Scientific Article

In Vitro Staining of Resin Composites by Liquids Ingested by Children

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Abstract: *Purpose:* The purpose of this study was to compare the influence of various children's drinks on the discoloration of dental resin composites. **Methods:** Ninety-six disks (3-mm thick, 10 mm in diameter) were prepared from 3 types of composite: (1) submicron; (2) nano; and (3) microhybrid. After polishing and obtaining baseline data, they were equally divided into 4 groups and immersed into 1 of 4 liquids at 37°C: (1) distilled water; (2) Kool-Aid Jammers (grape flavor); (3) Coca-Cola; or (4) snow cone syrup (banana flavor). On days 3, 6, 9, 12, and 15, the samples were measured again for color. On day 18, they were measured for both color and gloss. **Results:** Overall, the color change during the staining procedure was minimal (ΔE_{ab}<1.67) for all 3 composites, although it appeared that Tetric EvoCeram had the least discoloration. Using 3-way analysis of variance and linear regression analysis, only Estelite Σ in Coca-Cola showed a statistically significant linear relationship between discoloration and stain time. **Conclusions:** Three composites reacted differently in various staining solutions. During this study, the 4 solutions did not discolor any of the composites in a way that was clinically significant. Tetric EvoCeram may be the most stain resistant material among the 3 tested. (Pediatr Dent 2008;30:317-22) Received January 10, 2007 / Last Revision August 17, 2007 / Revision Accepted August 29, 2007

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Resin composite materials have been used for many years in dentistry with great success and high patient acceptance. A common problem encountered with these materials, after months and years of use and exposure to a variety of different foods and beverages, has been staining. This discoloration is a frequent reason for replacement.[1-3] The physical properties of composite resins are dependent on the nature of the resin matrix, filler particles, and the resin-filler interface. Physical properties also can be influenced by the chemical environment present in the mouth.[4] The changes in the oral environment that cause the staining can occur either intrinsically or extrinsically. Intrinsically, color can change due to physicochemical alteration of the resin material itself, within the resin matrix. Ultraviolet exposure, thermal changes, and humidity can all contribute to these intrinsic changes.[1,2,5] Color also can change extrinsically due to absorption of stains into the outer surface.[1,6,7]

There have been many studies on the color instability of composite resin materials.[1,8-21] In one such study, 10 composites were subjected to staining tests using different dyes under various physicochemical conditions. Some of the staining materials used were food dye, red vinegar, coffee, and erythrosine—with the latter 2 producing the most intense staining. The study noted that 3 of the 10 composites did not show any staining. It was also noted that polishing the composite material resulted in less overall staining.[5]

In multiple subsequent articles, composite resin material was found to show color changes. One such study demonstrated that restorative material, staining solutions, and immersion time were all statistically significant factors that affected color stability in these composites, with discoloration noted as early as day 7. Although all the resin types showed color changes, the light-cured composites were the least color stable and the coffee solution showed the most color change.[22] Another study again showed that the finishing and polishing system as well as the staining solution has an effect on the composite. Resin-based materials and unfilled resins were immersed into coffee, cola, or red wine after being finished and polished by 1 of 3 systems. The no treatment group showed the most color instability compared with the other 2 methods, and the resin composites exhibited a greater color change than the unfilled resin. In this report, red wine caused the greatest color change.[23] Finally, another study found a similar result in that different staining solutions showed color changes in 6 different types of resin composites and glass ionomer cements. Five different stains (red wine, coffee, tea, soy sauce, and cola) were evaluated. The outcome showed that all restorative materials tested were susceptible to discoloration by all 5 kinds of stains—especially coffee, red wine, and tea.[24]

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Thus, previous studies have found that red wine, coffee, and tea affect color changes. These 3 beverages, however, are mostly adult beverages that children in the United States rarely drink. Therefore, this study's purpose was to compare the influence of various children's drinks on the discoloration of different types of dental resin composites.

Table 1.   DENTAL RESIN COMPOSITES AND STAINING SOLUTIONS USED IN THE STUDY

	Brand name	Manufacturer, city, state, country	Lot no.	Expiration date
Dental composite resin	Estelite Σ	Tokuyama Dental Corp, Tokyo, Japan	UE42036S	2009-01
	Tetric EvoCeram	Ivoclar Vivadent Inc, Amherst, NY	J15941	2010-07
	Herculite XRV	Kerr USA, Orange, Calif	449827	2009-06
Staining solution	Kool-Aid Jammers (grape flavor)	Kraft, Northfield, Ill	FO4 0357CT773	2007-06
	Coca-Cola Classic	The Coca-Cola Co, Atlanta, Ga	DDE 20:51	2007-04
	Snow Cone Syrup (banana flavor)	Amerifoods Trading Co, Los Angeles, Calif	060705 15:50	2007-07

Methods

Ninety-six specimens were prepared from 3 composite resins (shade A2): (1) submicron; (2) nano; and (3) microhybrid composite (Table 1). The test specimens were made by injecting the resin into a split polytetrafluoroethylene mold 3 mm thick and 10 mm in diameter. The resin was carefully packed, ensuring that the mold was completely filled. Extra material was expelled by pressing down with a Mylar/glass slide. The specimen's lower side was light cured with a halogen curing light (model no. XL3000, 3M, St. Paul, Minn) according to the manufacturers' instructions. This surface was used as the working surface. The specimens were turned over and the opposite side was also light cured. The intensity of the curing light (500 mW/cm^2) was monitored with a radiometer (Kerr/Demetron, Danbury, Conn). The specimens were stored overnight in a 37ºC incubator.

Following the preparation of the composite resin specimens, they were polished with a high-speed carbide no. 7901 bur and Sof-Lex (3M ESPE) composite resin polishing discs according to the following procedure. The carbide finishing bur was used to replicate the initial finishing in clinical situations. They were then polished, in order, with a: 1) black coarse Sof-Lex disc for 90 seconds at a speed of 5,000 rpm; 2) dark blue medium disc for 30 seconds at a speed of 5,000 rpm; 3) medium blue fine disc for 30 seconds at a speed of 10,000 rpm; and 4) light blue superfine disc for 30 seconds at a speed of 10,000 rpm. A Rotary Master electric slow-speed handpiece (J Morita USA, Irvine, Calif) was used to control the speed of polishing. New Sof-Lex disks were used with each specimen.

Thirty-two specimens were made from each of the 3 composite types and divided into 4 groups of 8 for immersion into one of 4 liquids: (1) distilled water (as control); (2) Kool-Aid Jammers (grape flavor; Kraft, Northfield, Ill); (3) Coca-Cola Classic (The Coca-Cola Co, Atlanta, Ga); or (4) snow cone syrup (banana flavor; Amerifoods Trading Co, Los Angeles, Calif). Prior to staining, a glossmeter and chroma meter were accurately calibrated according to the manufacturers' recommendations and then the gloss and color of the composite disks were measured for baseline data.

The specimen gloss was measured with the glossmeter (Novo-Curve, Rhopoint Instrumentation, East Sussex, UK; precision=0.1; accuracy=0.5 GU; repeatability=1 GU[25] [GU= gloss unit, a standardized unit of measurement to calibrate all glossmeters; it is related to the amount of reflected light from a black glass standard with a defined refractive index][26]. A total of 4 values were read from each sample. To do this, the sample was turned 90º after each reading. An average value was used to represent the gloss of that specimen. Color was measured with a chroma meter (Minolta CR-221, Minolta Corp, Ramsey, NJ; short-term repeatability: chromaticity within ±0.0002; color difference {ΔE*ab}, standard deviation within 0.07; temperature drift=<ΔE*ab 0.05/ºC27) against a white background. CIE L*a*b* values were recorded with illumination D65.

After baseline measurement, the 8 samples were placed into a clean, dry Petri dish and then completely immersed in their previously assigned staining liquid. Following this immersion, the Petri dishes were covered and placed in an incubator at 37ºC. On days 3, 6, 9, 12, and 15, the samples were removed from the incubator, drained of liquid, lightly rinsed with distilled water, partially dried with compressed air, and then allowed to completely air dry. The specimens were handled with powder-free latex gloves, and they were only held at the edges. Once dry, they were measured again for color with the chroma meter. Following the readings, the composites were again immersed in fresh staining liquids and placed in the incubator. Day 18 was the final day, and the samples were cleaned, dried, and measured for both color and gloss.

Results

Figures 1 to 3 show the color changes over the course of the 18-day study of the 3 composite types with 4 staining solutions. Table 1 lists the brand names and all manufacturing data of the solutions and composites. Table 2 lists the means of gloss before and after staining. Overall, the color change during the staining procedure was minimal for all 3 composites tested, although it appeared that Tetric EvoCeram had the least discoloration, as shown in Figures 1-3.

Paired *t* tests were used to detect differences between before and after staining gloss values, and results are shown in Table 2. Surprisingly, distilled water decreased the gloss of 2 composite materials while the gloss of specimens in Kool-Aid did not change. Three-way analysis of variance was used to

Table 2.   GLOSS OF THE MATERIALS TESTED BEFORE AND AFTER STAINING WITH PAIRED *t* TEST RESULTS

| Material	Distilled water			Coca-Cola			Kool-Aid			Snow Cone Syrup		
	Before ±SD	After ±SD	*P*-value	Before ±SD	After ±SD	*P*-value	Before ±SD	After ±SD	*P*-value	Before ±SD	After ±SD	*P*-value
Estelite Σ	56.5±10.8	47.1±9.1	<.001	62.4±7.7	57.5±9.4	.07	62.7±8.0	64.0±7.1	.25	56.7±12.4	60.3±12.5	.33
Tetric EvoCeram	31.6±4.7	30.7±3.9	.33	36.2±11.3	29.0±8.4	.003	28.1±8.4	30.3±10.7	.25	27.3±5.1	25.6±6.7	.33
Herculite XRV	48.2±6.3	40.7±8.1	.02	38.1±8.5	24.8±4.9	<.001	32.6±4.1	30.6±5.7	.22	33.5±4.3	25.6±5.4	.002
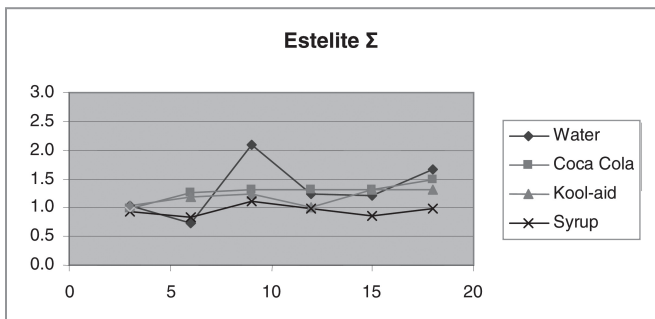
Figure 1. Color changes (ΔE*ab) of the submicron composite Estelite Σ in various staining solutions over time (days).
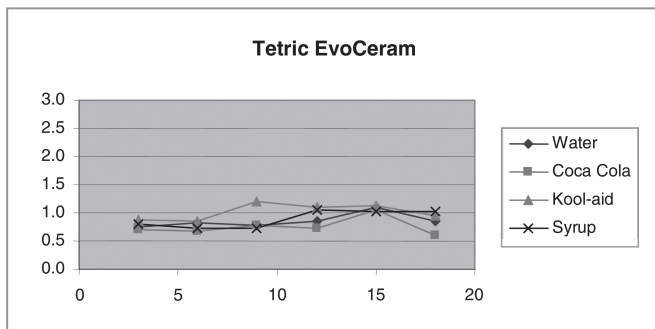

Figure 2. Color changes (ΔE*ab) of the nano composite Tetric EvoCeram in various staining solutions over time (days).
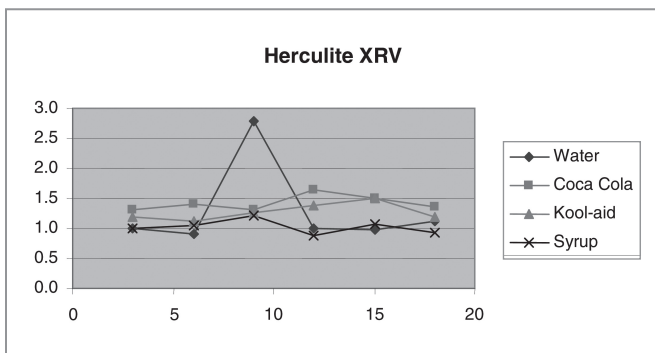

Figure 3. Color changes (ΔE*ab) of the microhybrid composite Herculite XRV in various staining solutions over time (days).

analyze the influence of material, stain solution, and stain time on color (Table 3) and gloss (Table 4). The main factors and/or the interactions between main factors had a significant influence on color change and gloss, as noted by the statistical significance of the *P*-values in Tables 3 and 4. Linear regression analysis was used to establish a statistical relationship between color change and stain time for each material/stain solution combination, and the results are shown in Table 5. The results revealed that only Estelite Σ in Coca-Cola showed a statistically significant linear relationship between discoloration and stain time. For all the other combinations, color did not change as stain time increased.

Discussion

Surface characteristics, such as color and gloss, are important esthetic components of an acceptable composite restoration. Thus, it was the intent of this study to measure the effect that various potentially staining solutions would have on these surface characteristics. To do this, the test composites were selected from different manufacturers. In addition, they represented 3 different types of composites (ie, submicron composite, nano composite, and a microhybrid composite). Microhybrid composites have an average filler particle size of approximately 1 μm or less. Therefore, these materials have high filler loading to yield high mechanical properties and low polymerization shrinkage while maintaining a smooth surface after polishing. This type of material is marketed as a universal or all-purpose composite.

Nano composites are a type of composite in which the primary filler size is in the nm range while the secondary filler clusters are in the μm range. Thus, this material has a better polishability and improved retention of the polish and gloss compared to microhybrid composites.[28] Tokuyama Dental Corporation developed a new type of filler for Estelite Σ. The filler produced by the sol-gel method has a spherical shape. The average particle size is 0.2 μm, with a narrow range from 0.1-0.3 μm. Therefore, this material is called a submicron composite.

All composites and natural teeth can have color changes over their lifetime. The staining solutions in this study did

Table 3.   SUMMARY OF 3-WAY ANALYSIS OF VARIANCE RESULTS FOR COLOR MEASUREMENT

Source term	Degrees of Freedom	Sum of squares	Mean square	F-ratio	P-value
A: Material	2	14.92	7.46	34.47	<.001
B: Stain	3	4.43	1.48	6.83	<.001
AB	6	5.54	0.92	4.27	<.001
C: Time	5	8.52	1.70	7.88	<.001
AC	10	5.10	0.51	2.63	.01
BC	15	11.95	0.80	3.68	<.001
ABC	30	12.99	0.43	2	.001
S (Error mean square)	504	109.03	0.22		
Total (adjusted)	575	172.48			
Total	**576**				

Table 4.   SUMMARY OF 3-WAY ANALYSIS OF VARIANCE RESULTS FOR GLOSS MEASUREMENT

Source term	Degrees of Freedom	Sum of squares	Mean square	F-ratio	P-value
A: Material	2	30230.39	15115.19	232.01	<.001
B: Stain	3	503.58	167.86	2.58	.06
AB	6	3229.90	538.32	8.26	<.001
C: Time	1	749.71	749.71	11.51	.001
AC	2	330.88	165.44	2.54	.08
BC	3	574.60	191.53	2.94	.03
ABC	6	283.86	47.31	0.73	.63
S (Error mean square)	168	10945.05	65.15		
Total (adjusted)	191	46847.96			
Total	**192**				

produce color changes in all 3 types of composites, although the changes were not clinically significant. A ΔE*ab range from 2.2 to 4.4 was considered to be a clinically acceptable color difference;[29] and a limit of ΔE*ab≤ 3.3 is interpreted as a clinically acceptable color change in many studies.[30,31] The 3 composites tested all had color changes less than this limit, so their changes were considered minimal. Tetric EvoCeram had the lowest color change of 3 types, as shown in Figures 1 through 3.

There were 2 reasons for choosing the beverages used in this study. First, although there have been several studies evaluating the ability of composites to resist staining from foods and beverages, most of these have involved the assessment of "adult" type beverages (ie, red wine, coffee, tea, etc.). Secondly, the choice of the beverages in this study attempted to represent diverse areas of the color spectrum. Kool-Aid is blue/purple, the snow cone syrup is yellow/orange, and Coca-Cola is brown. There are differences in consistency as well. Kool-Aid is a thin, watery solution, snow cone syrup is, in fact, syrupy, and Coca-Cola is carbonated. In addition to its staining potential, Coca-Cola has also been reported to have a corrosive effect on enamel and tooth structure.[32,33]

The esthetically pleasing color of a composite restoration is highly dependent on the careful selection of color and shade by the clinician. If the clinician does not select a composite that has the ability to remain stable under a variety of oral environments, however, the best clinical efforts will not result in a long-term esthetic restoration. To simulate oral conditions, the composite disks were finished and polished in a way that is commonly used in the clinical setting. In this study's experimental design, however, the disk area that was polished was flat and easy to reach. In a clinical situation, there may be surfaces that are difficult to polish due to diminished accessibility, and decreased treatment time due to patient behavior. Another study comparing polished samples to nonpolished ones could be looked at in future reports. Also with the flat disk, the composite was of uniform thickness. In a clinical restoration, there are areas of variable composite thickness, including margins, to consider. Since polymerization shrinkage may vary depending on the thickness of the composite, it would be interesting in future studies to evaluate a composite's resistance to stain in all areas of a restoration.

In addition to the previous changes that could be studied in the future, other modifications could be assessed as well. In this study, the samples were in contact with the undiluted staining solutions the entire time. In real-life clinical situations, however, the oral cavity would not replicate this. The solutions would be mixed with saliva, thus diluting them and causing further change in the pH of the oral liquids. Further reports on this topic could mix the staining solutions with a medium that would represent saliva. Thermocycling the samples could also be incorporated to simulate the natural oral environment.

All experimental specimens were stored in the staining solution at 37°C. Although this is appropriate for a controlled laboratory situation, it does not necessarily replicate oral conditions. Over time, clinical composite restorations are exposed to a wide range of temperatures, depending on the foods and beverages consumed. These changing oral temperatures may affect the physical properties of some composites.[34,35] In addition to temperature changes, color changes may be related to natural aging of composites in the mouth.[36] The extent to which variable temperatures or aging makes a difference in how

a composite resists staining is an important consideration, but not one that was addressed in this study. Finally, this study only tested the composites' resistance to staining for 18 days. The ability of the composites to resist staining over long periods of time should be evaluated further.

Conclusions
Based on this study's results, the following conclusions can be made:
1. Different dental composites reacted differently in various staining solutions.
2. Overall, during the time of the study, the 4 staining solutions tested did not discolor any of the composite materials in a way that was clinically significant.
3. Tetric EvoCeram was found to be the most stain-resistant material among the 3 dental composites tested, although this finding was not clinically significant.

Acknowledgments

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Abstract of the Scientific Literature

Streptococcus mutans growth on resin composites leads to altered surface integrity.

The aim of this study was to determine whether bacterial growth on composite resin results in surface changes and micro-hardness. Three different resin materials were tested and subjected to S. mutans in vitro. Resin material was formed into discs measuring 5mm in diameter and 1mm in thickness and light-cured. A total of 15 discs of each material were prepared, each receiving an application of 10 µL drop of bacteria suspension. Five discs from each resin material were incubated for 1 day to create a 1-day-old biofilm. Another 5 from each were incubated to create 1-week-old biofilms, while the final 5 discs from each resin were incubated for 1 month to create a 1-month-old biofilm. 15 control discs of each of the resin materials were incubated in sterile broth. One third were incubated for 1 day, 1/3 for 1 week, and 1/3 for 1 month. Discs were scanned to assess roughness for each grouping and surface changes while bacterial growth was also measured. Micro-hardness was assessed on another series of discs for each resin that were incubated for 1 month to develop a 1-month-old biofilm. Results from this study reveal that the growth of S. mutans leads to significant surface roughness but does not significantly affect the micro-hardness of composite resin.

Comments: This study indicates that over time dental biofilm, specifically S. mutans, can alter the surface of resin restorations leaving them roughened. Such surface irregularities are a potential niche for further bacterial colonization and biofilm growth, which can ultimately affect the longevity of composite resin restorations and contribute to recurrent caries. Careful consideration must be given before placing resin restorations, particularly in areas where marginal integrity of restorations is questionable. RJS

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