Original Article

pISSN, eISSN 0125-5614
M Dent J 2019; 39 (2) : 75-84

Effect of the surface roughness of composite resins on the water contact angle and biofilm formation

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Objectives: To establish the correlation among the surface roughness, contact angle and biofilm formation of composite resins after polishing with different grits abrasive discs.

Materials and Methods: Three different composite resins, including microhybrids (Filtek Z250), nanohybrids (Filtek Z250 XT) and nanocomposites (Filtek Z350 XT), were used in this study. Fifty discs of each composite resin were prepared and divided into the following 5 groups for polishing: non-polishing (control), coarse-grit abrasive discs, medium-grit abrasive discs, fine-grit abrasive discs, and superfine-grit abrasive discs. The surface roughness was determined using a contact profilometer. The water contact angle was determined using the sessile drop method. Finally, the biofilm formation was evaluated using a crystal violet assay for *S. mutans*. Data were analyzed using two-way ANOVA and Dunnett T3 multiple comparison. Additionally, the linear regression models were analyzed to determine the correlation among the surface roughness, water contact angle and biofilm formation.

Results: The roughest surfaces with low surface angles were the surfaces polished with coarse-grit abrasive discs, whereas the smoothest surfaces with high surface angles were the surfaces polished with superfine-grit abrasive discs for all composites. A large amount of biofilm was found in the groups polished with a coarse-grit abrasive disc. Correlations between surface roughness and contact angle ($r = 0.778$); surface roughness and biofilm formation ($r = 0.648$); and contact angle and biofilm formation ($r = 0.563$) were found.

Conclusions: There was a direct correlation among the surface roughness, water contact angle and biofilm formation of composite resins.

Keywords: biofilm formation, composite resins, contact angle, surface roughness

How to cite: Komalsingsakul A, Klaophimai A, Laovanitch Srisatjaluk R, Senawongse P. Effect of the surface roughness of composite resins on the water contact angle and biofilm formation. M Dent J 2019; 39: 75-84.

Introduction

Resin composites are universally used tooth-colored direct restorative materials, and they consist of three major components: a resin matrix, fillers and coupling agents. Resin composites can be categorized by their consistencies and by the size of the reinforced fillers. Focusing on the reinforced filler sizes, a macrofill has an average particle size of approximately 10-50 µm, which increases its mechanical properties. However, the macrofill is difficult to polish, and good surface smoothness cannot be obtained. Alternatively, a microfill is another formula of resin composite that consists of small particle sizes of approximately 40 nm. Consequently, true nanocomposites containing 40 nm silica dioxide and/or zirconium dioxide fillers were developed. The nanocomposite can produce a smoother surface, resulting in excellent long-term esthetics.

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Received : 25 February 2019 Accepted : 4 June 2019

However, nanocomposites have lower filler contents and weaker mechanical properties than older composites. The amounts of filler can be increased by adding highly filled, prepolymerized fillers (PPF) within the matrix. Additionally, a smaller particle size can be produced by further grinding conventional composites to yield small particle hybrid composites or midifills, which contain an average particle size slightly greater than 1 um, and they are also combined with a 40 nm microfiller. Further refinements in particle size through enhanced milling and grinding techniques produce resin composites of a submicron scale, which are typically particles with a size of approximately 0.4-1.0 µm. These composites are known as microhybrids. [1] The microhybrid resin composites are considered to be universal composites, as they can be used for both anterior and posterior restorations due to their high strength and polishability. Modification of microhybrids is performed by including more nanoparticles and PPF, thus producing a nanohybrid resin composite. [2] Resin composite fillers are made of silica, quartz, or ceramic. It is known that filler loading plays an important role in the mechanical properties of composites, such as flexural strength, modulus, diametral tensile strength, and fracture toughness. [3,4] The filler content of a composite is sometimes determined by the shape of the filler. Resin composites with prepolymerized fillers have been shown to have a low filler content and a low flexural strength and hardness. Resin composites containing round-shaped fillers were shown to have a high filler content and a high hardness and flexural strength. For mixed filler particles used as hybrid composites, there was no relationship between the filler content and flexural strength. [5]

Finishing and polishing of dental restorations are important steps to achieve longevity and esthetics in restoration. Different resin composites present different surface hardnesses, resulting in a nonuniform abrasion level after the polishing process. [6] The filler size of the resin composite also plays a major role in the nonuniform abrasion level after polishing. Large fillers are often associated with detachment of the filler and increased porosity of the restoration. [7,8] There is also a clear correlation between resin composite surface roughness and biofilm retention. Many studies showed that a rougher surface results in more biofilm and bacterial adherence. [9-11]

An increase in the wettability or the surface free energy of the dental resin composite is also an important factor that promotes plaque formation on dental materials. [12-15] Plaque formation can easily increase the risk of periodontal infections due to an increase in surface roughness and surface free energy, which could affect the initial adhesion and retention of oral microorganisms. [12] Thus, many studies about the different wettability of dental resin materials have concluded that a low wettability or high contact angle surface has less potential to be adhered by bacteria. [16-17] An increase in surface roughness can improve the surface wettability of the substrate, to become either more hydrophilic or hydrophobic compared to a smoother one. [18] Thus, the materials in dentistry should have good wettability on the tooth surface to result in sufficient adhesion, while the adhesion of bacteria should be prevented. A relationship between the surface roughness of polymers and the contact angles of liquids has also been demonstrated. A change or a different in surface roughness parameters of any materials, also resulted in a change or a different of contact angles or wettability. [19-21]

Microorganisms can colonize on non-shedding hard surfaces in the oral cavity, such as teeth, restorative materials and dentures, through adhesion, after which a biofilm is formed. The surface roughness and surface energy of the intraoral hard surfaces have major influences on microbial adhesion and biofilm formation. [12] Many studies have shown that a surface roughness of 0.2 µm is the minimum value that can promote the adherence of microbes. [22,23] Although the smoothest resin composite can be achieved through polymerization under a polyethylene matrix. The uppermost smoothest surface are composed of the unstable resin layer, which is clinically unacceptable due to a reduction in strength as well as the wear and stain resistance of the restoration. [24-26] Therefore, finishing and polishing of composite resins are required to maintain their longevity. However, the process led to a change in a surface roughness of composite resins. [27]

The purpose of this study was to establish the correlation among the surface roughness, contact angle and biofilm formation of composite resins after polishing with different grits abrasive discs.

Materials and methods

Three commercial resin composites (microhybrids, nanohybrids and nanocomposites) were used in this study. The details are shown in Table 1. All materials used a shade of A2. Fifty square-shaped specimens of each material were prepared using a silicone mold with dimensions of 5 mm X 5 mm X 2 mm. The material was placed into the mold, and both sides of the mold were covered with a celluloid matrix strip (Hawe Neos Dental, Bioggio, Switzerland) and then covered again with glass slides. Constant pressure was applied onto the glass slides. Both sides of the specimens were light-cured for 40 seconds using a light-curing unit (Bluephase, Ivoclar Vivadent AG, Schaan, Liechtenstein), with the curing tip touching the glass slide. The curing unit was set to a high power with an intensity of 1,200 mW/cm^2. All specimens were kept in distilled water at 37 °C for 24 hours. Then, 50 specimens of each material were prepared and randomly divided into 5 groups of ten. The surfaces of each group were further prepared with different grits abrasive discs (Table 2) under running water. The 5 subgroups were divided according to different polishing protocols, which were as follows. Group 1: unpolished surface used as a control; Group 2: polished with Sof-Lex coarse-grit disc in one direction for 20 seconds using a speed-controlled handpiece running at 10,000 rpm; Group 3: polished with Sof-Lex medium-grit disc in one direction for 20 seconds using a speed-controlled handpiece running at 10,000 rpm; Group 4: polished with Sof-Lex fine-grit disc in one direction for 20 seconds using a speed-controlled handpiece running at 10,000 rpm; and Group 5: polished with Sof-Lex superfine-grit disc in one direction for 20 seconds using a speed-controlled handpiece running at 10,000 rpm.

Table 1 Resin compositdetailsaccordingto the manufacturer data

Composite resin	Type	Resin matrix	Filler type	Mean particle size of filler	% of filler by volume	% of filler by weight	Lot no.	Manufacturer
Filtek Z250	Microhybrid	Bis-GMA, UDMA, Bis-EMA	Silica/zirconia	0.6 µm	60	82	N451981	3M ESPE Dental product, St. Paul, MN, USA
Filtek Z250XT	Nanohybrid	Bis-GMA, UDMA, Bis-EMA, PEGDMA, TEGDMA	Silica/zirconia Silica nanofillers Zirconia/ silica nanocluster	<3 µm 20 nm 0.6-1.4 µm	68	82	N509155	
Filtek Z350 XT	Nanocomposite	Bis-GMA, UDMA, Bis-EMA, TEGDMA	Silica nanofillers Zirconia/ silica nanocluster	20 nm 0.6-1.4 µm	55.6	72.5	N506768	

Table 2 Abrasive discs details according to the manufacturer data

Abrasive discs	Grit	Color	Compositions	Particles size (µm)	Manufacturer
Sof-Lex	Coarse	Dark orange	Aluminum oxide	100	3M ESPE Dental product, St. Paul, MN, USA
	Medium	Orange		40	
	Fine	Light orange		24	
	Superfine	Yellow		8	

The polished specimens were cleaned with an ultrasonicator (Vibraclean 300, MDT Biologic company, USA) in distilled water for 5 minutes and allowed to air-dry.

Determination of surface roughness

The surface area roughness (Sa) of all specimens was determined by 201 parallel tracings with an area of 1x1 mm^2 under a contact profilometer (TalyScan 150, Taylor Hobson LTD, Leicester, UK) with a speed of 1500 µm/s to result in a 3D-reconstructed image. The profilometer stylus had a tip radius of 2.0 µm. The data were then filtered with a cut-off of 0.08 mm (Gauss profile filter).

Determination of the water contact angle

Specimens from each group were randomly selected and used to determine the contact angle (θ) with the sessile drop method (Local-made apparatus, Center of nanoscience and nanotechnology research unit, Faculty of Science, Mahidol University, Thailand). The distilled water was used as a testing liquid. After placing 5 µl of the drop from the standardized dosing tube onto the polished surface of the specimen, each drop was immediately photographed (CCD-camera: VCC-5775P, Sanyo Electric Co. Ltd., Tokyo, Japan). The contact angle was then analyzed with image analyzing software (Drop analysis LB-ADSA, ImageJ software, Version 1.47, National Institutes of Health, USA).

Biofilm analysis

The biofilm quantification was done using the crystal violet assay for the adhesion of *S. mutans*. All specimens were sterilized in a UV chamber for 1.5 hours per side prior to the biofilm formation assays.

Unstimulated saliva were pool collected and centrifuge at 4000 x g for 15 min at 4 °C. The centrifuged saliva were diluted in PBS with ratio of 1:10, then undergone sterilization process using filtration devices at pore size of 0.2 µm. The specimen was placed in a 24-well culture plate and incubated with 1 ml filtrated saliva for 16 hours at 37 °C to form an acquire pellicle.

The cariogenic bacteria *S. mutans* (ATCC 25175) was cultured in brain-heart infusion broth (BHI) in a 5% CO_2 chamber at 37 °C for 24 hours. The bacteria were then cultivated in a brain-heart infusion broth supplemented with 5% sucrose to achieve the desired turbidity at a cell density of $1x10^8$ CFU/ml or 0.5 McFarland. After 16 hours of pellicle formation, the filtrated saliva was removed from the culture plate. Ten saliva-coated specimens from each subgroup were immersed and incubated with 1 ml of bacterial suspension in a 5% CO_2 chamber at 37 °C for 24 hours. After incubation, the specimens were washed with 1 ml distilled water 3 times and then transferred to a clean 24-well culture plate.

Specimens from each subgroup were used to determine biofilm formation using a crystal violet (CV) assay. The specimens were stained with 0.75 ml of 1% CV solution for 10 minutes at room temperature. The unbound dye was removed by washing the plates with phosphate buffered saline several times. The bound CV was extracted with destaining solution, which was composed of 70% ethanol and 30% acetone. The amount of biofilm was measured via the destained solution placed in a 96-well culture plate at an optical density (OD) of 595 nm using a microplate reader (Epoch, Biotek, USA).

Statistical analysis

To analyze the difference in the surface roughness, contact angle and biofilm formation among materials with different grits abrasive discs, the Komogorov-Smirnov test was used to determine normality, while Levene's test was used to determine the homogeneity of data. The difference in the means among groups was further analyzed with two-way ANOVA and Dunnett T3 multiple comparison, with a significance level of $\alpha = 0.05$.

Furthermore, the linear regression model was additionally analyzed to determine the correlations between roughness and contact angle; roughness and biofilm formation; and contact angle and biofilm formation, with a significance level of $\alpha = 0.05$.

Results

The surface roughness was determined with a contact profilometer. Mean values and standard deviations of Sa of three composite resins polished with 4 different grits abrasive discs are demonstrated in Table 3. The 3-D images from the contact profilometer of three composite resins after polishing are shown in Figure 1.

According to the two-way ANOVA, the type of composite resin and the different grit of abrasive discs had a significant effect on surface roughness ($p<0.01$), with a significant interaction ($p<0.01$).

The multiple comparisons test revealed that the smoothest surfaces were control surfaces and surfaces polished with a superfine-grit abrasive disc for the three composite resins. The roughest surfaces were the surfaces polished with a coarse-grit abrasive disc for all composite resins.

The mean values and standard deviations of the contact angle formed by distilled water on each composite resin are demonstrated in Table 4. The images from the sessile drop method of three composite resins after polishing with different grits abrasive discs are shown in Figure 2. The contact angle decreased in the groups polished with a coarse grit, while surfaces polished with a superfine grit demonstrated an increase in the contact angle that was comparable to that of control surfaces.

Two-way ANOVA revealed that the type of composite resin and the different grit of abrasive discs had a significant effect on the contact angle ($p<0.01$), with a significant interaction ($p<0.01$).

The multiple comparisons test revealed that the smoother surfaces included control surfaces and those polished with a superfine-grit abrasive disc, which demonstrated high contact angles that were statistically different between Z350 XT and the other two composite resins. Low contact angle values were found on the surfaces polished with a coarse-grit abrasive disc, which demonstrated a significant difference between Z350 XT and the other two composite resins.

The mean values and standard deviations of biofilm formation on each composite resin are demonstrated in Table 5. Two-way ANOVA demonstrated the effect of different grits abrasive discs ($p<0.01$) on biofilm formation. An effect of the type of composite resin ($p=0.88$) on the interaction between the factors ($p=0.156$) could not be found.

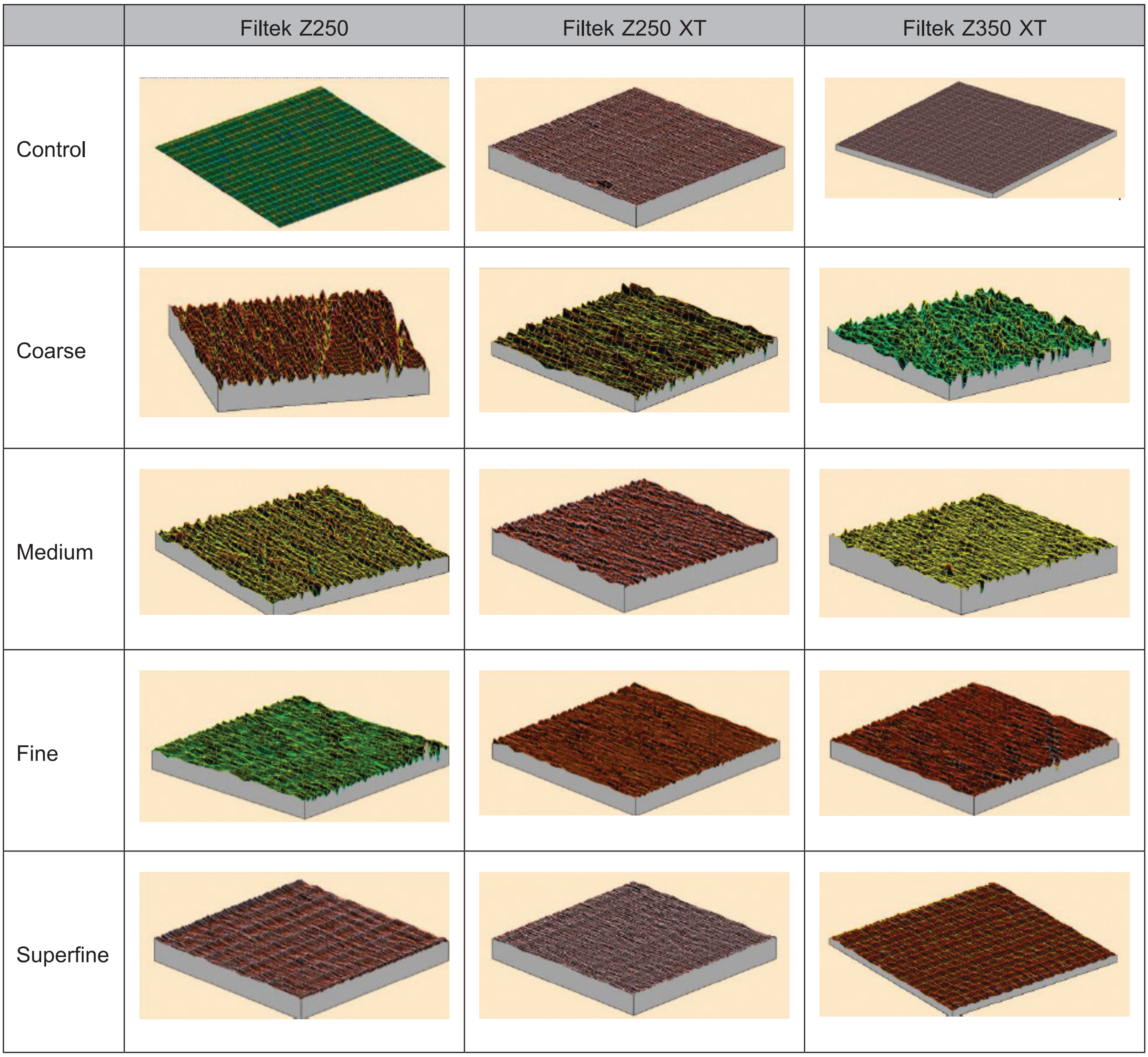

Figure 1 The 3-D images from the contact profilometer of three composite resins

Table 3 Means and standard deviations of surface roughness (Sa) after polishing with different grits abrasive discs

Groups	Control	Coarse	Medium	Fine	Superfine
Z250	0.0377 ± 0.0056^{a}	0.7849 ± 0.0651^{f}	0.3730 ± 0.0271^{e}	0.1178 ± 0.0129^{c}	0.0545 ± 0.0061^{a}
Z250 XT	0.0391 ± 0.0036^{a}	0.7797 ± 0.0586^{f}	0.3643 ± 0.0393^{e}	0.1053 ± 0.0140^{b,c}	0.0387 ± 0.0035^{a}
Z350 XT	0.0395 ± 0.0046^{a}	0.7902 ± 0.0940^{f}	0.2452 ± 0.0317^{d}	0.0738 ± 0.0135^{a,b}	0.0406 ± 0.0048^{a}

The data with the same superscript demonstrates no statistical difference

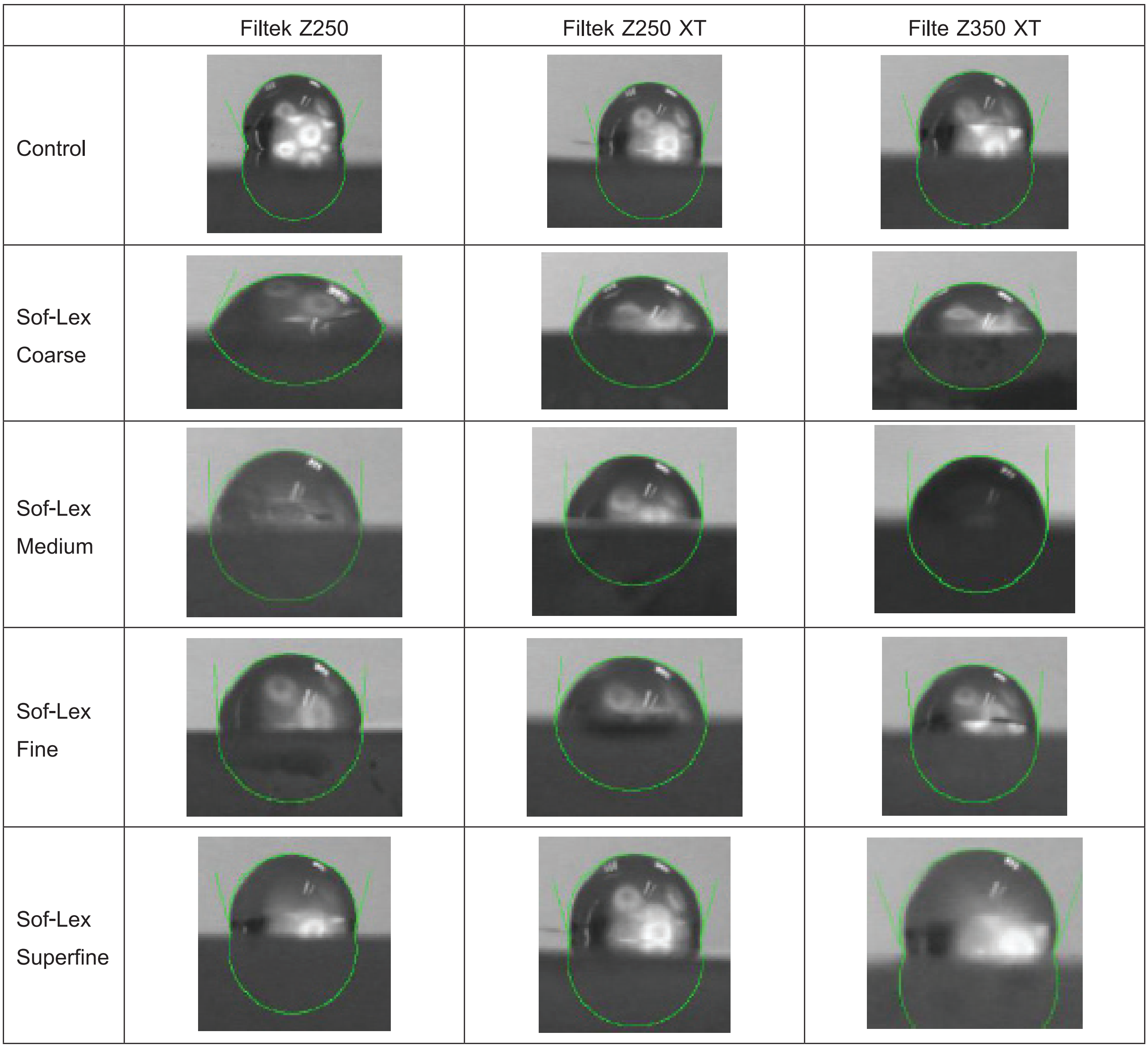

Figure 2 The images from the sessile drop method of the three composite resins

Table 4 Means and standard deviations of the contact angle of distilled water on the composite resin after polishing with different grits abrasive discs

Groups	Control	Coarse	Medium	Fine	Superfine
Z250	92.92 ± 0.87^{d}	75.59 ± 3.20^{a}	81.91 ± 1.58^{b}	87.08 ± 0.97^{c}	92.57 ± 2.80^{d}
Z250 XT	93.14 ± 1.74^{d}	76.21 ± 4.05^{a}	81.03 ± 0.69^{b}	87.46 ± 0.93^{c}	94.48 ± 3.14^{d}
Z350 XT	98.18 ± 3.81^{e}	81.43 ± 1.86^{b}	86.23 ± 2.83^{c}	97.30 ± 2.75^{e}	109.76 ± 3.15^{f}

The data with the same superscript demonstrates no significant difference

Table 5 Means and standard deviations of biofilm formation by crystal violet assay of composite resins after polishing with different grits abrasive discs

Groups	Control	Coarse	Medium	Fine	Superfine
Z250	0.52 ± 0.15^{a}	1.28 ± 0.19^{e}	$1.05 \pm 0.18^{c,d}$	$1.02 \pm 0.09^{c,d}$	0.98 ± 0.11^{c}
Z250 XT	0.48 ± 0.13^{a}	1.25 ± 0.12^{e}	$1.06 \pm 0.11^{c,d}$	$1.06 \pm 0.06^{c,d}$	0.95 ± 0.87^{c}
Z350 XT	0.52 ± 0.10^{a}	1.30 ± 0.18^{e}	1.12 ± 0.05^{d}	$1.06 \pm 0.08^{c,d}$	0.83 ± 0.11^{b}

The data with the same superscript demonstrates no significant difference

A large amount of biofilm was found in the groups polished with a coarse-grit abrasive disc, which showed a significant difference with other groups. A small amount of biofilm was found in the control groups, which was significantly different when compared to the other groups.

The data on surface roughness, contact angles and biofilm formation were used to determine whether there was a correlation between roughness and contact angle; roughness and biofilm formation; and contact angle and biofilm formation. The coefficients of the correlations and the linear regression models are shown in Table 6.

Discussion

Although the smoothest surface of the resin composite was obtained with the Mylar strip, the outermost layer contained a resin-rich layer that needed to be removed or polished, as it was unstable and less resistant to wear. [28,29] All finishing and polishing procedures decreased the smoothness obtained from these strips.

The surface morphology of the tooth-colored restorative materials after finishing and polishing was influenced by the size, hardness, type, and amount of filler particles in restorative materials, as well as by the hardness of the abrasive and the grit size of the polishing devices. [30,31] The larger the filler particles were, the rougher the surface was after finishing and polishing. [29] Furthermore, a higher hardness value of the material was also correlated with higher surface roughness. In addition, if the abrasive particle of the polishing systems was softer than the filler particle, only the soft resin matrix was removed. This resulted in remnant filler particles on the resin composite surface, thereby resulting in greater surface roughness. [32]

In this study, the surface roughness of all resin composites varied depending on the grit size of the abrasive discs, where a larger grit resulted in a rougher surface. Furthermore, this present study also showed that the surface roughness of Filtek Z350 XT was lower or equal to that of Filtek Z250 and Filtek Z250 XT in all polishing groups. This result corresponds to our expectations based on the different filler sizes in each resin composite (Filtek Z250: 0.01-3 μm, Filtek Z250 XT: 20 nm-3 μm). This phenomenon caused protrusion of the filler through the surface or its dislodgement after the loss of the resin matrix following the polishing procedure. Filtek Z350 XT is a true nanocomposite that has 20 μm-sized nanofillers, including silica dioxide and zirconium dioxide, and displays the same characteristics of filler particles as nanoclusters containing the same filler size as Filtek Z250.

Since resin composites have low polarity, the present study used distilled water as a highly polar liquid in order to create a contact angle value. A study by Wenzel showed that an increase in surface roughness can improve the surface wettability. [18] As a result of the present study, the water contact angle decreased on a rougher surface. Good wettability due to the higher surface area of resin composite was observed.

A study on the contact angle and surface free energy of resin-based dental restorative materials after chewing stimulation found an increase in the value of surface roughness of the material after chewing stimulation followed by a decrease in contact angle. [33]

Table 6 Correlation and regressive models

Correlations	r	r^2	Linear regressive model
Roughness (R) & Contact angle (C)	0.778	0.605	C = -24.63R + 95.33
Roughness (R) & Biofilm formation (B)	0.648	0.420	B = 0.645R + 0.799
Contact angle (C) & Biofilm formation (B)	0.563	0.317	B = -0.018C + 2.540

The analysis revealed a correlation between roughness and contact angle (r = 0.778); roughness and biofilm formation (r = 0.648); and contact angle and biofilm formation (r = 0.563).

Lower biofilm formation was found on the composite surfaces with low surface roughness caused by polishing with a superfine-grit abrasive disc. A surface that has low surface roughness and poor wetting by saliva would display the advantages of better stain resistance, color stability and greater resistance to plaque formation. [34]

As *S.mutans* are known to be a responsible microorganism causing dental caries, this study showed a lesser number of *S.mutans* biofilm on a smoother surface of composite resins. Hence, any composite resins placed in the oral cavity should be well polished to achieve a smooth surface and a low wettability or high contact angle, so *S.mutans* could not adhered well. A lower incidence of secondary caries could be expected.

Conclusion

A direct correlation among the surface roughness, water contact angle and biofilm formation of composite resins was observed in this study. Smoother surfaces of composite resins may result in the reduction of plaque accumulation.

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