©Operative Dentistry, 2011, **36-3,** 311-317

Streptococcus mutans Biofilm Adhesion on Composite Resin Surfaces After Different Finishing and Polishing Techniques

CA Pereira • E Eskelson • V Cavalli
PCS Liporoni • AOC Jorge • MA do Rego

Clinical Relevance

Biofilm adhesion to composite resin surface is modulated by the presence of human saliva pellicle, the type of composite resin used, and the finishing and polishing treatment performed.

Cristiane Aparecida Pereira, MSc, PhD student, Department of Biosciences and Oral Diagnosis, School of Dentistry of São José dos Campos, UNESP – Univ Estadual Paulista, São José dos Campos, Brazil

Evania Eskelsen, DDS, MSc, PhD, research scientist, Department of Dentistry, University of Taubaté, Taubaté, Brazil

Vanessa Cavalli, DDS, MSc, PhD, professor, Department of Dentistry, University of Taubaté, Taubaté, Brazil

Priscilla Christiane Suzy Liporoni, DDS, MSc, PhD, professor, Department of Dentistry, University of Taubaté, Taubaté, Brazil

Antonio Olavo Cardoso Jorge, DDS, MSc, PhD, chairman and professor, Department of Biosciences and Oral Diagnosis, School of Dentistry of São José dos Campos, UNESP – Univ Estadual Paulista, São José dos Campos, Brazil

*Marcos Augusto do Rego, DDS, MSc, PhD, Prof Dr, Departament of Dentistry, University of Taubaté, Brazil

*Corresponding author: R. Expedicionário Ernesto Pereira, 110, Taubaté, SP, Brazil, CEP: 12020–330, e-mail: marcosrego@uol.com

DOI: 10.2341/10-285-L

SUMMARY

This study evaluated *Streptococcus mutans* biofilm adhesion on the surface of three composite resins (nanofilled, Filtek Z350, 3M ESPE, Salt Lake City, UT, USA; nanohybrid, Vit-1-escence, Ultradent Products, South Jordan, UT, USA; and microhybrid, Esthet X, Dentsply, Milford, DE, USA) following different finishing and polishing techniques. Sixty standardized samples (6 × 3 mm) of each composite were produced and randomly divided into three finishing and polishing treatments (n=20): 1) control group: composite resin surface in contact with Mylar matrix strips with no finishing or polishing performed, 2) Sof-Lex aluminum oxide disc technique (3M ESPE, and 3) carbide bur finishing and Astrobrush polishing technique (Ultradent). Half the samples of each group were incubated in human saliva for 1 hour, and all the samples were subjected to *S mutans* (ATCC 35688) biofilm development.

The mean log of CFU/mL present in the *S mutans* biofilm was calculated, and data were statistically analyzed by three-way analysis of variance and the Tukey test ($p<0.05$). Human saliva incubation promoted a significant increase of bacterial adherence on all three of the composites' surfaces, regardless of the polishing treatment performed ($p<0.05$). Of the three, the nanofilled composite (Filtek Z350) had the lowest bacterial adherence with each of the finishing and polishing techniques despite the presence or absence of human saliva ($p<0.05$). Mylar matrix strips (control group) promoted the lowest bacterial adhesion on the surface of the microhybrid and nanofilled composites in the absence of human saliva.

INTRODUCTION

The development and refinement of composite resins intends to minimize and eliminate the critical drawbacks of the polymer-based materials such as polymerization contraction, fatigue, occlusal wear, organic matrix degradation, surface roughness, insufficient contour, and interdental contacts and fractures.[1,2]

Some clinical procedures, such as finishing and polishing, can improve the performance and longevity of these materials. The finishing procedure aims at an adequate anatomy, whereas polishing decreases surface roughness and minimizes the microgaps produced by the finishing instruments. In addition, roughness of the composites' surface also relies on the chemical composition and mechanical characteristics of these materials.[3-6]

Organic matrix and inorganic particles present different hardnesses and, as a consequence, undergo distinct wear when subjected to occlusal forces. Because of its lower hardness, the organic matrix degrades faster and exposes the inorganic fillers which are then dislodged by attrition. Therefore, the larger the particle size, the greater the roughness left by the inorganic filler.[7-11]

In view of the fact that the type, size, and quantity of inorganic filler influence the mechanical properties and polishing of polymer-based materials,[12] nanometric-sized composite fillers were developed in order to produce composites with improved mechanical and esthetic characteristics due to the reduced sized and wide distribution of the fillers.[6,10]

The abrasive particles of the polishing materials must exhibit hardness superior to that of the inorganic fillers of the composites to avoid excessive wear of the organic matrix.[5,13] In addition, the surface geometry and proper operatory handling also influence polishing of composites.[14] Aluminum oxide discs are available for finishing and polishing of resins; however, the shape of these instruments is inadequate for occlusal surfaces.[5,7,10,15-18] In order to reach and properly polish occlusal posterior surfaces, multifluted carbide burs, diamond burs, brushes, abrasive pastes, and silicon carbide burs are commonly used in clinical practice.[18-20]

A noticeably polished composite surface ensures esthetic attributes and significantly decreases the risk of initial bacterial adherence and subsequent colonization. It will also decrease periodontal disease, marginal discoloration, and secondary caries progress promoted specifically by *Streptococcus mutans* and *Streptococcus sobrinus*, the most prevalent streptococci in human dental caries.[7,10,11,21-24]

Based on the facts described, the aim of this study was to evaluate *S mutans* biofilm adherence on the surface of nanofilled, nanohybrid, and microhybrid composites submitted to different polishing and finishing techniques.

MATERIALS AND METHODS

Experimental Design

The factors under study were as follows:

- Composite resins (three levels: nanofilled, nanohybrid, and microhybrid)
- Finishing and polishing treatments (three levels: control, no finishing or polishing performed; aluminum oxide discs; and 30-blade carbide tungsten burs followed by silicon carbide brushes)
- Saliva incubation (two levels: with and without)

The experimental units consisted of standardized composite samples subjected to different surface treatments. The response variable was the mean CFU/mL present in the *S mutans* biofilm formed on the composite resin surface. Data were statistically analyzed by three-way analysis of variance (ANOVA) and the Tukey test ($p<0.05$).

Sample Preparation

Samples (n=60) of each composite were prepared in a cylindrical-shaped Teflon mold 6.0 mm in diameter and 3.00 mm in height. The mold was filled in a single increment with the nanofilled composite (Filtek Z350™, 3M/ESPE, Salt Lake City, UT, USA), the nanohybrid composite (Esthet X™,

Dentsply, Milford, DE, USA), or the microhybrid composite (Vit-l-escence™, Ultradent Products, South Jordan, UT, USA) and covered with a Mylar matrix strip. The top surface was cured for 40 seconds in a curing unit device (Optilux 401, Demetron/Kerr, Danbury, CT, USA) operating between 700 and 800 mW/cm^2. Samples were retrieved from the mold, the excess resin was removed with surgical blades, and samples were individually immersed in dark vials containing distilled water at 37°C for 24 hours. The composition and manufacturer of the composites tested are displayed in Table 1.

Polishing Treatment

Twenty samples of each composite (nanofilled, nanohybrid, and microhybrid) were submitted to one of three finishing and polishing techniques (n=20):

A) Control group—the surface remained intact after contact with the Mylar matrix strip, and no finishing or polishing procedures were performed.[10]
B) Aluminum oxide discs (Sof-Lex™, 3M ESPE).
C) 30-blade tungsten carbide burs (Beavers Dental, Morrisburg, ON, Canada) and silicon carbide brushes (Astrobrush™, Ivoclar, Vivadent, Schaan, Principality of Liechtenstein).

In group B, samples were polished with a sequence of four sandpaper discs (Sof-Lex coarse: 100 μm; medium: 29 μm; fine: 14 μm; and superfine: 5 μm) for 30 seconds each in a single direction. At each disc exchange, the composite surface was washed and air dried for 5 seconds. A new polishing disc was used after every fifth sample. Group C was polished with tungsten carbides for 30 seconds, washed and air dried for 5 seconds, and then finished with silicon carbide brushes for 30 seconds in a single direction. All samples from groups A, B, and C were placed into sterilized Petri dishes (24 wells, Costar, New York, NY, USA) and sterilized in a 20-kGy gamma radiation chamber (cobalt 60) for 6 hours (Embrarad, São Paulo, Brazil).

Human Saliva Incubation

Whole unstimulated saliva, was collected from six healthy subjects which was approved by the Ethical Committee of Research of the University of Taubaté (126/08). Human saliva donors presented low biofilm formation (as verified by the Silness and Löe Index) and a *S mutans* number lower than 10^6 colony-forming units per milliliter (CFU/mL) of saliva. Immediately after collection, the saliva was added to pooled saliva protease inhibitor phenyl methyl sulfonyl hydrofluoric and sodium azide (20 μL/mL of saliva). The pooled saliva was centrifuged for 20 minutes at 4°C at 20,000*g* (MPW-350R, Biosystems, Pinhais, Brazil) to remove debris, then frozen at −4°C until needed for the experiment. Before use, the pooled saliva was thawed and filtrated with a cellulose acetate filter with a pore diameter of 0.22 μm (Millipore membrane, São Paulo, Brazil). Half

Table 1: *Composition, type, manufacturer, and filler loading of the composite resins tested*

Composite	Composition[a]	Filler Loading (% Weight)
Filtek Z 350™/nanofilled/3M ESPE	Organic Matrix: Bis-GMA, Bis-EMA, UDMA with small amounts of TEGDMA. Inorganic filler: nonagglomerated/nonaggregated, 20 nm nanosilica filler, agglomerated zirconia/silica nanocluster (particles with filler size of 5-20 nm). The cluster particle size range is 0.6 to 1.4 microns.	78.5%
Esthet X™/nanohybrid/Dentsply, Caulk	Organic matrix: Urethane modified Bis-GMA dimethacrylate, TEGDMA, Bis-EMA. Inorganic filler: BAFG (0.6-0.8 μm and 0.02–2.5 μm) and silicon dioxide particles (10–20 nm).	78%
Vit-l-escence™/microhybrid/Ultradent Products	Organic matrix: Bis-GMA base. Inorganic filler: Glass-strontium-boron-aluminum-silicate (0.7 μm).	75%

Abbreviations: Bis-GMA, bisphenol glycidyl methacrylate; UDMA, urethane dimethacrylate; Bis-EMA, bisphenol A polyetheylene glycol diether dimethacrylate; TEGDMA, tetraethyleneglycol dimethacrylate; BAFG, inorganic bariumalumino fluoroborosilicate glass.
[a] *As disclosed by the manufacturer.*

the samples of each group were incubated in 1.5 mL of sterilized human saliva for 1 hour.

Biofilm Adhesion

A standard suspension of *S mutans* (ATCC 35688) containing 10^6 cells/mL was prepared. For this purpose, *S mutans* was seeded onto brain heart infusion (BHI) agar (Difco, Detroit, MI, USA) and incubated for 24 hours. All incubation was carried out at 37°C in a CO_2 chamber. After incubation, the growth was suspended in sterile physiological solution (0.9% sodium chloride (NaCl]), and the number of cells in suspension was counted in a spectrophotometer (B582, Micronal, São Paulo, Brazil). The parameters of optical density and wavelength used were, respectively, 0.620 and 398 nm. These parameters were previously established by means of a standard curve with CFU vs. absorbance. Adherence testing was performed in an aseptic environment in a laminar airflow chamber. The broth used for adherence was proposed by Gybbons and Nygaard[25] and contains 20 g tripticase, 2 g NaCl, 3 g K_2HPO_4, 2 g KH_2PO_4, 1 g K_2CO_3, 120 mg $MgSO_4$, 15 mg $MnSO_4$, and 50 g $C_6H_8O_7$ dissolved in 1000 mL of distilled water. The broth was sterilized by autoclaving at 121°C for 15 minutes. In each well of sterile 24-well polystyrene tissue culture plates was placed one specimen, 1.5 mL of broth, and 0.1 mL of standardized *S mutans* suspension. The plates were sealed and incubated at 37°C for 24 hours in a CO_2 chamber. Samples were then removed and washed twice with sterile physiological solution (0.9% NaCl) in order to remove loosely bound material. Following this, the samples were placed in tubes with 3 mL of sterile physiological solution (0.9% NaCl) and sonicated (Sonoplus HD 2200, 50 W, Bandelin Electronic, Berlin, Germany) for 30 seconds to disperse the biofilms. The suspension obtained was diluted 10, 100, and 1000 times, and aliquots of 0.1 mL were seeded in duplicate onto BHI agar and incubated for 48 hours at 37°C in a CO_2 chamber. After the incubation, the plates with 30 to 300 typical colonies of *S mutans* were counted in a colony counter (Phoenix CP-600, São Paulo, Brazil), and mean values of CFUs were obtained (CFU/mL). Mean values of CFU/mL were converted into logarithmic ($\log_{10}$) values and analyzed by three-way ANOVA and the Tukey test. A p value <0.05 was considered to indicate a statistically significant difference.

RESULTS

Mean and standard deviation values of the CFU/mL ($\log_{10}$) of *S mutans* within the biofilms formed are displayed in Table 2. The presence of saliva increased bacterial adhesion for all composites tested, regardless of the finishing and polishing treatment performed ($p<0.05$). The nanofilled composite (Filtek Z350™) presented the lowest bacterial adhesion among composites, regardless of the groups (A, B, or C) or the presence/absence of human saliva ($p<0.0001$).

In the absence of human saliva, Mylar matrix strips (group A) promoted the lowest bacterial adhesion in the nanofilled and microhybrid composites (Filtek Z350™ and Vit-l-escence™), whereas for nanohybrid resin (Esthet X™), groups A and C were similar and showed lower bacterial adhesion than group B.

In the presence of saliva, bacteria adhesion in nanohybrid and microhybrid composites (Esthet X™ and Vit-l-escence™) were similar for all finishing and polishing treatments performed (A, B, or C; $p>0.0518$). In contrast, nanofilled composite (Filtek Z350™) exhibited the lowest bacterial

Table 2: *Mean and standard deviation of* Streptococcus mutans *adherence (log CFU/mL) on composite resins submitted to different polishing treatments with and without saliva incubation*[a]

Composite Resins	Without Saliva Incubation			With Saliva Incubation		
	A (Mylar Matrix)	B (Sof-Lex™)	C (Astrobrush™)	A (Mylar Matrix)	B (Sof-Lex™)	C (Astrobrush™)
Filtek Z350™	4.47 ± 0.22Aa	5.32 ± 0.08Ab	5.46 ± 0.07Ab	5.00 ± 0.08Aa*	5.70 ± 0.09Ab*	5.77 ± 0.07Ab*
Esthet X ™	5.54 ± 0.09Ba	5.83 ± 0.05Bb	5.55 ± 0.04Ba	5.90 ± 0.06Bb*	5.97 ± 0.05Bb*	5.91 ± 0.05Bb*
Vit-l- escence™	5.54 ± 0.08Ba	5.77 ± 0.09Bb	5.76 ± 0.07Cb	5.91 ± 0.04Bb*	5.99 ± 0.05Bb*	6.02 ± 0.05Cb*

[a] *Different letters indicate differences among groups according to three-way analysis of variance and Tukey test (p<0.05). Uppercase letters compare columns, and lowercase letters compare rows. Asterisks indicate differences between incubated and nonincubated saliva samples.*

adhesion when no finishing or polishing was performed (group A).

DISCUSSION

Clinical procedures such as finishing and polishing of restorations are important to improve the performance and longevity of resin composites. Besides the esthetic considerations, polishing is performed in order to minimize surface roughness and crevices created by the finishing instruments, thus increasing the clinical longevity of restorations and minimizing biofilm formation.[3-5] As a consequence, the polished surface of a composite decreases the possibility of periodontal disease, marginal discoloration, and secondary caries.[7,10]

The results of the present study demonstrated that the inherent characteristics of the composite resin tested, the finishing and polishing techniques performed, and the presence of human saliva influence *S mutans* adhesion to the composite surface.[9,21,22]

In the absence of human saliva, Mylar matrix strips (group A) produced the lowest bacterial adhesion on the nanofilled and microhybrid surfaces, whereas nanohybrid resin exhibited lower adhesion for both group A and group C. These results are in accordance to previous findings in which the composite surface polymerized against Mylar matrix strips exhibited flatter surfaces compared to finished and polished composites.[16,26,27]

Although polyester matrix strips promote the smoothest surface in the absence of saliva, most clinical situations require bulk removal of excess composite. Additionally, finishing and polishing techniques are necessary to remove the monomer-rich surface layer of the composite material.[28] This eventually eliminates the organic matrix, exposing and dislodging the filler particles, thus increasing the surface roughness of polymer-based materials.[16]

Among finishing and polishing techniques, it has been reported that the Sof-Lex™ aluminum oxide discs technique provides a smoother surface on composites compared to carbide bur finishing followed by the Astrobrush™ technique.[27] In the present study, however, polishing with aluminum oxide discs (group B) promoted surface roughness similar to polishing with brushes (group C), except for nanohybrid composite (Esthet X™) without saliva incubation. This indicates that polishing treatment C was the best treatment alternative for this composite in this particular situation.

It must be kept in mind that although the Sof-Lex™ technique promoted surface bacterial adhesion similar to the Astrobrush™ technique for some groups, the use of discs is limited because of the geometry of the surface to be polished. Aluminum oxide discs are unable to efficiently create, finish, and anatomically polish contoured surfaces, especially posterior occlusal surfaces.[27]

A lower *S mutans* biofilm adhesion rate is observed on nanofilled resin surface in the presence or absence of human saliva, confirming that biofilm adhesion varies according to filler size, shape, distribution, and matrix monomer.[6,9,10,21,22] The inorganic filler of Filtek Z350™ is a combination of nonagglomerated 20-nm nanosilica filler and loosely bound agglomerated zirconia/silica nanoclusters, which consists of agglomerates of primary zirconia/silica particles with a filler size of 5-20 nm. The reduced size and the wide distribution of the fillers reduces roughening after finishing and polishing, consequently decreasing *S mutans* adherence.

The samples of our study incubated in human saliva exhibited a significant increase of *S mutans* biofilm growth to nanofilled, nanohybrid and microhybrid composites and demonstrated the powerful ability of salivary components to modulate biofilm adhesion as oral bacteria adhere to receptors of the host origin in saliva pellicle.[29]

The selection of *S mutans* to promote biofilm adhesion is based on the fact that these microorganisms are recognized as the major etiological agent of human dental caries, are harbored in mature plaque, and represent a significant amount of the oral streptococci in caries lesions.[9] *S mutans* adherence to enamel surface and to restorative materials is a preliminary condition to biofilm formation and can eventually promote secondary caries and periodontal diseases.[21,24]

This study observed that *S mutans* biofilm adhesion to composite resin surfaces is influenced by the composition of composites, finishing and polishing performed, and the presence of saliva pellicle.

The quality and amount of adhered biofilm are important to the success of the esthetic restorations on a long-term basis. The initial adherence and subsequent colonization of bacteria on the surface of composite resins is the key of the pathogenesis of the secondary caries promoted particularly by *S mutans* and *S sobrinus*. Therefore, further *in situ* studies should be executed to confirm the results of this in vitro observation.

CONCLUSIONS

Within the limitations of this *in vitro* study, the following were concluded:

1. Human saliva pellicle increased *S mutans* adhesion to all composite restorative materials tested.
2. Nanofilled composite resin presented the lowest bacterial adhesion among composites.
3. Mylar matrix strips promoted the smoother surface among polishing treatments in the absence of human saliva.

Acknowledgement

The author Cristiane A. Pereira is grateful to FAPESP for the scholarships provided (Process 2010/00879-4).

(Accepted 1 March 2011)

REFERENCES

1. Manhart J (2004) Direct composite restorations in posterior region: A case history using a nanohybrid composite *Dentistry Today* **23(11)** 66-70.
2. Turssi CP, Faraoni-Romano JJ, de Menezes M & Serra MC (2007) Comparative study of the wear behavior of composites for posterior restorations *Journal of Materials Science: Materials in Medicine* **18(1)** 143-147.
3. Neme AL, Frazier KB, Roeder LB & Debner TL (2002) Effect of prophylactic polishing protocols on the surface roughness of esthetic restorative materials *Operative Dentistry* **27(1)** 50-58.
4. Baseren, M (2004) Surface roughness of nanofill and nanohybrid composite resin and ormocer-based tooth-colored restorative materials after several finishing and polishing procedures *Journal of Biomaterials Applications* **19(2)** 121-134.
5. Sarac D, Sarac YS, Kulunk S, Ural C & Kulunk T (2006) The effect of polishing techniques on the surface roughness and color change of composite resins *Journal of Prosthetic Dentistry* **96(1)** 33-40.
6. Uçtaşli MB, Arisu HD, Omurlu H, Eliguzeloglu E, Ozcan S & Ergun G (2007) The effect of different finishing and polishing systems on the surface roughness of different composite restorative materials *Journal of Contemporary Dental Practice* **8(2)** 89-96.
7. Barbosa SH, Zanata RL, Navarro MF & Nunes OB (2005) Effect of different finishing and polishing techniques on the surface roughness of microfilled, hybrid and packable composite resins *Brazilian Dental Journal* **16(1)** 39-44.
8. Jung M, Eichelberger K & Klimek J (2007) Surface geometry of four nanofiller and one hybrid composite after one-step and multiple-step polishing *Operative Dentistry* **32(4)** 347-355.
9. Ikeda M, Matin K, Nikaido T, Foxton RM & Tagami J (2007) Effect of surface characteristics on adherence of *S. mutans* biofilms to indirect resin composites *Dental Materials Journal* **26(6)** 915-923.
10. Attar N (2007) The effect of finishing and polishing procedures on the surface roughness of composite resin materials *Journal of Contemporary Dental Practice* **8(1)** 27-35.
11. Koh R, Neiva G, Dennison J & Yaman P (2008) Finishing systems on the final surface roughness of composites *Journal of Contemporary Dental Practice* **9(2)** 138-145.
12. Yap AU, Yap SH, Teo CK & Ng JJ (2004) Finishing/polishing of composite and compomer restoratives: Effectiveness of one-step systems *Operative Dentistry* **29(3)** 275-279.
13. Reis AF, Giannini M, Lovadino JR & Ambrosano GM (2003) Effects of various finishing systems on the surface roughness and staining susceptibility of packable composite resins *Dental Materials* **19(1)** 12-18.
14. Choi MS, Lee YK, Lim BS, Rhee SH & Yang HC (2005) Changes in surface characteristics of dental resin composites after polishing *Journal of Materials Science: Materials in Medicine* **16(4)** 347-353.
15. Liporoni PCS, Paulillo LA, Cury JA, dos Santos Dias CT & Paradella TC (2003) Surface finishing of resin-modified glass ionomer *General Dentistry* **51(6)** 541-543.
16. Turkun LS & Turkun M (2004) The effect of one-step polishing system on the surface roughness of three esthetic resin composite materials *Operative Dentistry* **29(2)** 203-211.
17. Uçtaşli MB, Bala O & Güllü A (2004) Surface roughness of flowable and packable composite resin materials after finishing with abrasive discs *Journal of Oral Rehabilitation* **31(12)** 1197-1202.
18. Venturini D, Cenci MS, Demarco FF, Camacho GB & Powers JM (2006) Effect of polishing techniques and time on surface roughness, hardness and microleakage of resin composite restorations *Operative Dentistry* **31(1)** 11-17.
19. Reis AF, Giannini M, Lovadino JR & dos Santos Dias CT (2002) The effect of six polishing systems on the surface roughness of two packable resin-based composites *American Journal of Dentistry* **15(3)** 193-197.
20. Celik C & Ozgünaltay G (2009) Effect of finishing and polishing procedures on surface roughness of tooth-colored materials *Quintessence International* **40(9)** 783-789.
21. Montanaro L, Campoccia D, Rizzi S, Donati ME, Breschi L, Prati C & Arciola CR (2004) Evaluation of bacterial adhesion of *Streptococcus mutans* on dental restorative materials *Biomaterials* **25(18)** 4457-4463.
22. Eick S, Glockmann E, Brandl B & Pfister W (2004) Adherence of *Streptococcus mutans* to various restorative materials in a continuous flow system *Journal of Oral Rehabilitation* **31(3)** 278-285.
23. De Munck J, Van Landuy K, Peumans M, Poitevin A, Lambrechts P, Braem M & Van Meerbeek B (2005) A critical review of the durability of adhesion to tooth tissue: Methods and results *Journal of Dental Research* **84(2)** 118-132.
24. Lobo MM, Gonçalves RB, Ambrosano GMB & Pimenta LAF (2005) Chemical or microbiological models of secondary caries development around different dental

restorative materials *Journal of Biomedical Materials Research. Part B, Applied Biomaterials* **74(2)** 725-731.

25. Gybbons RJ & Nygaard M (1968) Synthesis of insoluble dextran and its significance in the formation of gelatinous deposits by plaque-forming streptococci *Archives of Oral Biology* **13(10)** 1249-1262.
26. Ozgünaltay G, Yazici AR & Görücü J (2003) Effect of finishing and polishing procedures on the surface roughness of new tooth-coloured restoratives *Journal of Oral Rehabilitation* **30(2)** 218-224.
27. Gedik R, Hürmüzlü F, Coşkun A, Bektaş OO & Ozdemir AK (2005) Surface roughness of new microhybrid resin-based composites *Journal of the American Dental Association* **136(8)** 1106-1112.
28. Wassel RW, McCabe JF & Walls AW (1994) Wear characteristics in a two-body wear test *Dental Materials* **10(4)** 269-274.
29. Ahn SJ, Ahn SJ, Wen ZT, Brady LJ & Burne RA (2008) Characteristics of biofilm formation by *Streptococcus mutans* in the presence of saliva *Infection and Immunity* **76(9)** 4259-4268.