Microhardness and color monitoring of nanofilled resin composite after bleaching and staining

Isabel Cristina G. Bandeira de Andrade¹, Roberta Tarkany Basting², José Augusto Rodrigues³, Flávia Lucisano Botelho do Amaral², Cecília Pedroso Turssi², Fabiana Mantovani Gomes França²

ABSTRACT

Objectives: The present study aimed to investigate the effect of staining solutions on microhardness and shade changes of a nanofilled resin composite, which had been previously in contact with bleaching agents. Materials and Methods: A total of 135 disk-shaped specimens (10 mm × 2 mm) were fabricated with a nanofilled resin (Filtek Supreme) and photocured with a Light Emission Diode (LED) unit and then allocated into three groups to be bleached with 10% or 16% carbamide peroxide (CP) bleaching agents or a 35% hydrogen peroxide (HP) product. Following bleaching, specimens within each group were subdivided into three groups to be immersed in coffee, red wine or distilled water. Microhardness and color were monitored at baseline, after bleaching and after staining. Results: Analysis of variance for split-plot design showed lower microhardness values when the composite had been in contact with HP (P < 0.0001). The specimens immersed in red wine and coffee provided lower microhardness values than those immersed in distilled water, regardless of the bleaching agent to which the composites were previously exposed. Kruskal Wallis and Dunn tests demonstrated that the composite was lighter after bleaching with a 35% HP agent (P < 0.0500). Conclusion: The composite was darker as a result of being immersed either in red wine or coffee, regardless of the bleaching agent.

Key words: Bleaching agents, color change, laboratory research, microhardness, staining solutions, nanofilled composites

INTRODUCTION

Dental resin composites have borne witness to remarkable refinements in recent years, especially due to filler size reduction, which has enhanced the polishability and wear resistance of these materials.¹ An important factor driving the achievements made in advanced resin composite properties has been nanotechnology.¹

However, despite the ongoing improvements in resin composites, a concern remains about their chemical and enzymatic degradation in the oral environment.² Apart from the degradation caused by saliva,³ by foodstuffs⁴ and by beverages,⁵ bleaching procedures can potentially cause softening⁶,⁷ and increased surface roughness⁸ to resin composites, depending on the resin composite and on the bleaching agent used.

Contradictory data has been reported regarding the effect of bleaching agents on microhardness and surface roughness of resin composites. Although the authors of some investigations have noticed a reduction in surface microhardness of resin composites after exposure to
bleaching agents,\cite{6,7} authors of other papers have described no change or increase in surface microhardness of composites after bleaching.\cite{8,9} In regard to surface roughness, the application of bleaching agents to resin composites has been reported, in previous studies, as not changing or increasing surface roughness.\cite{9}

Bleached resin composites have been observed to stain more easily than unbleached counterparts, probably as a result of softening and increased surface roughness.\cite{10} Although the literature is still conflicting regarding the susceptibility of bleached and unbleached composites to staining, an important issue is that staining solutions can also soften resin composites.\cite{5} In fact, red wine and coffee, commonly used as staining solutions in the literature, have been proven to decrease microhardness of resin composites.\cite{11} Therefore, both bleaching agents and beverages with staining capacity can decrease the microhardness of resin composites, including nanostrucutured materials.\cite{6}

Considering the softening effect produced by staining solutions, it is important to evaluate whether resin composites that had been previously in contact with bleaching agents would be affected by further softening and darkening as a result of being immersed in different staining solutions.\cite{8,12} This study aimed at elucidating this issue by evaluating the effect of staining solutions on microhardness and on the shade changes of a nanofilled composite resin previously in contact with bleaching agents.

**MATERIALS AND METHODS**

Table 1 states the main materials used, the staining solutions and their compositions, pH values, manufacturers, batch numbers and manufacturers’ directions. Figure 1 describes the experimental design schematically.

**Specimen preparation**

A total of 135 disk-shaped specimens were

| Table 1: Nanofilled composite resin, bleaching agents and their composition, manufacturers, batch numbers, directions and pH values |
|---|---|---|---|---|---|
| Material/Solution | Composition | Manufacturer | Batch number | Use mode | pH |
| Nanofilled Composite Resin Filtek Supreme XT-shade A2E (3M ESPE, St Paul, MN, USA-FS) | Resin matrix: Bis-GMA, UDMA, TEGDMA e Bis-EMA Filler type: Nanoclusters (0.6 and 1.4 um), nanoparticle, zirconia, (5-20 nm) and silica (20 nm) Filler content (%): 78.5% (weight), 59.5% (volume) | 3M ESPE-St Paul, MN, USA | 7 EE 7 EU | Single increment 2 mm thick 20 s of photoactivation | - |
| Whitness perfect 10%-FGM Produtos Odontológicos, Joinville, SC, Brazil (10% carbamide peroxide agent-10% CP) | CP 10%, carbopol, glycol, water, potassium nitrate and sodium fluoride | FGM, Joinville, SC, Brazil | 7898032–323050 | 4 h daily for 2 weeks | Initial: 5.7 After 4 h of use: 6.13 |
| Whitness Perfect 16%-FGM Produtos Odontológicos, Joinville, SC, Brazil (carbamide peroxide agent 16%-16% CP) | 16% CP, carbopol, glycol, water, potassium nitrate and sodium fluoride | FGM, Joinville, SC, Brazil | 7898032–320080 | 4 h daily for 2 weeks | Initial: 5.64 After 4 h of use: 6.11 |
| Whitness HP 35%-FGM Produtos Odontológicos, Joinville, SC, Brazil (35% hydrogen peroxide agent-35% HP) | 35% HP, thickener, dye, glycol, load and distilled water | FGM, Joinville, SC, Brazil | 7898032–323036 | 3 sessions every 7 days, with 3 15 min applications per session | Initial: 5.51 After 15 min of use: 5.26 |
| Coffee (Melitta São Paulo, Brazil) | Coffee grounds Water | Melitta, São Paulo, SP, Brazil | BJ612TR | 16 g coffee 200 ml of water/ day at 55°C | 5.5 |
| Red wine | Cabernet Sauvignon grapes Alcohol 12.5% | Concha Y Toro, Santiago, Chile | L2CA2338 L1C41269 | 200 ml/day at 25°C | 4.2 |
| Distilled water | - | - | - | 200 ml/day at 25°C | 6.8 |

Bis-GMA: bisphenol A diglycidyl methacrylate, MTAD: Mixture of Tetracycline isomer, citric acid and detergent, UDMA: Urethane dimethacrylate, TEGDMA: Triethyleneglycol dimethacrylate
prepared with a nanofilled resin composite (Filtek Supreme XT - 3M ESPE, St. Paul, MN, USA), which was inserted with a metal spatula (Minelli no. 1 - Golgran, São Paulo, SP, Brazil) in circular acrylic molds (10 mm diameter × 2 mm thick). A mylar strip (Dentart - Polidental, São Paulo, Brazil) was placed on top of and pressed flat with a microscope slide plus a 500 g weight for 30 s. The composite was photocured with a LED unit (Radii-Cal SDI - Melbourne, Victoria, Australia - 1,200 mW/cm²) for 20 s.

Following preparation, specimens were stored in relative humidity at 37°C for 24 h. The finishing and polishing steps were performed with Al₂O₃ abrasive disks (Sof-Lex Pop On - 3M ESPE, St Paul, MN, USA) of decreasing coarseness. The specimens were then stored at 37°C for another 24 h.

### Bleaching Treatment

The specimens were randomly divided into three groups (n = 45) to be subjected to one of the bleaching agents. An individual acetate tray was fabricated in a vacuum-forming machine (P7/Bio-Art Equip Odontológicos Ltda., São Carlos, SP, Brazil) for each specimen, which would be then exposed to 10% or 16% carbamide peroxide (CP) bleaching agents [CP - Whiteness Perfect FGM Produtos Odontológicos, Joinville, SC, Brazil - Table 1]. These bleaching agents were applied for 4 h/day, for 14 days, simulating the at-home bleaching technique. In regard to the group exposed to the 35% hydrogen peroxide (HP) agent (HP - Whiteness HP 35% FGM Produtos Odontológicos, Joinville, SC, Brazil), the product was applied with a disposable applicator (Microbrush - Vigodent, Rio de Janeiro, RJ, Brazil) for 45 min (three sequential 15-min applications) once a week for three consecutive weeks.

When the bleaching time was completed, the specimens were rinsed thoroughly with distilled water and stored in distilled water at 37°C.

### Immersion in staining solutions

When the bleaching procedures were completed, specimens of each group were allocated into three subgroups (n = 15) to be immersed daily in 200 mL of either coffee (Melitta, São Paulo, Brazil) or red wine (Concha Y Toro, Santiago, Chile), as described in Table 1. Distilled water served as the negative control.

Specimens were immersed in staining solutions or distilled water for 3 h/day, at room temperature, over 40 days.[10] After every 3-h period of immersion time, the specimens were rinsed thoroughly with distilled water and stored in distilled water at 37°C.

### Color testing

Color shade was measured using a spectrophotometer (VITA Easyshade - VITA Zahnfabrik, Bad Säckingen, Germany) and the data...
were expressed based on the VITA shade guide and then converted into scores, as can be seen in Table 2.\textsuperscript{[13,14]}

Color shade was recorded on three different occasions: baseline, after bleaching and after staining.

**Microhardness testing**

Three Knoop microhardness indents were carried out on the top surface of each specimen, using a HVS-1000 microhardness tester (Panambra, São Paulo, SP, Brazil), under a 50-g load, applied for 15 s. Measurements were performed at the baseline, following bleaching and after staining.

**Statistical analysis**

Microhardness data were analyzed using an analysis of variance (ANOVA) for split-plot design and Tukey’s test, whereas the color shade recordings were submitted to Kruskal-Wallis and Dunn’s tests. The significance level was set at 5%. Statistical procedures were performed with SAS 6.11 (SAS Institute Inc., Cary, NC, USA).

**RESULTS**

Means and standard deviations (SD) of microhardness values and color shade recordings of the nanofilled resin composite at baseline, after bleaching and after staining, are shown in Tables 3 and 4, respectively.

In regard to the microhardness data, ANOVA for split-plot design showed no significant interaction between the bleaching agent and the staining solution ($P = 0.4384$). Bleaching agents significantly influenced the microhardness values of the nanofilled resin composite ($P = 0.0003$). Lower microhardness values were noticed when the composite had been in contact with 35% HP ($P < 0.0001$). No difference was noticed between the microhardness values produced by the 10% and 16% CP bleaching agent. Overall, there was a significant reduction in the microhardness values from the baseline to the post-staining condition ($P < 0.0001$). The microhardness values of the nanofilled resin composite that had been previously exposed to the bleaching agents were lower when red wine or coffee were used as staining solutions, when compared to distilled water.

In terms of color change, as examined spectrophotometrically, Kruskal Wallis and Dunn tests demonstrated that the nanofilled resin composite was lighter after bleaching with the 35% HP agent ($P < 0.0500$). Specimens became significantly darker after staining ($P < 0.0500$). Regardless of the bleaching agent, no difference was observed for the color shade recorded for the nanofilled resin composite as a result of being immersed either in red wine or coffee, both of which produced a darker color than distilled water.

**DISCUSSION**

The results of the present study showed that the bleaching agents significantly influenced nanofilled resin composite microhardness, which was reduced...
Andrade, et al.: Microhardness and color change of nanofilled composite

when the resin composite was exposed to the 35% HP bleaching agent. The adverse effects of this agent at high concentrations (such as 35% or 38%) have been demonstrated in previous in vitro studies.\[7,8\] It has been speculated that hydrogen-peroxide-based bleaching agents may have high oxidation and reduction capability, thus generating free radicals.\[7\] These radicals have been thought to degrade the resinous matrix\[15\] and disrupt the filler/matrix interface, effects which cause increasing water sorption and filler detachment\[16\] and which, in turn, augment effects which cause increasing water sorption and capability, thus generating free radicals.\[7\] These agents may have high oxidation and reduction capacity of peroxides may cleave the composite polymer-chains.\[7\] In this context, resin composites may have also impaired such physical properties as the microhardness.

The effects caused by 10% CP and CP 16% agents on the surface microhardness of the nanofilled resin composite were significantly lower than those produced by the 35% HP. It is still controversial whether these bleaching agents can affect restorative materials deleteriously.\[17\] While some studies have shown significant reduction in Knoop microhardness after at-home bleaching,\[15\] others have demonstrated the opposite.\[9,10\] Differences in protocols of daily use and in bleaching agents may explain such contradictory findings. For example, Malkondu et al.\[18\] in their study have reported a significant decrease in nanofilled composite after a 6 h/day bleaching period with 10% CP (as opposed to the 4-h period used in the current study). The authors here cited hypothesized that the bleaching agent had sufficient time to diffuse into the high molecular weight organic matrix of the nanofilled composite. It is worth noting, however, that these investigations were conducted in in vitro conditions, in which specimens are commonly stored in distilled water. An in situ study\[19\] demonstrated that bleaching with 15% CP had no effect on nanofilled composite microhardness. This may be explained by the dilution of the bleaching agent by saliva. However, this effect needs to be confirmed in further in situ studies. The specimens immersed in distilled water (control) showed decreased microhardness, which probably occurred because of previous exposure to bleaching agents.

Data analysis also showed a decrease in microhardness after the bleached specimens were stained. This finding may be attributed to the composition of the nanofilled resin composite,\[6\] the inherent characteristics of the staining solutions and the storage protocol adopted.\[20\] In this study, red wine and coffee produced lower microhardness values in the resin composite than distilled water. No difference was found between the microhardness values obtained as a result of using red wine or coffee. Although the red wine had a lower pH (4.2) than the coffee (pH 5.5) and an alcoholic strength of 12.5% – overall factors (lower pH and average alcohol by volume) which seem to affect the resin matrix crosslink, the filler/matrix interface and the filler itself\[21\] – the coffee was used at a temperature above 37°C, a condition that may have accelerated the resin composite degradation process.\[22\]

Color change was evaluated with the VITA Easyshade spectrophotometer, which allows tooth shade to be determined based on the VITA Classical scale.\[13\] The data from the this study shows that no significant difference existed between the color of the specimens at the baseline and in post-bleaching conditions, except when 35% HP was used, which made the nanofilled composite lighter. This may be attributed to a higher proportion of hydrogen and the more acidic pH of the 35% bleaching gel, as compared to the other home-use gels tested in this study. Conversely, CP agents did not change the color of the resin composite. In fact, color changes of composites have not been clinically detectable after the application of 10% CP agents.\[23\]

The assessment of color stability of resin composites following contact with different immersion media has shown that composites are not inert in the oral environment.\[24\] The results of color evaluation showed that, after immersion in coffee and red wine, specimens which had been previously exposed to bleaching agents were significantly darker than those exposed to distilled water.

It has been reported that color changes may occur in different restorative materials after bleaching followed by immersion in staining solutions, including red wine and coffee.\[25\] Apart from the pigments contained in red wine and coffee, the alcoholic content of the red wine (12.5% by volume) and the coffee temperature used in this study may have softened the organic matrix of the composite and allowed dye absorption.\[19\]

In this study, the effect on nanofilled resin caused by bleaching followed by staining was evident, i.e. microhardness decreased after bleaching and the samples became lighter when subjected to 35% HP. Immersion in red wine and coffee darkened the samples.
CONCLUSION

Resin composite previously in contact with bleaching agents showed softening and darkening as a result of being stained in red wine and coffee.

CLINICAL SIGNIFICANCE

Nanofilled composite resin may undergo changes in microhardness and color after bleaching and staining.

REFERENCES

1. Ferracane JL. Resin composite – State of the art. Dent Mater 2011;27:29-38.
2. Carvalho FG, Sampaio CS, Fucio SB, Carlo HL, Correr-Sobrinho L, Puppin-Rontani RM. Effect of chemical and mechanical degradation on surface roughness of three glass ionomers and a nanofilled resin composite. Oper Dent 2012;37:509-17.
3. Larsen IB, Munksgaard EC. Effect of human saliva on surface degradation of composite resins. Scand J Dent Res 1991;99:254-61.
4. Wongkhanee S, Patanapiradej V, Maneenut C, Tantibrojn D. Effect of acidic food and drinks on surface hardness of enamel, dentine, and tooth-coloured filling materials. J Dent 2006;34:214-20.
5. Soares-Geraldo D, Scaramucci T, Steagall-Jr W, Braga SR, Sobral MA. Interaction between staining and degradation of a composite resin in contact with colored foods. Braz Oral Res 2011;25:369-75.
6. Okte Z, Villalita P, García-Godoy F, Lu H, Powers JM. Surface hardness of resin composites after staining and bleaching. Oper Dent 2006;31:623-8.
7. Hannig C, Duong S, Becker K, Brunner E, Kahler E, Attin T. Effect of bleaching on subsurface micro-hardness of composite and a polyacid modiﬁed composite. Dent Mater 2007;23:198-203.
8. de Andrade IC, Basting RT, Lima-Arsati YB, do Amaral FL, Rodrigues JA, França FM. Surface roughness evaluation and shade changes of a nanofilled resin composite after bleaching and immersion in staining solutions. Am J Dent 2011;24:245-9.
9. García-Godoy F, García-Godoy A, García-Godoy F. Effect of bleaching gels on the surface roughness, hardness, and micromorphology of composites. Gen Dent 2002;50:247-50.
10. Polydorou O, Hellwig E, Ausschill TM. The effect of at-home bleaching on the microhardness of six esthetic restorative materials. J Am Dent Assoc 2007;138:978-84.
11. Yu H, Pan X, Lin Y, Li Q, Hussain M, Wang Y. Effects of carbamide peroxide on the staining susceptibility of tooth-colored restorative materials. Oper Dent 2009;34:72-82.
12. Celik C, Yüzügüllü B, Erkut S, Yazici AR. Effect of bleaching on staining susceptibility of resin composite restorative materials. J Esthet Restor Dent 2009;21:407-14.
13. Browning WD. Use of shade guides for color measurement in tooth-bleaching studies. J Esthet Restor Dent 2003;15 Suppl 1:S13-20.
14. Kihn PW, Barnes DM, Romberg E, Peterson K. A clinical evaluation of 10 percent vs. 15 percent carbamide peroxide tooth-whitening agents. J Am Dent Assoc 2000;131:1478-84.
15. Bailey SJ, Swift EJ Jr. Effects of home bleaching products on composite resins. Quintessence Int 1992;23:489-94.
16. Curtis AR, Palin WM, Fleming GJ, Shortall AC, Marquis PM. The mechanical properties of nanofilled resin-based composites: Characterizing discrete ﬁller particles and agglomerates using a micromanipulation technique. Dent Mater 2009;25:180-7.
17. Attin T, Hannig C, Wiegand A, Attin R. Effect of bleaching on restorative materials and restorations – A systematic review. Dent Mater 2004;20:852-61.
18. Malkondu Ö, Yurdagüven H, Say EC, Kazazoğlu E, Soyman M. Effect of bleaching on microhardness of esthetic restorative materials. Oper Dent 2011;36:177-86.
19. Yu H, Li Q, Hussain M, Wang Y. Effects of bleaching gels on the surface microhardness of tooth-colored restorative materials in situ. J Dent 2008;36:261-7.
20. Almeida GS, Poskus LT, Guimarães JG, da Silva EM. The effect of mouthrinses on salivary sorption, solubility and surface degradation of a nanofilled and a hybrid resin composite. Oper Dent 2010;35:105-11.
21. Gordan VV, Patel SB, Barrett AA, Shen C. Effect of surface ﬁnishing and storage media on bi-axial ﬂexure strength and microhardness of resin-based composite. Oper Dent 2003;28:560-7.
22. Lu H, Roeder LB, Lei L, Powers JM. Effect of surface roughness on stain resistance of dental resin composites. J Esthet Restor Dent 2005;17:102-8.
23. Canay S, Cehreli MC. The effect of current bleaching agents on the color of light-polymerized composites in vitro. J Prosthet Dent 2003:89:474-8.
24. Durner J, Spahl W, Schweikl H, Hickel R, Reichl F. Eluted substances from unpolymerized and polymerized dental restorative materials and their Nernst partition coefﬁcient. Dent Mater 2010;26:91-9.
25. Topcu FT, Sahinkesen G, Yamanel K, Erdemir U, Oktay EA, Ersahan S. Inﬂuence of different drinks on the colour stability of dental resin composites. Eur J Dent 2009;3:50-6.