IN VITRO STAINING OF NANOCOMPOSITES EXPOSED TO A COLA BEVERAGE

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ABSTRACT

The objective of this in vitro study was to assess the color change of resin nanocomposites exposed to a cola beverage at different times. Seventy-five cylinders (10 mm x 2 mm) were prepared from each of 4 resin-based composite materials, 3 nanocomposites (Filtek Supreme, Tetric EvoCeram, Premise) and 1 microhybrid (Filtek Z250), to give a total of 300 specimens. Specimens in each group were divided into 5 subgroups (n=15) and immersed in Coca Cola Classic beverage (Coca Cola Co., Atlanta, GA, USA). Color was measured at time 0 (subgroup 1) and after 24 hrs (subgroup 2), 48 hrs (subgroup 3), 1 wk (subgroup 4), and 2 wks (subgroup 5) by using a spectrophotometer (Color Eye 7000, Gretag Macbeth LLC, New Windsor, NY, USA). The mean color change (ΔEab^*) for each material subgroup was calculated at each time interval. Data were analyzed using 2-way ANOVA, with the level for statistical significance set at α=0.05. Significant color differences were observed among all materials at all time periods, in particular after 2 weeks ($P<0.05$). Values reported after 2 weeks for the nanocomposites were clinically unacceptable ($\Delta E >3.3$). Filtek Z250 showed the least color change among the materials at different times. Based on the results, it can be concluded that a microhybrid resin composite showed the least color change and remained clinically acceptable after continuous exposure to a cola beverage.

Key words: *Nanocomposite; Staining; Cola; Color change.*

INTRODUCTION

Resin-based composite materials have been manufactured and developed for the restoration of teeth to meet patients' growing esthetic demands. These materials are classified as 'heterogeneous microfills', and possess overall filler loadings of 60-70 vol%.[1,2] A common problem encountered with such materials after daily exposure to a variety of media is stain and/or alteration of the surfaces of dental restorations. The discoloration is a frequent reason for restoration replacement.[3,4]

Modification of fillers in conventional resin-based composites has improved their mechanical properties and esthetic performances. One of the most significant modifications in recent years has been the application of nanotechnology to resin composites. Nanotechnology is based on the production of functional materials and structures in the range of 1-100 nm using various physical and chemical methods. Resin nanocomposites (NCs) have many advantages such as reduced polymerization shrinkage,[5] increased mechanical properties [6], improved optical characteristics [5], better gloss retention and diminished wear.[7]

Previous *in vitro* studies have investigated the discoloration effects of coffee, tea and wine that are associated with restoration staining. Chan *et al.*[8] concluded that the greatest degree of resin composite staining occurred during the first week. One other *in vitro* study found that nanocomposite material changed color more than a microhybrid composite when tested using red wine or coffee.[3] Though previous surveys have reported the widespread use of cola beverages by children,[9,10] few *in vitro* studies have evaluated the effects of common soft drinks such as cola beverages on the staining of restorative dental materials.[11-13] Therefore, the aim of the present *in vitro* study was to evaluate the color change of different resin nanocomposites exposed to a cola beverage at different

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immersion times. The null hypothesis tested was that there was no significant change in color for the different restorative materials tested at different times during the study.

MATERIALS AND METHODS

Resin Composite Specimens

Four resin-based composite materials were used in this study (Table 1). Three-hundred disk-shaped specimens (75/material) from A2 color shade materials were prepared in a cylindrical Teflon mold (10 mm x 2 mm). Materials were handled according to the manufacturers' instructions. Specimens were covered with Mylar strips and pressed between glass plates before polymerization using a halogen light (Astralis 10, Ivoclar Vivadent, Schaan, Liechtenstein) with a light intensity of 750mW/cm^2, using a 40 s exposure. The distance between the light source and specimen was standardized by using a 1.0 mm thick glass slide. All samples were immersed and stored in distilled water for 24 hrs at 37° C to ensure complete polymerization. The top surface of each specimen was polished using fine polishing disks (Sof-Lex, 3M ESPE) with a slow-speed handpiece.

Staining Procedure

Specimens in each group of 75/material were divided randomly into five equal subgroups. Randomization was carried out by using the random numbers method and, 15 specimens per subgroup were selected. Specimens in each subgroup were immersed in vials containing 10 ml of Coca Cola Classic beverage (Coca Cola Co., Atlanta, GA, USA), which was changed daily. Baseline color values for all groups were recorded at time 0 before immersion (subgroup1), and after 24 hrs (subgroup 2), 48 hrs (subgroup 3), 1 wk (subgroup 4), and 2 wks (subgroup 5) of immersion.

Color Testing

Color of the specimens was measured using a spectrophotometer (Color Eye 7000, Gretag Macbeth LLC, New Windsor, NY, USA) against a white background using CIELAB relative to a standard illuminant. In this scheme, color is measured in three coordinate dimensions of L*(lightness), a* green-red (-a*=green; +a*=red), and b* blue-yellow (-b*=blue; +b*=yellow) [12]. The total color score (E*) is computed for all three spectral values, and obtained by the formula: $E^*=(L^*+a^{*2}+b^{*2})^{1/2}$ The total color change is described by Delta E (ΔEab^*) as has been described previously.[3]

Color change was calculated as follows: $\Delta Eab^*=[(\Delta L_*)^2+(\Delta a_*)^2+(\Delta b_*)^2]^{1/2}$. Three initial readings were taken for each specimen at three separate non-overlapping areas to ensure a representative assessment, and the mean values calculated. A limit of $\Delta Eab^* \leq 3.3$ was interpreted as a clinically acceptable difference in this study.[8] All measurements were performed by the same operator.

Statistical Analysis

All statistical analyses were carried out using SPSS statistical software (V.16, SPSS, Chicago, IL, USA). The materials tested (Filtek Z250, Filtek Supreme, Tetric EvoCeram, and Premise) and the measurement times (0 time, 24 hrs, 48 hrs, 1 wk, 2 wks) were independent variables. The color change (ΔE) at different times of immersion in Coca Cola beverage was the dependent variable. Two-way analysis of variance (ANOVA) was used for the statistical analysis with the probability for statistical significance set at $\alpha=0.05$.

RESULTS

The mean color changes (ΔE) for all subgroups and times are shown in Table 2. The 2-way ANOVA found that the treatment time accounted for 45.3%, the material effect accounted for 17.6%, and the interaction accounted for 30.7% of the total variance ($P<0.0001$).

At time = 0 hr

There was no significant difference between Filtek Z250 (FZ) and EvoCeram (EV) (P=0.98), with mean ΔE of 0.42 and 0.44, respectively. Similarly, there was no significant difference between Premise (Pr) and Filtek Supreme (FS) (P=0.73), with mean ΔE of 1.10 and 1.19, respectively. There was a significant difference between the two types of resin composite materials ($P<0.05$).

At time = 24 hrs

There was no significant difference between FS and EV (P=0.99), and FZ presented the lowest mean ΔE of 0.62. A significant difference was found between FS and Pr (P=0.001), with Pr having the highest mean ΔE of 1.62.

TABLE 1: NAME AND CHARACTERISTICS OF MATERIALS USED IN THE STUDY

Resin composite	Composition	Type and Batch No.	Shade	Filler content (%)
Filtek Z250 (3M ESPE, St Paul, MN, USA)-**FS**	**Matrix**: Bis-phenol A diglycidylmethacrylate (Bis-GMA), urethane dimethacrylate (UDMA), bisphenol A polyethylene glycol, diether dimethacrylate. **Filler**: zirconia/silica (without silane) (0.19-3.3 µm).	Microhybrid Batch No.: 280922	A2	W/W =79 V/V =60
Filtek Supreme (3M ESPE, St Paul, MN, USA)-**FZ**	**Matrix**: Bis-phenol A diglycidylmethacrylate (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA), urethane dimethacrylate (UDMA), bisphenol A polyethylene glycol, diether dimethacrylate. **Filler**: silica nanofillers (5-75 nm), zirconia/silica nanoclusters (0.6-1.4 µm).	Nanofilled Batch No.: 20070831	A2B	W/W =78.5 V/V =59
Tetric EvoCeram (Ivoclar Vivadent, Schaan, Liechtenstein)-**EV**	**Matrix**: Dimethacrylates, additives, catalysts, stabilizers, pigments. **Filler**: Barium glass, ytterbium trifluoride, mixed oxide, prepolymers.	Nanohybrid Batch No.: L52963	A2	W/W =82.5 V/V =68
Premise (Kerr Hawe, Bioggio, Switzerland) **-Pr**	**Matrix**: Ethoxylated bis-phenol A dimethacrylate, triethylene glycol dimethacrylate (TEGDMA), light-cure initiators and stabilizers. **Filler**: Prepolymerized filler (30- 50 µm), barium glass (0.4 µm), silica nanoparticles (0.02 µm).	Trimodal nanofilled Batch No.: 2762829	A2 Dentin	W/W =84 V/V =69

* W/W: Weight/weight
V/V: Volume/volume

TABLE 2: MEAN COLOR CHANGE (“E ± SD) FOR THE RESIN COMPOSITES TESTED AT DIFFERENT TIMES (T) OF IMMERSION IN COLA SOLUTION (N=15/SUBGROUP/TIME)

Resin composite	T = 0 ΔE (SD)	T = 24 hrs ΔE (SD)	T = 48 hrs ΔE (SD)	T = 1 wk ΔE (SD)	T = 2 wks ΔE (SD)
Filtek Z250 (FZ)	0.42 (02)[a,*]	0.62 (0.31)[a]	0.95 (0.42)[a]	1.26 (0.34)[a]	1.89 (0.64)[a]
Filtek Supreme (FS)	1.19 (0.35)[b]	1.21 (0.69)[a]	2.54 (0.61)[b]	2.54 (0.09)[b]	3.71 (0.62)[b]
Tetric EvoCeram (EV)	0.44 (0.21)[a]	0.65 (0.39)[a]	0.98 (0.50)[a]	1.79 (0.59)[c]	4.42 (2.15)[c]
Premise (Pr)	1.10 (0.68)[b]	1.62 (0.12)[b]	1.74 (0.38)[c]	3.16 0.38)[d]	11.73 (0.56)[d]

*Same superscript letters indicate no significant differences among materials, at the same time period.
SD = Standard Deviation.
Values > 3.3 are clinically unacceptable.

At time = 48 hrs

The highest mean ΔE was for FS (2.54), while the lowest was for FZ (0.95). No significant difference was noted between EV and FZ (P=0.99), with mean ΔE of 0.95 and 0.98, respectively. A significant difference (P=0.001) was seen between these two materials (EV and FZ) and the other two materials (Pr and FS), with mean ΔE of 1.74 and 2.54, respectively.

At time = 1 wk

Significant differences were observed among all groups (P=0.001). The lowest mean ΔE was for FZ (1.26) and highest mean ΔE was for Pr (3.16).

At time = 2 wks

The mean ΔEs for all groups showed a wide range, with significant differences present among all materials (P=0.001). FZ showed the lowest mean ΔE (1.89), followed by FS (3.72), EV (4.42), and Pr (11.73).

DISCUSSION

Discoloration of tooth-colored resin-based materials may be caused by intrinsic and extrinsic factors. Intrinsic factors involve discoloration of the resin material itself, such as the alteration of the resin matrix structure and the interface of the matrix and fillers[4], with the formation of colored degradation products. Extrinsic factors for discoloration include staining by absorption of colorants as a result of contamination from different beverages. Chan et al[8] investigated the staining potential of coffee, tea and cola. They reported that the greatest amounts of discoloration occurred after one week and extended into the second week, which is in agreement with the results of the present study. Discoloration was due to absorption of colorants by the tested materials.[15,16] It was reported that stain sorption was closely related to water sorption,[17] and that most of the water sorption was observed during the first week.[18] A low staining susceptibility was generally related to a low water absorption rate or low resin content. The structure of the composite and characteristics of the filler particles may have a direct impact on the surface smoothness and susceptibility to extrinsic staining. Color stability is directly related to the resin phase of resin composites. Urethane dimethacrylate (UDMA) has been found to be more stain resistant than Bis-phenol A diglycidylmethacrylate (Bis-GMA), or triethylene glycol dimethacrylate (TEGDMA).[19]

FZ is a microhybrid resin composite with a filler loading of 60% by volume and, therefore, the low discoloration rate of FZ is probably related to its high inorganic content, which results in a lower water sorption rate. As reported by the manufacturer, FS is a NC having a primary 20 nm silica filler and loosely-bonded nanoclusters of zirconia/silica particles ranging from 0.6-1.4 μm. Its resin matrix is composed of Bis-GMA, UDMA, Bis-EMA and TEGDMA.[4] The higher susceptibility of FS to stain as compared to FZ could be attributed to the filler size, morphology, rate of degradation, and the potential porosity of the nanoclusters.[20] FZ and FS have an essentially identical matrix composition, apart from the addition of TEGDMA to the resin matrix of FS that also may explain its higher discoloration which is in concordance with recent study.[21]

Water infiltration degrades siloxane bonds by hydrolysis, to initiate debonding of the filler at the resin matrix interface.[22-24] Water sorption and water uptake are dependent on the constituents of the methacrylate resin matrix, the morphology and dispersion of the filler, and the properties of the filler/matrix interface.[25-27] This dependence may explain the high discoloration obtained with Pr after one week and two weeks, as the TEGDMA resin matrix can absorb staining substances easily.[28,29] Furthermore, the present study showed that, when compared to Pr and FS, EV generally had a better resistance to staining that might have been due to the omission of TEGDMA from its composition.

While differences of even 1 or 2 units in ΔEab* may indicate some perceptible stain,[30] a limit of $\Delta E^*ab \leq 3.3$ is interpreted as a clinically acceptable color change in many studies.[14, 31] In the present study, the values reported were clinically acceptable except after two weeks, as by then the three NCs showed a $\Delta E^*ab > 3.3$ while FZ had color changes less than this limit. Coca Cola is a brown carbonated beverage, and gains its color through the addition of caramel. Caramel exhibits colors ranging from palest yellow to deepest brown, and is made by heating sugar or glucose in the presence of an alkali or mineral acid. [32] In addition to its staining potential, cola beverage has been reported as having a corrosive (erosive) effect on enamel and tooth structure.[33, 34] The negative b^* values for NCs immersed in cola solution indicate that the specimens may have become more translucent. The sensitivity of water sorption and the solubility behavior of NCs as influenced by time and pH appear closely related to the hydrophilicity of the resin matrix.[35,36] However, any effect of pH on color change was not examined in the present study.

It is important to point out that the colorimeter employed, analyses light reflected from the specimen surface. If the specimen is translucent, the light may travel through the entire thickness of the specimen before it is reflected from the background. For resin-bonded composite materials, the tooth-like color results from the fillers and pigment additives, which contribute to the higher L^*, a^* and b^* values. In addition, these additives attenuate the intensity of the light as it travels through the specimen. Therefore, the color measurement of NCs is dominated by the bulk of the specimens.[5] Longer light pathways within NCs would reduce the effect of any stain on the colorimeter reading.

The clinical relevance of the present study depends on how much color change is considered as being perceptible. Though the color change limit of $\Delta E^*ab \leq 3.3$ is considered as being clinically acceptable[8,31], other studies reported that observers did not believe that restorations with a ΔE as high as 3.3 and 3.7 required replacement,[8,37] even though the color differences were definitely perceptible.

CONCLUSIONS

The observed results led to rejection of the null hypothesis that "there was no significant change in color of the different restorative materials tested at different time intervals". Furthermore, the following conclusions can be made:

1. All of the resin-based composite materials tested showed statistically significant color changes after immersion in Coca Cola Classic beverage.
2. The three nanocomposites were more prone to discoloration with time and, after two weeks; the mean values reported were clinically unacceptable.
3. The microhybrid composite Filtek Z250 was the most resistant material to discoloration, with a clinically acceptable appearance.

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