



***In situ* evaluation of micro and nanofilled load on biofilm retention of composite resins submitted to different surface treatments**

André Afif Elossais¹, Gleice Gomes dos Reis¹, Luis Fernando Benitez Macorini², Pedro Gregol da Silva³, Anna Thereza Peroba Rezende Ramos⁴, Gabriel Galvão³, Keren Cristina Fagundes Jordão Basso⁴, Andrea Abi Rached Dantas⁴

¹Department of Dental Materials, School of Dentistry, University Center of Grande Dourados, Dourados, MS, Brazil, ²Department of Biomedicine, School of Biomedicine, University Center of Grande Dourados, Dourados, MS, Brazil, ³Department of Endodontics, University Center of Grande Dourados, Dourados, MS, Brazil, ⁴Department of Restorative Dentistry, State University Paulista Julio de Mesquita Filho - UNESP, School of Dentistry, Araraquara, Brazil

Keywords

Composite resin, plaque retention, surface properties

Correspondence

André Afif Elossais, Rua Balbina de Matos, 2121, Dourados, MS, 79824-900, Brazil.
Tel.: +55 67 92364440. Email: ellossais1976@hotmail.com

Received October 06, 2014;
Accepted November 19, 2014

doi: 10.15713/ins.sjod.16

How to cite the article:

Elossais AA, dos Reis GG, Macorini LF, da Silva PG, Ramos AT, Galvão G, Jordão Basso KC, Dantas AA. *In situ* evaluation of micro and nanofilled load on biofilm retention of composite resins submitted to different surface treatments. *Sci J Dent* 2015;2:12-16.

Introduction

The dental restoration performed by the dentist aims to restore the anatomical, functional and aesthetic normality to the destroyed teeth from carie, trauma, congenital malformation, iatrogenic and possible combinations among these factors. However, the failure or success of any cosmetic restoration depends on the chosen material,^[1,2] as well as color stability and its physicochemical properties.^[3,4]

The restorations success depends on smooth exposed surface preservation without any pathological alteration with

Abstract

Introduction: Finishing and polishing materials did not develop as the composites improved in these last years. The nanometric load particles were firstly and only incorporated in the polishing pastes formulation in this *in vitro* and *in situ* study.

Objective: This study evaluated the biofilm retention of two different composite resins - Vit-I-escence™ (microhybrid) and Filtek™ Z350 XT (Nanofilled) after finishing and polishing technique, quantification and *in situ* comparison of the polishing pastes effectiveness with micrometric loads (Enamelize™ and Diamond Polish Paste™) and Nanofilled load - Lummina-E (Alumina and Diamond).

Materials and Methods: Ten volunteers were selected for the *in situ* biofilm evaluation, a palatal device made of acrylic resin was confectioned, and each intra-oral device had fixed six specimens from each experimental group. After exposition to 20% saccharosis, the biofilm from each specimen was extracted in NaOH 1.0 M and quantified by absorbance spectrophotometer. The data were submitted to analysis of variance, followed for multiple comparisons of averages for the ad hoc de Dunn test, both at 1% significance level.

Results: Vit-I-escence™ resin showed lower biofilm retention than Filtek Z350™ XT in all the tested groups. The lowest biofilm retention was after aluminum oxide paste (Lummina-E - Alumina) polishing.

Conclusion: The Vit-I-escence™ (microhybrid) composite resin exhibited lower biofilm retention than Filtek™ Z350 XT (nanofilled) resin among all the tested groups. The lowest *in situ* biofilm retention occurred at the surfaces treated with Lummina - E Alumina (folded-based aluminum oxide) nanometric prototype.

periodontal structures. There is an unceasing search for a compatible restorative material to the adjacent tooth structures once the composite resin retains more biofilm percentage than the dental surface.^[5,6]

After some time, the chewing and tooth brushing cause abrasion exposing inorganic particles^[7] producing a roughened surface^[8] regardless of finishing and polishing procedure. The dental materials industry has been concerned about composites development with smaller^[9] and more regular particles, in order to improve the surface smoothness,^[6,10-13] promote lower biofilm

retention^[14] and obtain better optical properties with higher esthetic quality, longer restorations durability especially in roughness and superficial brightness.^[15]

Hence, the mechanical and physicochemical properties of composites must be known once the load particles structure and features have a direct impact on the surface smoothness and extrinsic staining susceptibility; as well the finishing and polishing procedures may influence the composite surface quality. Thus, the bigger the particle load, the greater the surface roughness.^[12,13,16] Consequently, the biofilm retention will be higher on the great roughness surfaces.^[17,18]

The oral environment is greatest responsible for the composites chemical degradation, the presence of biofilm favors the restorative material surface staining due to the production of organic acids^[19-21] regardless the presence of abrasive forces and compression in the oral cavity. These acids promote higher susceptibility to restoration softening and surface texture alteration. Human saliva contains cholesterol esterase and active hydrolases, increasing the composites biodegradation.^[22] There are also glycoproteins and mucin that form the acquired pellicle and favor the bacterial colonization on tooth surfaces.^[23]

The purpose of this study was to evaluate two types of composite resin, microhybrid and nanofilled, submitted to different finishing and polishing techniques using an *in situ* methodology.

Materials and Methods

Two different light-curing composite resins, suitable for direct esthetic restorations were selected. This study was performed an *in situ* methodology with volunteers from both genders, undergraduate students of dentistry school. The employed composites and their features are described in Table 1.

The specimens were made using a stainless steel device with cylinders (2 mm height × 6 mm diameter). Polyester matrix strips (K Dent - Quimidrol[®] - Joinville, SC, Brazil) were used at the light cure time of the last resin increment and at the bottom portion in order to standardize the surface texture.

The specimens were fixed in glass plates, submerged in distilled water inside plastic containers at 37°C ± 1°C (Cz, model 480 Es, Olidef[®], Ribeirir Preto, SP, Brazil). After 24 h, the specimens were removed from the water, dried with air blow and the surface finishing was performed. The polishing and final gloss materials are shown in Table 2.

The specimens were polished with Sof-Lex Pop On[™] sequential discs, intermittently, following only one direction and at a low speed with water and after 30 s, it was discarded. Then, the specimens were washed with air/water spray to remove debris, dried with air spray, and submitted to another lower granulation disc, total of four disks and polishing for 2 min for each sample. After polishing, the specimens were subjected to final polishing and gloss, associating polishing discs and abrasive pastes for the final gloss. Thus, a total of 120 specimens were divided into 12 experimental groups, 6 groups to Vit-l-escence[™] resin and 6 groups to Filtek[™] Z350 XT resin, subjected to surface

polishing consecutive steps. Table 3 presents Vit-l-escence[™] and Filtek[™] Z350 XT resin experimental groups.

Ten volunteers, aged between 18 and 30, healthy, presenting a suitable control of oral care, low caries index and normal salivary flow, were selected for the *in situ* biofilm retention evaluation. Dental impressions were taken from the upper arch, and a model made of stone plaster was obtained. A palatal acrylic plate was made from this model with six specimens, each one was from the experimental groups, were fixed one millimeter below the resin surface to facilitate the biofilm retention [Figure 1]. A 20% sucrose solution was dripped eight times a day over the specimens to stimulate biofilm formation.

The specimens of each intra-oral device day over the specimens to stimuli *in situ* stage was at the first 7 days; 10 volunteers used the intra-oral device with six specimens made of Vit-l-escence[™] resin, the specimens were removed at the end of the 7th, following a sequence of specimens day schedule of the intra-oral device [Figure 2] for biofilm quantification using a spectrophotometer (Model 700S - FEMTO[™] Industry and

Table 1: Composite resins and manufacturer's specifications

Composite resin	Vit-l-escence [™] (M ₁)	Filtek [™] Z350 XT (M ₂)
Load particle	Microhybrid	Nanopatterned
Polymeric matrix	Bis-GMA	Bis-GMA, Bis-EMA and TEGDMA
Particle type and size	0.7 µm colloidal silica	20 nm non-agglomerated primary silica 5-20 nm zirconia/silica agglomerates from 0.6-1.4 µm
% load particle (weight)	56.0	78.5
Shade	A2	A2E
Manufacturer	Ultradent Corporation (Chicago, USA)	3M – ESPE Dental products (St. Paul, USA)
Lot	B5L7Y	N111499/6018A2



Figure 1: Intra-oral device used in this study

Table 2: Polishing materials and manufacturer's specifications

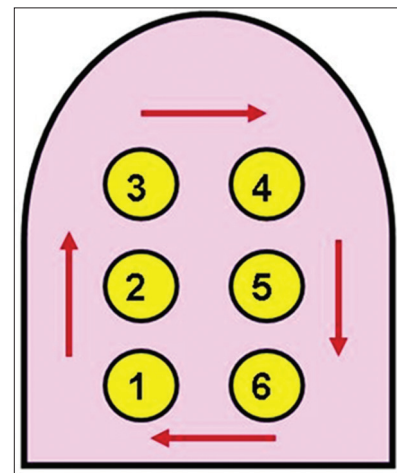
Material	Classification	Particle grit	Granulation	Manufacturer	Lot Reference
Sof-Lex Pop On™	Polyester abrasive disk	Aluminum oxide	Coarse: 17.10 µm Medium: 7.10 µm Fine: 5.72 µm Ultra fine: 1,68 µm	3M – ESPE Dental Products St. Paul, USA	24054 2380B
Diamond Flex™	Synthetic fabric silicon polyester disk	No abrasive	-	FGM Odontológica Joinville, Brasil	010604-6 145
Diamond Polish Paste™	Abrasive paste	Diamond	0.5 µm	Ultradent Corporation Chicago, USA	5XW1 998
Enamelize™	Abrasive paste	Aluminum oxide	0.7 µm	Cosmedent Inc. Chicago, USA	034832 242-4
Lummina – E Diamond	Abrasive paste	Diamond	<100 nm	Experimental product	-
Lummina – E Alumina	Abrasive paste	Aluminum oxide	<100 nm	Experimental product	-

Table 3: Vit-I-escence™ and Filtek™ Z350 XT experimental groups

Group	Composite resin	Surface treatment
G ₁ (n=10)	Vit-I-escence™	Polyester matrix
G ₇ (n=10)	Filtek™ Z350 XT	
G ₂ (n=10)	Vit-I-escence™	Polyester matrix+Sof-Lex Pop On™
G ₈ (n=10)	Filtek™ Z350 XT	
G ₃ (n=10)	Vit-I-escence™	Polyester matrix+Sof-Lex Pop On™+Diamond Polish Paste™
G ₉ (n=10)	Filtek™ Z350 XT	
G ₄ (n=10)	Vit-I-escence™	Polyester matrix+Sof-Lex Pop On™+Enamelize™
G ₁₀ (n=10)	Filtek™ Z350 XT	
G ₅ (n=10)	Vit-I-escence™	Polyester matrix+Sof-Lex Pop On™+Diamond Polish Paste™+Lummina – E Diamond
G ₁₁ (n=10)	Filtek™ Z350 XT	
G ₆ (n=10)	Vit-I-escence™	Polyester matrix+Sof-Lex Pop On™+Enamelize™+Lummina – E Alumina
G ₁₂ (n=10)	Filtek™ Z350 XT	

Trade Instruments, SSing) and six specimens of Filtek™ Z350 XT resin.

To quantify the biofilm, each specimen was placed into identified microtube with 1.5 ml of sodium hydroxide (NaOH) - 1.0 M and mechanically shaken inside the high frequency shaker tubes (Shaker - Model MA 563, orbital™ - TECNAL Laboratory Equipment, Piracicaba, SP, Brazil) for 10 s. The tubes remained under stirring for 3 h, afterwards the tubes were centrifuged (Microcentrifuge –iModelo SPIN 1, Incibr1,™S Instrumentacib Cienttment Inddtmen e ComComme Limitada, S mitada, cibrBrasil) for 10 min at the speed of 16000 rpm. The precipitate was discarded and the remaining was subjected to absorbance spectrophotometer reading (Modelo 700S 700S d™Ind0S 70 e ComCom 7 de Instrumentos, S trumentos, oBrasil) at the wavelength of 280 nm; and in order to calibrate the equipment, NaOH solution - 1.0 M with the specimen without biofilm was used.

**Figure 2:** Sequence of specimens of each intra-oral device

Statistical methodology

The absorbance data from the spectrophotometer were used for the *in situ* biofilm evaluation. The data were submitted to statistical analysis of variance by multiple comparisons of averages for the *ad hoc* Dunn test, both at the significance level of 1%. Intervals of 95% confidence for the population means were used in order to quantify the difference between the roughness means from the different experimental groups.

Results

Table 4 shows the results from the Vit-I-escence™ resin specimens submitted to six polishing steps: G₁ reControl (Standardization - K Dent I Quimidrol™ polyester strips); G₂ - olyeste- Quimidrol™ polyester strips + Sof-Lex Pop On™Abrasive Discs; G₃ - iscs; GDQuimidrol™ polyester strips + Sof-Lex Pop On™Abrasive Discs; + Diamond Polish Paste™; G₄ - Gscs; - Quimidrol™ polyester strips + Sof-Lex Pop On™Abrasive Discs+ Enamelize™;

Table 4: M and SD of the biofilm absorbance spectrophotometry results from Vi-l-escence™ resin after different polishing techniques

Experimental groups					
G ₁	G ₂	G ₃	G ₄	G ₅	G ₆
0.0030±0.0044 ^a	0.0421±0.0222 ^b	0.0173±0.0186 ^{a,b}	0.0096±0.0114 ^{a,b}	0.0029±0.0073 ^a	0.0017±0.038 ^a

Superscript letters indicate similarity statistics (α=0,05). M: Mean, SD: Standard deviation

Table 5: M and SD of the biofilm absorbance spectrophotometry results from Filtek™ Z350 XT resin after different polishing techniques

Experimental groups					
G ₇	G ₈	G ₉	G ₁₀	G ₁₁	G ₁₂
0.0122±0.0217	0.0434±0.0277	0.0296±0.0296	0.0252±0.0250	0.0214±0.0176	0.0197±0.0151

M: Mean, SD: Standard deviation

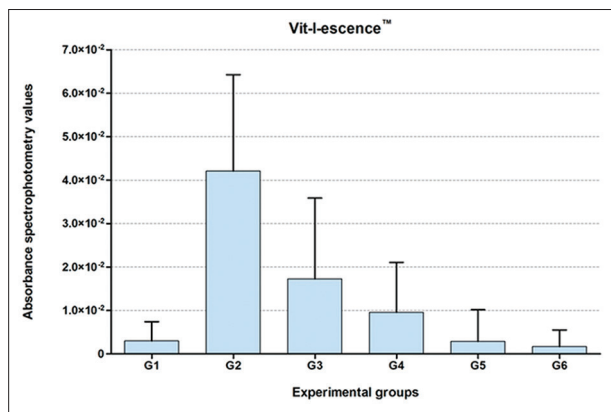


Figure 3: Mean and standard deviation of the absorbance spectrophotometry results from the Vit-l-escence™ resin after different polishing techniques

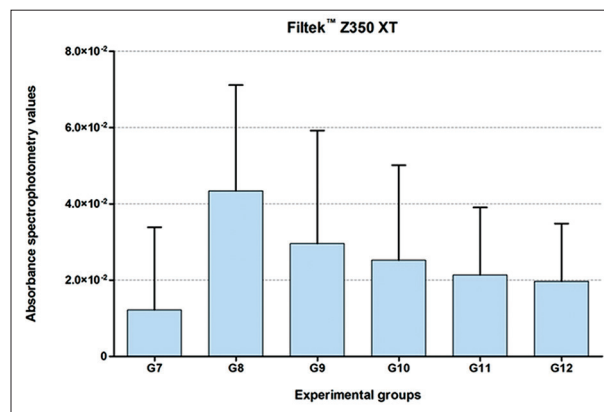


Figure 4: Mean and standard deviation of the absorbance spectrophotometry results from the Filtek™ Z350 XT resin after different polishing techniques

G₅ – Gmeliz –mQuimidrol™ polyester strips + Sof-Lex Pop On™ Abrasive Discs + Diamond Polish Paste™ + Lummina ummina na amond Po₆ – mmina nQuimidrol™ polyester strips + Sof-Lex Pop On™ Abrasive Discs + Enamelize™ + Lummina ummina eName.

The *ad hoc* Dunn test was performed for multiple comparisons among the experimental groups and revealed significant differences among them ($P < 0.0001$), the values are graphically shown in Figure 3.

The results from Filtek™ Z350 XT resin specimens subjected to the same steps of polishing cited above, the average of each group, and the standard deviation values are graphically presented in Figure 4. Statistical analysis revealed no significant differences among the tested groups ($P = 0.1214$), and Table 5 presents the mean values and standard deviation from each group.

Vit-l-escence™ and Filtek™ Z350 X resin specimens subjected to the same polishing technique were compared. The mean and standard deviation from the obtained results are in Figure 5.

The results from Table 4 can be explained by the difference among the inorganic constituents from the composites.

Discussion

The Filtek™ Z350 XT nanofilled or nanoagglomerated composite resin present relatively smaller filler loader, which tend to

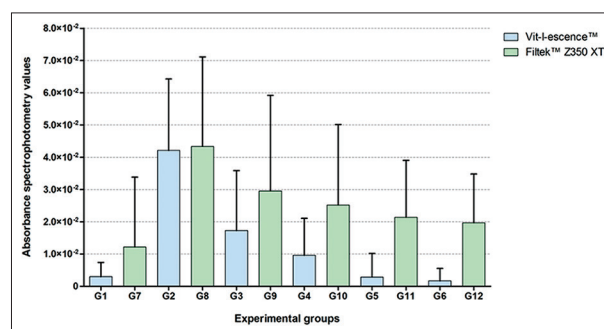


Figure 5: Mean and standard deviation of the absorbance spectrophotometry results from Vi-l-escence™ and Filtek™ Z350 XT resins after different polishing techniques

cluster in larger agglomerates due to the repulsive and cohesion force of from nanometric load^[9,24] - when compared to Vit-l-escence™ microhybrid resin. Another important factor is the Knoop hardness of inorganic load filler from composite resins. Composite with silica load filler - Vit-l-escence™ - are less resistant to wear than quartz or zirconia - Filtek™ Z350 XT composites.^[25]

The polishing from Sof-Lex On™ disks system presented significantly higher biofilm retention average than the averages from the all other groups at 1% level, from each material analyzed

[Tables 4 and 5]. The lowest roughness average among the materials at 1% level or less was found in M1 (Vit-I-escence™) and the highest in M2 (Filtek™ Z350 XT) composites. This difference from disks system is explained by the size of the impregnated abrasive particles. The Sof-Lex Pop On™ system specification and features show great difference between its granulation and the load particle size from composites inorganic filler - M1 (Vit-I-escence™) and M2 (Filtek™ Z350 XT), tested in this experiment. The greater the Knoop hardness difference between the substrate and abrasive agent, higher is the potential for wear, while the lower the discrepancy between the abrasive particles size from the discs and the substrate to be worn, damage occurrence such as risks and grooves on the worn surface is decreased.

Although the™ Diamond Paste Polish (0.5 µm) and Enamelize™ (0.7 µm) have abrasive particles with different size and Knoop hardness - Diamond Paste Polish™ (D = 7000-10000 kg/mm²) and Enamelize™ (D = 2100 kg/mm²), they were statistically similar, since both pastes exhibited satisfactory absorbance values, due to the wear potential (Knoop hardness × particle size).

Only Group 6 (G6) - polishing with abrasive paste Lummina - E Alumina- exhibited statistically lower values of biofilm retention than the control group (G1) - K Dent - Quimidrol™ Strip Polyester. The results were similar in Group 5 (G1 and G5). These facts are due to the formation of a microcrystalline layer of ionic repulsion that hinders the biofilm adhesion.

Conclusion

The Vit-I-escence™ (microhybrid) composite resin exhibited lower biofilm retention than Filtek™ Z350 XT (nanofilled) resin among all the tested groups. The lowest *in situ* biofilm retention occurred at the surfaces treated with Lummina - E Alumina (folder-based aluminum oxide) nanometric prototype.

References

1. Türkün LS, Türkün M. The effect of one-step polishing system on the surface roughness of three esthetic resin composite materials. *Oper Dent* 2004;29:203-11.
2. Jung M, Sehr K, Klimek J. Surface texture of four nanofilled and one hybrid composite after finishing. *Oper Dent* 2007;32:45-52.
3. Yap AU, Yap SH, Teo CK, Ng JJ. Comparison of surface finish of new aesthetic restorative materials. *Oper Dent* 2004;29:100-4.
4. Venturini D, Cenci MS, Demarco FF, Camacho GB, Powers JM. Effect of polishing techniques and time on surface roughness, hardness and microleakage of resin composite restorations. *Oper Dent* 2006;31:11-7.
5. Zee KY, Samaranyake LP, Attström R. Scanning electron microscopy of microbial colonization of rapid and slow dental plaque formers *in vivo*. *Arch Oral Biol* 1997;42:735-42.
6. Da Costa J, Ferracane J, Paravina RD, Mazur RF, Roeder L. The effect of different polishing systems on surface roughness and gloss of various resin composites. *J Esthet Restor Dent* 2007;19:214-24.
7. Janus J, Fauxpoint G, Arntz Y, Pelletier H, Etienne O. Surface roughness and morphology of three nanocomposites after two different polishing treatments by a multitechnique approach. *Dent Mater* 2010;26:416-25.
8. Joniot S, Salomon JP, Dejous J, Grégoire G. Use of two surface analyzers to evaluate the surface roughness of four esthetic restorative materials after polishing. *Oper Dent* 2006;31:39-46.
9. Zhang J, Coombs N, Kumacheva E. A new approach to hybrid nanocomposite materials with periodic structures. *J Am Chem Soc* 2002;124:14512-3.
10. Berger SB, Palialol AR, Cavalli V, Giannini M. Surface roughness and staining susceptibility of composite resins after finishing and polishing. *J Esthet Restor Dent* 2011;23:34-43.
11. Chung KH. Effects of finishing and polishing procedures on the surface texture of resin composites. *Dent Mater* 1994;10:325-30.
12. Jung M, Eichelberger K, Klimek J. Surface geometry of four nanofiller and one hybrid composite after one-step and multiple-step polishing. *Oper Dent* 2007;32:347-55.
13. Guler AU, Duran I, Yucel AC, Ozkan P. Effects of air polishing powders on the surface roughness of composite resins. *J Dent Sci* 2010;5:136-43.
14. Bollen CM, Lambrechts P, Quirynen M. Comparison of surface roughness of oral hard material to the threshold surface roughness for bacterial plaque retention: A review of the literature. *Dent Mater* 1997;13:258-69.
15. Ereifej NS, Oweis YG, Eliades G. The effect of polishing technique on 3D surface roughness and gloss of dental restorative resin composites. *Oper Dent* 2013;38:9-20.
16. Stanford WB, Fan PL, Wozniak WT, Stanford JW. Effect of finishing on color and gloss of composites with different fillers. *J Am Dent Assoc* 1985;110:211-3.
17. Padovani G, Fúcio S, Ambrosano G, Sinhoret M, Puppini-Rontani R. *In situ* surface biodegradation of restorative materials. *Oper Dent* 2014;39:349-60.
18. Pereira CA, Eskelson E, Cavalli V, Liporoni PC, Jorge AO, do Rego MA. *Streptococcus mutans* biofilm adhesion on composite resin surfaces after different finishing and polishing techniques. *Oper Dent* 2011;36:311-7.
19. de Gee AJ, Wendt SL, Werner A, Davidson CL. Influence of enzymes and plaque acids on *in vitro* wear of dental composites. *Biomaterials* 1996;17:1327-32.
20. Geiger S, Ravchanukayev M, Liberman R. Surface roughness evaluation of resin modified glass-ionomers polished utilizing poly(acrylic acid) gel. *J Oral Rehabil* 1999;26:704-9.
21. de Paula AB, de Fúcio SB, Alonso RC, Ambrosano GM, Puppini-Rontani RM. Influence of chemical degradation on the surface properties of nano restorative materials. *Oper Dent* 2014;39:E109-17.
22. Santerre JB, Shajii L, Tsang H. Biodegradation of commercial dental composites by cholesterol esterase. *J Dent Res* 1999;78:1459-68.
23. Koulourides T, Chien MC. The ICT *in situ* experimental model in dental research. *J Dent Res* 1992;71:822-7.
24. Rai R, Gupta R. *In vitro* evaluation of the effect of two finishing and polishing systems on four esthetic restorative materials. *J Conserv Dent* 2013;16:564-7.
25. Anusavice JK. Materiais de acabamento e polimento. In: Anusavice JK, editor. *Materiais Dentatários de Phillips*. Rio de Janeiro: Elsevier; 2005. p. 411-35.