Changes in Translucency of Resin Composites after Storage in Salivary Esterase

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ABSTRACT

Background: Dental resin composites are degraded by salivary enzymes, but the enzymatic influence on the translucency of resin composites has not been determined.

Purpose: The purpose of this study was to evaluate the changes in translucency of resin composites after storage in the salivary enzyme esterase (ETE, porcine liver esterase, 400 mU/mL) compared with those in phosphate-buffered saline (PBS).

Materials and Methods: The colors of specimens of three brands of resin composites of various shades were measured after polymerization and polishing (baseline), and after immersion in PBS or ETE for 9 weeks; measurements were determined over white and black backgrounds according to the CIELAB color scale (established by Commission Internationale de l'Eclairage). The final specimen thickness was 1.75 mm. Translucency parameter (TP) was obtained by calculating the color difference between the specimen over a white background and that over a black background. Two-factor, repeated-measures analysis of variance was used to compare differences.

Results: TP values varied among and within different shade designations and also among different brands of resin composites. TP values were significantly changed after immersion in PBS and ETE and were influenced by the brand of resin composites, but they were not influenced differently by the immersion solutions of PBS and ETE ($p = .05$).

CLINICAL SIGNIFICANCE

Translucency of dental resin composites is an important esthetic consideration. Based on the results of this study, the influence of salivary esterase on the changes in translucency of dental resin composites is not significantly different from that of phosphate-buffered saline. Therefore, it can be concluded that the enzymatic effects of saliva do not adversely alter the translucency of dental resin composites.

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Dental resin composites are not chemically inert at the material-biologic interface. Several studies have investigated the process of biodegradation of resin composites in the presence of salivary enzymes.[1–3] Since plaque-covered resin restorations have been reported to be susceptible to pronounced surface staining, they may also be susceptible to softening caused by the organic acids pro-

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duced in dental plaque.[4] Cholesterol esterase and pseudocholinesterase have been reported to degrade dental resin composites, and human saliva has been shown to contain esterase-like activity (human saliva–derived esterase activity [HSDEA]) similar to cholesterol esterase and pseudocholinesterase.[5] The microhardness of bisphenol A glycidyl methacrylate (BIS-GMA) polymers has decreased after treatment with porcine liver esterase.[1] The surface of BIS-GMA/triethylene glycol dimethacrylate (TEGDMA) polymers treated with porcine liver esterase in activities equivalent to those found in human saliva exhibited a reduced microhardness.[6]

Normally, light penetrates through the enamel into dentin before being reflected outward. This effect affords the lifelike esthetic vital characteristic of natural teeth.[7] Since natural enamel has inherent translucency, esthetic restorations should reproduce the translucency of teeth.[8,9] The thickness and surface texture of restorative materials can also influence the esthetics.[10] Translucency of esthetic restorative material has usually been determined with a translucency parameter (TP).[11] High variability in translucency changes after curing and subsequent water submersion has been observed in resin composites, with some products increasing and others decreasing the TP.[12] Therefore, the translucency of dental esthetic materials is an important esthetic parameter that can be changed by curing and aging.

Although the change of mechanical properties,[1] the amount of biodegradation products,[3] and color changes of resin composites by salivary enzymes have been evaluated previously,[13] the enzymatic influence on the translucency of resin composites has not yet been determined. The purpose of this study was to elucidate the influence of porcine liver esterase, which has similar activity to that of human salivary esterase,[5] on the translucency change of dental resin composites.

MATERIALS AND METHODS

Three to five shades of three brands of light-cured resin composites were studied; these are outlined in Table 1. A2 shade was included to compare between composites, and other shades were included to compare the difference by shade within each composite. The compositions presented for the resin composites are based on the manufacturers' brochures and Internet sites. The resin matrix of Filtek Supreme (FS) is composed of 5 to 15% bisphenol A polyethylene glycol diether dimethacrylate, 1 to 10% BIS-GMA, 5 to 15% urethane dimethacrylate, and < 5% TEGDMA. Point 4 (P4) contains 20 to 35% methacrylate ester monomers. Renew (RN) contains 15 to 40% ethoxylated bisphenol A dimethacrylate and 3 to 7% TEGDMA.

Resin composites were packed into a polytetrafluoroethylene mold (10 mm in diameter and 1.8 mm thick) on a cover glass. After packing the composites, another cover glass was pressed on the top of the specimens with a 5 kg load for 3 minutes to achieve a uniform thickness of the specimens. After removing the load, the specimens were light cured for 40 seconds per area (a total of 120 s) by dividing each specimen in three areas with a light-curing unit (Spectrum 800, Dentsply/Caulk, Milford, DE, USA) with an intensity setting of 400 mW/cm^2. The irradiated areas were overlapped, and light curing was performed on one side only. The output of the light was checked with a radiometer (Curing Radiometer, Demetron/Kerr, Orange, CA, USA). After curing, the specimens were polished with wet, 1,500-grit silicon carbide papers on both sides.

Thickness was measured at five points (the center and four points on the periphery) of each specimen with a micrometer (Mitutoyo, Tokyo, Japan), and a mean value was calculated. The mean thickness of polished specimens was 1.75 mm. Ten specimens were prepared for each shade of material. Of these, five specimens were immersed in 37°C, 0.01 M phosphate-buffered saline (PBS, Sigma, St. Louis, MO, USA), and the other five were immersed in 37°C, PBS containing 400 mU/mL porcine liver esterase (ETE, Sigma). All solutions were

TABLE 1. RESIN COMPOSITES USED IN THIS STUDY.

Material	Shade	Filler Content and Size*	Batch No.	Manufacturer
Filtek Supreme	YT WE A2 B2 D2	57.7–59.5 vol% (72.5–78.5 wt%) aggregated zirconia/silica cluster filler of 0.6–1.4 μm, and a nonaggregated 20 nm or 75 nm silica filler	3AF 2AC 2AA 2AA 3AB	3M ESPE, St. Paul, MN, USA
Point 4	T1 XL2 A2	59 vol% (77 wt%) inorganic filler of 0.04 μm	203822 203899 201C30	SDS/Kerr, Orange, CA, USA
Renew	A1 A2 A3 B1 C2	73 wt% glass filler of 0.7 μm, and 1–20% amorphous silica	0200005667 0200009126 0200011336 0100014457 0300002256	Bisco, Schaumburg, IL, USA

*The compositions are based on the manufacturers' brochures and Internet sites.

filtered with a 0.22 μm filter (Nalge Nunc, Rochester, NY, USA) before use for sterilization. One large batch of each solution was made, which was then used to refresh the individual specimen test vials. The solutions were changed daily throughout the experimental period to keep the activities of the enzyme and ions constant.

In the present study, the concentration of porcine liver esterase was adjusted to 10 times higher than that in normal saliva to reduce the experimental period, the efficiency of which was confirmed in a previous study performed in our laboratory in which a higher concentration of porcine liver esterase decreased the microhardness of the surfaces of dental resin composites significantly.[13] The effect of this enzyme dissolved in PBS on dental resin composites has been established in previous studies.[6,13,14]

Color was measured after immersion in distilled water for 1 day (baseline) and after immersion in PBS or ETE for 9 weeks. Three repeated measurements were made for each specimen. Measurements were made after blotting, according to the CIELAB color scale relative to the Commission Internationale de l'Eclairage (CIE) standard illuminant D65 over a white background (L* = 96.68, a* = –0.18, and b* = –0.22) and a black background (L* = 1.15, a* = –0.11, and b* = –0.50) on a reflection spectrophotometer (CM-3500d, Minolta, Osaka, Japan) with specular component excluded geometry.[15–17] L* represents the value of an object, a* is the measurement along the red-green axis, and b* is the measurement along the yellow-blue axis. Blotting was done by holding a tissue paper against the surface of a specimen for 1 second to maintain the surface condition similar to that of a clinical condition. The aperture diameter of the spectrophotometer measuring port was 8 mm. Illuminating and viewing conditions of this instrument were CIE diffuse/10° geometry. The software used was SpectraMagic (Version 1.01, Minolta, Osaka, Japan).

TP was obtained by calculating the color difference between the specimen on a white background and on a black background with the following equation:

$$TP = [(L_B^* - L_W^*)^2 + (a_B^* - a_W^*)^2 + (b_B^* - b_W^*)^2]^{\frac{1}{2}}$$

where the subscript *B* refers to the color coordinates over the black background and the subscript *W* refers to those over the white background.[11]

Two-factor, repeated-measures analysis of variance (ANOVA) was used to compare differences in immersion in PBS versus ETE among the resin composites tested with the independent variables of resin composite (regardless of shade) and immersion solution (SPSS 11.0, SPSS, Chicago, IL, USA; $p = .05$). Within each composite, the influence of shade and immersion solution on the TP values was also analyzed by two-factor, repeated-measures ANOVA.

RESULTS

TP values of specimens are presented in Table 2. The range of TP values at baseline was 14.3 to 38.5 in FS, 10.5 to 23.1 in P4, and 12.1 to 18.4 in RN. After the 9-week immersion in PBS or ETE, the range of mean TP values was 12.8 to 39.0 in FS, 9.9 to 22.2 in P4, and 12.3 to 17.7 in RN. TP values were significantly changed after immersion in PBS and ETE.

TP values were influenced by the brand of resin composites but were not influenced differently by the immersion solutions of PBS and ETE, and there was no significant interaction between resin composite and immersion solution based on two-factor, repeated-measures ANOVA ($p = .05$). Changes in TP were similar in both solutions, and there was no general trend in the changes of TP by the immersion solution. Mean TP values of 10 specimens of A2 shade (PBS and ETE) at baseline were 17.1 in FS, 18.4 in P4, and 15.3 in RN. Although the shade designation of the three resin composites was the same A2 shade, TP values varied among composites.

Within FS composite specimens, shade and immersion solution influenced the TP values significantly, and there was a significant interaction between shade and immersion solution based on two-factor ANOVA ($p = .05$). Within P4 composite specimens, shade influenced TP values significantly; immersion solution did not influence significantly, but there was a significant interaction between shade and immersion solution based on two-factor ANOVA ($p = .05$). Within RN composite specimens, shade and immersion solution influenced TP values significantly, and there was a significant interaction between shade and immersion solution based on two-factor ANOVA ($p = .05$).

TABLE 2. TRANSLUCENCY PARAMETER VALUES OF RESIN COMPOSITES.

Material	Shade	Solution*	Baseline TP (SD)	TP after 9 Weeks (SD)
Filtek Supreme	YT	PBS	38.5 (0.4)	39.0 (0.4)
		ETE	38.5 (0.5)	38.9 (0.9)
	WE	PBS	15.8 (0.3)	14.9 (0.3)
		ETE	16.1 (0.8)	15.3 (0.7)
	A2	PBS	17.2 (0.7)	16.0 (0.6)
		ETE	16.9 (0.7)	15.9 (0.7)
	B2	PBS	20.6 (0.4)	18.8 (0.7)
		ETE	22.1 (0.6)	19.9 (0.7)
	D2	PBS	14.3 (0.3)	12.8 (0.4)
		ETE	15.1 (0.8)	13.7 (1.0)
Point 4	T1	PBS	22.3 (1.5)	20.4 (1.5)
		ETE	23.1 (0.3)	22.2 (0.4)
	XL2	PBS	10.6 (0.6)	9.9 (0.7)
		ETE	10.5 (0.4)	10.1 (0.2)
	A2	PBS	18.7 (0.5)	17.4 (0.6)
		ETE	18.2 (0.5)	17.4 (0.3)
Renew	A1	PBS	18.0 (0.3)	17.3 (0.5)
		ETE	17.2 (0.3)	16.7 (0.3)
	A2	PBS	15.5 (1.3)	15.1 (1.5)
		ETE	14.9 (0.5)	14.6 (0.6)
	A3	PBS	15.8 (0.4)	15.4 (0.3)
		ETE	14.8 (0.4)	14.7 (0.4)
	B1	PBS	18.4 (0.7)	17.7 (0.8)
		ETE	17.9 (0.6)	17.3 (0.5)
	C2	PBS	12.9 (0.4)	12.9 (0.4)
		ETE	12.1 (0.3)	12.3 (0.3)

TP = translucency parameter.
*Immersion solution: PBS = 0.01 M phosphate-buffered saline; ETE = 400 mU/mL porcine liver esterase added to PBS.

DISCUSSION

There have been studies on the effects of salivary enzymes on the properties of resin composites. Enzymes in human saliva have been reported to be able to influence the esthetic parameters such as shade and translucency of resin composites.[18] Since TEGDMA and BIS-GMA oligomers were completely hydrolyzed by human saliva–derived esterase within a 25-hour incubation, it was concluded that saliva contained esterase activity that could readily catalyze the biodegradation of commercial resin composites.[5] Enzymes in human saliva were capable of softening the surface of dimethacrylate polymers presumably by inducing a hydrolysis of methacrylate ester bonds.[6] The mean activity of esterase in human saliva was found to correspond to about 40 mU of porcine liver esterase per milliliter, and porcine liver esterase catalyzed the hydrolysis of several acrylates. Esterase gave rise to the liberation of methacrylic acid from BIS-GMA/TEGDMA polymers, and it was estimated that a TEGDMA polymer would be hydrolyzed faster than a BIS-GMA polymer.[2]

TP values for the bleached shades of resin composites varied from 2.0 to 7.1.[19] In the present study, the difference in translucency by the brand of composites may be due to the difference in the resin matrix and/or the size, amount, and distribution of fillers. Although the primary particle sizes were 5 to 20 nm and 75 nm for FS, 40 nm for P4, and 700 nm for RN, the mean TP values of A2 shade at baseline were 17.1 for FS, 18.4 for P4, and 15.3 for RN. These values changed to 16.0 for FS, 17.4 for P4, and 14.9 for RN after the 9-week immersion. Therefore, there was only a small effect on TP values by filler size, although filler sizes were different by more than 10 times. Rather TP values varied by the shade of resin composite specimens within each brand of composite. Particle size may influence the absorption and scattering of light.[20] When the filler particle size is smaller than the wavelength of visible light (400–700 nm), refraction of light within the composite is different from that within large-particle composites. However, the exact mechanism of light refraction within dental resin composites is still unclear. TP values of A2 shade resin composites FS, P4, and RN were 16.9 to 17.2, 18.2 to 18.7, and 14.9 to 15.5, respectively. These values might reflect differences in filler size and filler type, such as zirconia/silica filler, glass filler, and amorphous silica. Further study on the influence of the differences in refractive index between the resin matrices and fillers of resin composites should be performed.

In the present study, the effect of porcine liver esterase on the translucency change was not significant based on two-factor ANOVA. Since esterase molecules are large compared with the polymer network, it seems that the only reactions of ETE that occurred were surface diffusion and degradation. Therefore, there might be no significant difference of the translucency compared with that in PBS. However, if the specimens were stored in a staining solution after treatment with ETE, the esterase might be able to generate active polymer sites that could bind with stains. Such reactions could have major effects on esthetics. Further study on this subject is needed.

The influence of dental materials on the activity of esterase isolated from human saliva has been studied. Eugenol has been shown to be a strong competitive inhibitor of the esterase, and eugenol-containing materials, some resin composites, and some dental amalgams were in vitro inhibitors of the esterases.[21] Thus, it was necessary to determine the stability of HSDEA in the presence of resin composite to ascertain that sufficient enzyme activity levels were maintained throughout the course of the experiment.[5] Since the porcine liver esterase dissolved in PBS (ETE) significantly degraded the resin composites and reduced their surface hardness,[1,6,13,14] the efficiency of ETE solution was previously confirmed.

In the present study, TP values were calculated to evaluate the translucency of resin composites. Although direct measurement of light trans-

mittance could be adopted to evaluate translucency of dental composite materials,[8,9] this method requires a more complicated apparatus. TP values correspond directly to the common visual assessment of translucency.[11,12,22] In the present study, TP values of A2-designated composites were significantly different among the brands of composite; also, TP values were significantly different among the shades within the same brand of composite. After immersion in solutions, TP values generally decreased for FS and P4; however, TP values were not changed for RN.

Translucency of resin composites can be affected by the degree of polymerization. The reduction in translucency was caused by the increased polymerization of the resin matrix and corresponding change of the refractive index of the resin matrix. Increased polymerization produced a greater difference in refractive indices between the resin matrix and the inorganic filler.[22] For all the shades of RN composite, decreases in translucency were not significant after immersion in PBS or ETE. This result might suggest that this composite was fully cured before immersion, or that the hydrophobicity of resin matrix limited the diffusion of solutions into materials. The influence of porcine liver esterase on the changes of translucency was not different from that of PBS in the present study.

It has been reported that various resin composites have different levels of water sorption depending on the type of monomer.[23] Therefore, water sorption might lead to differences in translucency changes among the composites. In the present study, the changes of TP values after immersion in porcine liver esterase were not different from those in PBS, based on ANOVA ($p < .05$). This result might have two explanations. First, the enzymatic action was limited to the surface; therefore, the effect of enzyme on TP change was negligible. Second, general water sorption rather than enzymatic action was the cause for the change in TP.

Changes of translucency of resin composites that occurred after light exposure with and without water storage were previously evaluated. The change of translucency in water was found to be significantly higher than that in dry storage.[24] These results may account for the lack of changes in TP values after immersion in PBS and ETE.

With regard to the translucency of human enamel, the translucency of wet enamel was higher than that of dehydrated enamel.[25] To minimize the effect of water content on the optical properties in the present study, color was measured immediately after blot drying the specimens that had been immersed in distilled water for 24 hours.

From this study, although TP values varied among and within different shade designations and among different brands of composites, the changes in TP values were similar for PBS and ETE solutions. The influence of salivary esterase (ETE, porcine liver esterase, 400 mU/mL) on the changes in translucency of dental resin composites was not significantly different from that of phosphate-buffered saline.

DISCLOSURE

The authors do not have any financial interest in the companies whose materials are discussed in this article.

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COMMENTARY

CHANGES IN TRANSLUCENCY OF RESIN COMPOSITES AFTER STORAGE IN SALIVARY ESTERASE

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The authors have tackled an interesting research issue, that of long-term degradation of dental composites caused by salivary esterase interactions. There are two key points to emphasize in considering this issue: (1) What are all possible events that may be involved with resin composite changes over time? (2) What is the relative importance of the salivary esterase contribution to those changes?

As with any restorative material, there are quite a few possible intraoral events that can occur along the restoration surfaces. It is convenient to categorize these events in terms of physical, mechanical, chemical, and biologic ones,[1] with salivary esterase effects being considered chemical events. Imagine the restorative surface as schematically portrayed in Figure 1. Although interactions may occur throughout the "bulk restorative material," most likely the external surfaces will be more affected. These surfaces (or interfaces) involve the passage of materials from the outside to the inside and from the inside to the outside. Under most circumstances, surfaces are not clean. They are coated with saliva, other intraoral fluids, absorbed materials (such as stains), and acquired coatings (such as biofilms).

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