Original Article

The effect of saliva substitute on the color stability of three different nanocomposite restorative materials after 1 month: An *in vitro* study

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Abstract

Context (Background): The color stability of an esthetic material like composite resin may be hampered, due to the constant presence of oral microflora, saliva, and the frequent intake of food. However, as the oral cavity has a dynamic environment, the color stability of these restorative materials has been a challenge to dentistry.

Aim: The aim of this study was to evaluate the effect of a saliva substitute (SS) on the color stability of three different nanocomposite restorative materials.

Materials and Methods: A total of 66 disc-shaped samples of test materials were prepared using a polycarbonate mold measuring 15 mm diameter and 2 mm height and were randomly assigned to three groups of 22 samples each based on the test material used: Group I – Estelite Sigma Quick, Group II – Solare sculpt, and Group III – Beautifil II LS. They were further divided into two subgroups A and B with 11 samples each based on the immersion solutions, namely distilled water (DW) and saliva substitute (SS), respectively. The samples were immersed for 30 days. Baseline and post immersion color analysis were done with a spectrophotometer. The CIE L*a*b* values were measured and CIE ΔE values obtained were tabulated. Data were analyzed using the two-way-ANOVA test and Tukey's multiple *post hoc* test.

Results: The mean color difference (ΔE) among the three different nanocomposite restorative materials ranged from 4.0 ± 0.26 to 10.62 ± 1.92. Group I showed the lowest color change (7.80 ± 0.55 and 4.00 ± 0.26), followed by Group III (8.59 ± 0.29 and 6.24 ± 0.66) and Group II (10.62 ± 1.92 and 6.85 ± 0.46) when immersed in SS and DW, respectively.

Conclusion: All the specimens when immersed in SS showed greater discoloration than compared to the specimens immersed in DW. Group I showed greater resistance to color change compared to other groups.

Keywords: Color stability; distilled water; nanocomposites; saliva substitute; spectrophotometer

INTRODUCTION

Saliva plays an important role in protecting and moistening the oral hard and soft tissues. Decreased salivation promotes severe oral side effects such as difficulty in phonetics, deglutition, eating, reduced antibacterial activity, lack of remineralization, reduced buffer capacity, and rampant caries of teeth.[1,2] Xerostomia is derived from a Greek word, meaning "xeros" (dry) and stoma (mouth) which is usually used to indicate the condition of not having enough saliva to keep the mouth moist. The more accurate term for measurable and objectifiable changes in reduced salivary function are "salivary gland hypofunction" for a reduction in saliva output and "salivary gland dysfunction"

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Date of submission : 31.08.2020
Review completed : 21.10.2020
Date of acceptance : 29.01.2021
Published : 05.07.2021

Access this article online
Quick Response Code:
Website: www.jcd.org.in
DOI: 10.4103/JCD.JCD_444_20

How to cite this article: Rai AV, Naik BD. The effect of saliva substitute on the color stability of three different nanocomposite restorative materials after 1 month: An *in vitro* study. J Conserv Dent 2021;24:50-6.

for a more general alteration in physiologic salivary gland function.[3-5]

Several conditions that can lead to hyposalivation are Sjogren's syndrome, psychological problems (stress and anxiety), and chemotherapy or radiation therapy for head-and-neck carcinomas.[6-9] A large number of medications such as tricyclic antidepressants, antipsychotics, benzodiazepines, atropinics, beta-blockers, antihistaminics, H^2-receptor antagonists, diuretics and angiotensin-converting enzyme inhibitors, anti-HIV protease inhibitors, and omeprazole cause dry mouth as an adverse effect.[8,9]

The restoration of carious teeth in patients with xerostomia, especially for those who have undergone cervicofacial radiotherapy, will be very challenging and adhesive restorative materials are often recommended.[10,11] Advancements in restorative resins and bonding science have resulted in the development of resin-based composite materials which are the most commonly used anterior esthetic restorative materials in contemporary dentistry. Any esthetic restorative material must simulate the natural tooth in their color, translucency, and texture. These materials must maintain color stability for longer periods of time.[12] The degree of color change can be influenced by a number of factors such as incomplete polymerization, dimension of filler particles, resin matrix, water sorption, chemical reactivity, diet, oral hygiene, and surface smoothness of the restoration. The introduction of nanocomposites has displayed improved properties comparable or superior to that of microfill and microhybrid composite resins.[13]

Patients suffering from xerostomia are often prescribed with saliva substitutes which are applied by them frequently in order to lubricate the tissues.[14] Due to their constant contact, they may affect the physical, mechanical, and biological integrity of restorative materials. However, there are no documented studies evaluating the effect of saliva substitute on the composite restorative materials. The purpose of the study was to evaluate the effect of a saliva substitute on the color stability of three different nanocomposite restorative materials. This study was begun by considering the null hypothesis stating that there is no significant difference between any groups and subgroups.

MATERIALS AND METHODS

Sample preparation

Sixty-six disc-shaped samples, 22 of each material approximating the same shade (A2), were prepared using polycarbonate molds of 15 mm internal diameter and 2 mm height. The specimens were made by placing the composite restorative materials into the mold over a glass slab sandwiched between two Mylar strips using the Teflon-coated plastic filling instrument; the smoothest surfaces were obtained by curing the materials against the Mylar strip. The excess composite material was removed and the samples were light cured according to the manufacturer's instructions. The tip of the light source was placed in contact with the plastic base and cover, which had the same thickness. Therefore, the distance between the light source and the specimen was standardized. All composite discs were retrieved from the molds, then visually inspected for surface defects and those with visible flaws were discarded. The defects might be encountered from inadequate material or inadequate packing force. Later, the samples were stored at 37°C in an incubator within the lightproof containers for 24 h to achieve rehydration. After rehydration, the samples were rinsed and dried with filter paper, and the baseline color measurements were performed using a spectrophotometer (Datacolour, Lawrenceville, New Jersey, USA).

Immersion in test solutions

The samples were randomly assigned to three groups of 22 samples each

- Group I – Estelite Sigma Quick (Tokuyama, Japan)
- Group II – Solare sculpt (GC, USA)
- Group III – Beautifil LS II (Shofu, India).

Each group was further randomly divided into two subgroups A and B of 11 samples each. The samples of subgroups IA, IIA, and IIIA were immersed in distilled water (DW); subgroups IB, IIB, and IIIB were immersed in saliva substitute, [Wet Mouth, ICPA, India] for 30 days. The solutions were changed every day.

Color analysis

Baseline color analysis was done before immersing the samples in the test solutions with a spectrophotometer (Datacolour, Lawrenceville, New Jersey, USA) utilizing a small-area view with ultraviolet value at 0 and mode set to reflectance. Single-type measurement was made with a small aperture of 9 mm. The sample was placed in the port, the door was closed, and CIE $L^*a^*b^*$ values were obtained. Later, the samples of Group IA, IIA, and IIIA were immersed in DW; samples of Group IB, IIB, and IIIB were immersed in SS. All the samples were stored at 37°C and 100% humidity in an incubator for 30 days. After the immersion period, the samples were removed, washed with DW, and wiped gently with filter paper for further spectrophotometric analysis. The CIE $L^*a^*b^*$ values were measured as mentioned previously. Three consecutive readings were taken from each specimen. The color difference ΔE was calculated from the mean ΔL^*, Δa^*, and Δb^* values for each specimen using the following formula:[12]

$$\Delta E\,(L^*a^*b^*) = ([\Delta L^*]^2 + [\Delta a^*]^2 + [\Delta b^*]^2)^{1/2}$$

The CIE ΔE values obtained were tabulated.

Table 1: Summary of color change (ΔE) in three main groups (1, 2, and 3) and two subgroups (A and B)

Groups with subgroups	*n*	Mean±SD	SE
Group 1 with subgroup A	11	4.00±0.26	0.08
Group 1 with subgroup B	11	7.80±0.55	0.16
Group 2 with subgroup A	11	6.85±0.46	0.14
Group 2 with subgroup B	11	10.62±1.92	0.58
Group 3 with subgroup A	11	6.24±0.66	0.20
Group 3 with subgroup B	11	8.59±0.29	0.09

SD: Standard deviation, SE: Standard error

Table 2: Comparison of three main groups (1, 2, and 3) and two subgroups (A and B) with mean color change (ΔE) by two-way ANOVA

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	*F*	*P*
Main effects					
Groups	2	88.70	44.35	55.7136	0.0001*
Subgroups	1	180.70	180.70	227.0030	0.0001*
Two-way interaction effects					
Groups×subgroups	2	7.54	3.77	4.7343	0.0123**
Error	60	47.76	0.80		
Total	65	324.69			

*$P<0.05$

RESULTS

The mean values and standard deviations of color changes are given in Table 1. The color change exhibited by all the three groups was significantly different after 30 days. The mean color change in subgroup B was significantly greater than the mean color change in subgroup A. Also, the mean color change in Group I was significantly less compared to the mean color change in Group III and Group II. When the color change was compared between the groups and subgroups using a two-way ANOVA test; it was found that there were statistically significant differences with $P < 0.05$ [Table 2]. For further analysis, when Tukey's multiple *post hoc* test was applied between three groups and two subgroups, it was found that there were statistically significant differences between Group I Subgroup A with Group I Subgroup B, Group II Subgroup A and B, Group III Subgroup A and B; Group II Subgroup B with Group I Subgroup B and Group II Subgroup A; Group III Subgroup A with Group I Subgroup B and Group II Subgroup B; Group III Subgroup B with Group II Subgroup A and B and Group III Subgroup A [Table 3].

The mean values of ΔL^*, Δa^*, and Δb^* for all the groups and subgroups are given in Table 4. When a two-way ANOVA test was applied to compare between the three main groups and two subgroups, the mean values between the groups and subgroups and their interactions were found to be significantly linking with each other.

ΔL^* (brightness) values

There was a statistically significant difference between groups, subgroups, and their interactions with $P < 0.05$. Positive ΔL^* indicates that the specimens became lighter, whereas negative ΔL^* indicates that the specimens became darker. All the groups and subgroups showed positive ΔL^* values. Their F value between groups was 67.351, between subgroups was 165.5744, and between interactions was 8.0388.

Δa^* (change along red-green axis) values

There was no statistically significant difference between groups, subgroups, and their interactions. Positive Δa^* indicates a shift toward red color, whereas negative Δa^* indicates a shift toward green color. All the groups and subgroups showed negative Δa^* values. Their *F* value between groups was 0.3920, between subgroups was 0.2364, and between interactions was 0.0493.

Δb^* (change along yellow-blue axis) values

There was a statistically significant difference between subgroups with $P < 0.05$. Positive Δb^* indicates shift toward yellow color, while negative Δb^* indicates shift toward blue color. The subgroup B of all the groups showed negative Δb^* values, whereas subgroup A of all the groups showed positive Δb^* values. Their *F* value between groups was 0.8565, between subgroups was 4.6584, and between interactions was 0.70483.

DISCUSSION

Increasing esthetic demands from patients has resulted in an increasingly widespread use of resin-based composites in dental practice. The physical, mechanical, esthetic properties and clinical behavior of composite resins depend on their structure.[15] Despite its clinical acceptance in satisfying esthetic demands, previous studies have revealed that composite resins are susceptible to various degrees of discoloration which may be attributed to either intrinsic or extrinsic factors. Intrinsic factors involve alterations or changes in the chemical structure of the composite resins under physical and chemical conditions, whereas extrinsic factors are mainly due to the surface staining from absorption or adsorption of exogenous substances. Rough composite surfaces cause plaque accumulation, which may lead to gingival and periodontal inflammation.[16] Moreover, it increases the scattering of light striking on the restorative materials which impairs its esthetic appearance.[17] It is also reported that matrix, filler composition and content, minor pigment addition, initiation components, and filler coupling agents affect the color of esthetic restorative materials.[16]

In this study, the color change between the three different nanocomposites when immersed in SS and DW

Table 3: Pairwise comparison of interactions of three main Groups (1, 2, and 3) and two subgroups (A and B) with mean color change (ΔE) by Tukey's multiple *post hoc* procedures

Groups with subgroups	Group 1 with subgroup A	Group 1 with subgroup B	Group 2 with subgroup A	Group 2 with subgroup B	Group 3 with subgroup A	Group 3 with subgroup B
Mean	4.00	7.80	6.85	10.62	6.24	8.59
SD	0.26	0.55	0.46	1.92	0.66	0.29
Group 1 with subgroup A	-					
Group 1 with subgroup B (*P*)	0.0001*	-				
Group 2 with subgroup A (*P*)	0.0001*	0.1416	-			
Group 2 with subgroup B (*P*)	0.0001*	0.0001*	0.0001*	-		
Group 3 with subgroup A (*P*)	0.0001*	0.0018*	0.5956	0.0001*	-	
Group 3 with subgroup B (*P*)	0.0001*	0.3113	0.0005*	0.0001*	0.0001*	-

*$P<0.05$. SD: Standard deviation

Table 4: Comparison of three main groups (1, 2, and 3) and two subgroups (A and B) with mean ΔL^*, Δa^*, Δb^* by two-way ANOVA

Groups with subgroups	Summary	Mean±SD		
		ΔL^*	Δa^*	Δb^*
Group 1 with subgroup A		2.38±1.28	−0.88±1.94	0.00±0.72
Group 1 with subgroup B		6.67±0.26	−1.34±3.02	−0.36±2.45
Group 2 with subgroup A		6.32±0.54	−0.53±2.09	0.03±1.12
Group 2 with subgroup B		9.99±1.98	−0.91±3.59	−1.23±2.30
Group 3 with subgroup A		5.40±0.21	−0.45±1.72	1.27±2.68
Group 3 with subgroup B		7.28±0.69	−0.48±1.75	−0.70±3.20
Between groups	*F*	67.3519	0.3920	0.8565
	P	0.0001*	0.6774	0.4298
Between subgroups	*F*	165.5744	0.2364	4.6584
	P	0.0001*	0.6286	0.0349*
Between interactions	*F*	8.0388	0.0493	0.70483
	P	0.0008*	0.9519	0.49824

*$P<0.05$. SD: Standard deviation

was evaluated at the baseline and after 1 month. The null hypothesis of the present study was rejected since the analysis of the results showed that the immersion of specimens in SS caused color change, which varied according to the three different nanocomposite restorative materials used. ΔE^* values ranged between the lowest 4.0 ± 0.26 and the highest 10.62 ± 1.92. Group I showed the lowest color change (7.80 ± 0.55 and 4.00 ± 0.26), followed by Group III (8.59 ± 0.29 and 6.24 ± 0.66) and Group II (10.62 ± 1.92 and 6.85 ± 0.46) when immersed in SS and DW, respectively.

To evaluate discoloration, visual or instrumental techniques can be used. The color evaluation by visual comparison may not be a reliable method as there might be inconsistencies inherent in color perception and specifications among observers.[12] The use of instrumental methods to quantify tooth colors, such as spectrophotometers, colorimeters, and digital image analysis could potentially eliminate the subjective aspects of color assessment[18] and hence has been reported to be a reliable technique in dental material studies.[12] The American Dental Association recommends the use of the CIE $L^*a^*b^*$ color differential system in assessing the chromatic differences, as it characterizes the color based on human perception.[19]

In the present study, the color evaluations were carried out using a spectrophotometer in reflectance mode according to the International Commission on Illumination (usually abbreviated as CIE for its French name, Commission Internationale de l'éclairage), where color was expressed by the CIE $L^*a^*b^*$ lab system. It designates color according to 3 spatial coordinates, L^*, a^*, b^*, where L^* represents the brightness (value) of a shade, a^* represents the amount of red-green color, and b^* represents the amount of yellow-blue color. L^* coordinates are located along a vertical axis that ranges from a value of 0 (dark) to 100 (light). The a^* and b^* coordinates revolve on axes around L^*. Absolute measurements are made in L^* a^*b^* coordinate and color changes are calculated as ΔE.[12]

In principle, if a material is completely color stable, no color difference will be detected after its exposure to the testing environment ($\Delta E = 0$). The human eye cannot detect ΔE values of <1.5, although this value is measurable with the help of a spectrophotometer. Based on the previous reports, ΔE values equal to or greater than 3.3 were considered as clinically perceptible in the present study.[12]

To minimize the edge loss effect, the diameter of the specimens prepared in the present study was 15 mm, greater than the aperture size of the instrument (3 mm × 8 mm). According to the manufacturer, the thickness of composite discs should be limited to 2 mm as it is the accepted thickness for the incremental technique of composite application. A matrix strip was used in this study to mimic the clinical procedures for restoring anterior teeth, as it provides the smoothest surface when compared to other finishing and polishing procedures. Moreover, finishing and polishing procedures cannot be standardized for all composite discs with regard to the direction, force, and time per stroke.[20]

An increase in the stiffness of enamel and dentine near the DEJ will be observed postradiotherapy. This increased stiffness is hypothesized due to a radiation-induced decrease in the protein content, with a much greater reduction in the enamel as compared to dentin. These changes in mechanical properties and chemical composition can contribute to DEJ biomechanical failure and enamel delamination that occurs postradiotherapy.[21] A clinical study on xerostomia patients with radiation caries has shown that resin composite and resin-modified GIC (RMGIC) have significantly better scores for marginal adaptation and structural integrity over the long term in patients who were amenable to fluoride application.[10] In these cases, resin composites could be placed, making the best use of their esthetic properties, adhesive potential, sealing ability, longer clinical retention, and better wear resistance.

To minimize oral discomfort, patients are routinely prescribed with salivary substitutes or oral mucosal lubricants when they do not respond to pharmacological, gustatory, or masticatory stimulation.[22] Salivary substitutes can be based on different substances such as animal mucin, carboxymethyl-cellulose (CMC), xanthan gum, and aloe vera.[23] They can be categorized into solutions based on CMC, mucin, and linseed. Most commercially available salivary substitute is based on CMC. It is a polymer derived from natural cellulose and used in the salivary substitute formulation as a thickening agent. CMC produces crystal clear gel products and thickens the aqueous phase as well as renders water retention and film formation.[24]

These formulations are applied on the oral tissues for various time intervals depending on the extent of discomfort. Since these restorative materials will be in constant contact with salivary substitutes, it may have deleterious effects on the restorative materials. One of the properties evaluated here is the color stability of various nanocomposite restorative materials. Salivary substitutes may cause discoloration of these restorative materials on prolonged usage. In this study, a CMC-based salivary substitute was selected. This interval was chosen assuming that salivary substitute may be in contact with oral tissues and restorations for approximately 12 h to maintain the wetness. Hence, immersion for 30 days consisting of 720 h would correspond to 60 days of intraoral application.

The introduction of nanotechnology enabled the production of functional materials and structures in the size ranging from 0.1 to 100 nm using various chemical and physical processes. Nanotechnology revolutionized the field of dentistry through the development of resin materials with more encouraging mechanical properties.[25,26] Based on the concept of nanotechnology, a class of composites referred to as nanocomposites were developed and marketed.[27] Nanocomposites combine the good mechanical strength of hybrids and the excellent polishing ability of microfills.[26] In addition, nanocomposites demonstrate high wear resistance, improved optical characteristics, reduced water sorption, and reduced polymerization shrinkage.[26,27]

In this study, Estelite Sigma Quick showed the least discoloration on immersion in SS and DW. This might be because of the presence of 0.2 µm supranano monodispersing spherical filler (Si-Zr). These fillers easily produce extremely high surface gloss. Filler particle diameters are relatively uniform, and the particle size can be controlled by filler synthesis reaction times. With the Radical Amplified Photo-polymerization (RAP) technology, restorative resins require only 10 s of light exposure for complete polymerization.

Beautiful II LS shows less color change when compared to Solare sculpt. This might be because of the presence of bioactive Giomer technology. It is a universal hybrid composite and attributes release/recharge of fluoride, strontium, and four other beneficial ions. It is reported that Giomer consists of additional discrete nanofillers (10–20 nm), which makes it possible to incorporate larger filler content of 68.6 vol%. They also contain multifunctional prepolymerized filler complex with unique proprietary silanol resin formulation that imparts remarkable mechanical properties, higher wear resistance, exceptional and lasting surface luster. This was in agreement with a previous study by Hotwani *et al*.,[16] who demonstrated that Giomer material showed greater resistance to staining as compared to RMGIC at all time periods for 4 weeks.

Solare sculpt has unique, homogeneous, prepolymerized nanofillers with high density and uniform dispersion silane treatment technology. Three-hundred nanometer strontium glass fillers are homogeneously dispersed for high flexural strength and wear resistance. The least color resistance of the Solare sculpt might be due to its increased resin content and high porosity of these restorative materials.[28] Thus, there is an increase in water sorption in the case of GC Solare Sculpt. The result of the present study is in accordance with the previous study by Manohar and Jeevanandan,[29] who demonstrated that Solare sculpt showed maximum color change in all the tested children's beverages.

In previous studies, the excellent color stability and surface luster of the nanofilled and nanohybrid composite resins were mainly reported.[25,30,31] Reddy *et al*. reported that nanofilled composite resin showed less color change than microhybrid and hybrid composite resins.[32] Nasim *et al*. reported that this finding can be expected, because nanocomposites with smaller particle sizes will have a smoother surface and will retain less surface stains.[12] Reis *et al*. reported that high filler content in resin composites can decrease the monomer content and thus enhance color stability, whereas higher resin volume is reported to cause greater discoloration.[33]

A study by Topcu *et al*. found that nanocomposites showed the least discoloration than compared to microhybrid and hybrid composite materials.[34] A study by Yu *et al*. found that bleached nanocomposite restorative materials exhibited best color stability than compared to RMGIC.[35] Another study by Rao YM *et al*. found that nanofilled composite was found to be highly color stable than compared to microfilled composites and GIC.[36]

It has been reported that composite restorative resins can absorb water and other fluids with pigments, resulting in staining. It is expected that water acts as a medium for the penetration of stains into the resin matrix. It is reported that stain sorption is closely related to water sorption, which is caused by a high resin content and lack of coupling agent. Mainly, water sorption occurs by the direct absorption into the resin matrix and further diffuses into the microvoids within the polymer. Second, water absorbs on the filler surface, as the inorganic particles are incapable of absorbing water molecules, thereby accumulating at the filler–matrix interface. Water sorption hydrolyses the chemical bond between the resin matrix and filler particle causing filler dislodgment and microcrack formation. The loss of fillers leads to a rough surface that is easily eroded. The microcracks allow for further water diffusion, which acts as a vehicle for stain penetration, hence causing discoloration.[37]

In the present study, composite discs were in contact with the undiluted SS. However, in the oral cavity, saliva dilutes and buffers all oral liquids. Further studies on this topic could mix the SS with a medium that would represent saliva. All specimens were stored in the staining solution at 37°C. Although this is appropriate for a controlled laboratory situation, it does not replicate oral conditions. Thermocycling could be incorporated to simulate the oral environment.

CONCLUSION

Under the limitations of the present study, it was observed that SS affected the color stability of the tested nanocomposite restorative materials and also their color changes were above the clinically perceptible range ($\Delta E > 3.3$). However, Group I (Estelite Sigma Quick, Tokuyama) showed the greatest resistance to color changes. Therefore, in clinical practice, patients should be aware of the staining effects of the SS, while practitioners also should take into consideration the staining susceptibility of the resin composites by paying special attention to achieve a perfect surface finish by polishing.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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