and warrants further studies.

### **Bibliography**

1. Antonson SA., *et al.* "Comparison of different finishing/polishing systems on surface roughness and gloss of resin composites". *Journal of Dentistry* 39.1 (2011): e9-17.
2. Gönülol N and Yılmaz F. "The effects of finishing and polishing techniques on surface roughness and color stability of nanocomposites". *Journal of Dentistry* 40 (2012): 64-70.
3. Sirin Karaarslan E., *et al.* "Effects of different polishing methods on color stability of resin composites after accelerated aging". *Dental Materials Journal* 32 (2013): 58-67.
4. Bollen CM., *et al.* "Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: a review of the literature". *Dental Materials* 13 (1997): 258-269.
5. Busscher HJ., *et al.* "Biofilm formation on dental restorative and implant materials". *Journal of Dental Research* 89 (2010): 657-665.
6. Bernardo M., *et al.* "Survival and reasons for failure of amalgam versus composite posterior restorations placed in a randomized clinical trial". *Journal of the American Dental Association* 138 (2007): 775-783.
7. Park JW., *et al.* "The effects of surface roughness of composite resin on biofilm formation of *Streptococcus mutans* in the presence of saliva". *Operative Dentistry* 37 (2012): 532-539.
8. Schweikl H., *et al.* "Salivary protein adsorption and *Streptococcus gordonii* adhesion to dental material surfaces". *Dental Materials* 29 (2013): 1080-1089.
9. Kolenbrander PE., *et al.* "Oral multispecies biofilm development and the key role of cell-cell distance". *Nature Reviews Microbiology* 8 (2010): 471-480.
10. Glauser S., *et al.* "Bacterial colonization of resin composite cements: influence of material composition and surface roughness". *European Journal of Oral Sciences* 125.4 (2017): 294-302.
11. Mei L., *et al.* "Influence of surface roughness on streptococcal adhesion forces to composite resins". *Dental Materials* 27 (2011): 770-778.
12. Derchi G., *et al.* "Bacterial adhesion on direct and indirect dental restorative composite resins: An in vitro study on a natural biofilm". *Journal of Prosthetic Dentistry* 117 (2017): 669-676.

13. Yuan C., *et al.* "Effects of surface properties of polymer-based restorative materials on early adhesion of *Streptococcus mutans* *In vitro*". *Journal of Dentistry* 54 (2016): 33-40.
14. Bradford MM. "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding". *Analytical Biochemistry* 72 (1976): 248-254.
15. Spencer P, *et al.* "Proteins, pathogens, and failure at the composite-tooth interface". *Journal of Dental Research* 93 (2014): 1243-1249.
16. De Fúcio SB, *et al.* "Analyses of biofilms accumulated on dental restorative materials". *American Journal of Dentistry* 22 (2009): 131-136.
17. Shvero DK, *et al.* "Antibacterial effect of polyethyleneimine nanoparticles incorporated in provisional cements against *Streptococcus mutans*". *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 94 (2010): 367-371.
18. Ohsumi T, *et al.* "Residual structure of *Streptococcus mutans* biofilm following complete disinfection favors secondary bacterial adhesion and biofilm re-development". *PLoS One* 10 (2015): e0116647.
19. Kim DH and Kwon TY. "In vitro study of *Streptococcus mutans* adhesion on composite resin coated with three surface sealants". *Restorative Dentistry and Endodontics* 42 (2017): 39-47.
20. Delius J, *et al.* "Label-free quantitative proteome analysis of the surface-bound salivary pellicle". *Colloids and Surfaces B: Biointerfaces* 152 (2017): 68-76.
21. Svendsen IE and Lindh L. "The composition of enamel salivary films is different from the ones formed on dental materials". *Biofouling* 25 (2009): 255-261.

**Volume 19 Issue 9 September 2020**

**©All rights reserved by Nir Sterer, *et al.***