

Could Different Formulations of Grape Seed (*Vitis vinifera*) Influence the Physical Properties of Conventional Glass Ionomer Cement?

Abstract

Objective: The aim is to evaluate the ability of different formulations of grape seed (GS) to influence the physical properties of conventional glass ionomer cement (GIC). **Materials and Methods:** Five groups were considered; Group I: Unmodified GIC (control), II: 3% v/v GS oil-modified GIC, III: 5% v/v GS oil-modified GIC, IV: 3% v/v ethanolic extract of GS (EEGS)-modified GIC and V: 5% v/v EEGS-modified GIC. Assessment parameters were; compressive strength, shear bond strength, surface roughness, water sorption and solubility and color difference. A representative specimen of each group was used for being analyzed by the Fourier transformation infrared spectroscopy. Analysis of variance was used to compare the results, followed by a Tukey *post hoc* test ($P < 0.05$).

Results: 3% v/v GS oil-modified GIC only exhibited a significant increase in its compressive strength and shear bond strength. Concurrently, there was a significant decrease in surface roughness, water sorption and solubility for 3% v/v GS oil-modified GIC group ($P < 0.05$). The least color change was for 3% v/v GS oil-modified GIC, which is a clinically acceptable change. **Conclusions:** 3% v/v GS oil-modified conventional GIC is an optimistic formulation of a restorative material with enhanced physical properties and agreeable esthetic.

Keywords: Chemical structure, color stability, compressive strength, glass ionomer cement, grape seed, shear bond strength, surface roughness, water sorption and solubility

Introduction

Glass ionomer cements (GICs) have many applications in the oral cavity.^[1] This is due to their favorable characteristic properties like chemical adhesion to enamel and dentin,^[2] fluoride release, coefficient of thermal expansion and modulus of elasticity comparable to dentin^[3] and biocompatibility.^[4] Even though conventional GICs possess certain restrictions such as susceptibility to dehydration,^[5] high solubility, slow setting rate,^[6] low flexural strength, low fracture toughness,^[7] low wear resistance, brittleness, and water sensitivity.^[8]

In a trial to increase GICs mechanical properties, metallic powders had been incorporated to reinforce GIC powder, but these products negatively influenced esthetics and bonding to enamel.^[9] Moreover, resin-reinforced GIC has remarkably higher flexural strength^[10] but lower compressive^[11] as well as dental pulp irritation in comparison to conventional GIC.^[12]

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Grape seed (GS) is a natural material derived from *Vitis vinifera* with many biomedical and dental applications. It possesses antioxidant, antimicrobial, and anti-inflammatory characteristics.^[13] It is able to strengthen the collagenous tissues by developing crosslinks, thus decreasing dentine degradation. Furthermore, it enhances collagen synthesis.^[13]

In addition, the bleached enamel treated with GS extract acquires significantly higher bond strength when compared to other scavenging agents as sodium ascorbate.^[14] GS extract can potentially remineralize tooth surfaces through the deposition of minerals.^[15] Recently, GS extract has been suggested to develop the antifungal activity of acrylic soft liners and enhance its adhesion to denture base material.^[16]

Based on the promising criteria of GS, this investigation was conducted to assess its influence in either oil or ethanolic extract forms on the chemical structure and physical properties of conventional GIC. The null hypothesis was that the GS does not have an impact on conventional GIC characteristics.

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Materials and Methods

Material preparation and experimental design

A conventional glass ionomer restorative material (GC Corporation, Tokyo, Japan) has been used in the study. GS powder (Nutra Manufacturing, Greenville, South Carolina, USA) was employed to prepare an ethanolic extract of GS (EEGS) of 10% concentration. This formulation, besides GS oil (NOW foods, Glen Ellyn Rd., Bloomingdale, IL 60108, USA) were blended separately with the liquid of GIC in ratios of 3 and 5% (v/v) and kept on magnetic stirrer for 24 h. The resulting five groups were as follows; Group I: Unmodified GIC (control), Group II: 3% (v/v) GS oil-modified GIC, Group III: 5% (v/v) GS oil-modified GIC, Group IV: 5% (v/v) EEGS-modified GIC, Group V: 5% (v/v) EEGS-modified GIC. The assigned groups were experimented for compressive strength, shear bond strength, surface roughness, color difference, and water sorption and solubility tests. Fourier transformation infrared spectroscopy (FTIR) was used for chemical structure analysis. A total number of 150 specimens were prepared for evaluation of the previously mentioned parameters; 25 specimens for each test; 5 specimens for each group.

Chemical structure characterization

FTIR analysis using a spectrometer (Nicolet iS10, America) was applied to inspect the chemical structure and the formation of intermolecular bonds. Specimens of each group were ground into fine powder, blended with KBr and compressed into a clear homogenous disc employed to detect chemical groups. The FTIR data were recorded in the range of 500-4000 cm^{-1} .

Estimation of compressive strength

Twenty-five cylindrical-shaped specimens of 6 mm length and 4 mm diameter were prepared in a split Teflon mold. Compressive strength was evaluated using a Universal Testing Machine (Model 3345, Instron Corporation, Canton, MA, USA). A compressive load was applied at a crosshead speed of 1 mm/min until the material fractured. Compressive strength (MPa) was calculated through the following equation:^[17]

$$C_s = 4P/\pi D^2$$

where P is the maximum applied load at fracture (N) and D is the diameter of the specimen (mm).

Estimation of shear bond strength

Twenty-five extracted human third molar teeth (surgically removed due to impaction) were collected from the out-patient clinic at the Faculty of Dentistry, Mansoura University, Egypt. Teeth were thoroughly washed under distilled water, scaled using a sharp hand sickle scaler and stored in a solution of 1% chloramine-T at 4°C to inhibit microbial growth. Teeth occlusal surfaces were removed below the dentino-enamel junction using diamond

bur to expose a flat dentin surface. Smoothing of the flat dentinal surface was achieved with silicon carbide paper and their roots were mounted in self-cured acrylic resin blocks. Conditioning of the exposed dentin surface with polyacrylic acid for 20 s and bonding of GIC to the conditioned dentin surface was performed using split Teflon mold 4 mm × 4 mm. GIC powder was mixed with the different liquid formulations separately according to the manufacturer recommendations. The specimens were stored in distilled water at room temperature for 48 h.

The shear bond strength was determined using the Universal Testing Machine (Model 3345, Instron Corporation, Canton, MA, USA) at a crosshead speed of 1 mm/min with the shearing load directed on the bonding interface, and the shear bond strength (MPa) was calculated as follows:^[18]

$$\tau = P/\pi r^2$$

where; τ is shear bond strength, P is the load at failure (N), and r is the radius of the specimen (mm).

Analysis of surface roughness

A sectional Teflon mold (8 mm diameter × 2 mm thickness) was utilized to prepare disc-shaped specimens for surface roughness (Ra) evaluation. The surface roughness of each specimen was determined using a surface profilometer (Mitutoyo Surf Test SJ 210 Analyzer; Mitutoyo Corp, Japan). The diamond stylus was moved crossway the specimen surface at a speed of 0.5 mm/s and a tracing length of 8 mm. This procedure was repeated at five different locations of each specimen surface and the average value was considered to be the mean roughness value (μm).

Estimation of water sorption and solubility

Specimens were prepared in a split Teflon mold of 10 mm diameter and 3 mm thickness. The prepared specimens were stored in a desiccator containing calcium sulfate at $23 \pm 1^\circ\text{C}$ for 24 h period. Afterward, the specimens were weighed using an electronic precise balance (TS4000, Ohaus, Pine Brook, NJ, USA). Weighing was repeated until reaching constant mass and recorded as m_1 . Later, the specimens were kept in a glass vial containing 100 mL artificial saliva; the vial was stored in an incubator at 37°C , weight was checked and recorded as m_2 . Before weighing, gentle drying with a filter paper was performed. Finally, redessiccation and reweighing of the specimens were performed till obtaining a constant mass and recording it as m_3 . The water sorption (W_{SP}) and solubility (W_{SL})($\mu\text{g}/\text{mm}^3$) were calculated as follows;^[19]

$$W_{\text{SP}} = m_2 - m_1 / V$$

$$W_{\text{SL}} = m_3 - m_1 / V$$

where m_1 is the specimen weight before immersion, m_2 is the specimen weight after immersion and m_3 is the specimen weight after redessiccation and V is the volume of the specimen.

Analysis of the color difference

A split Teflon mold of 8 mm diameter × 2 mm thickness was used to prepare the specimens. The color coordinates (L^* , a^* , b^*) for each group specimens were assessed using the Vita Easyshade spectrophotometer (Vita Zahnfabrik H. Rauter GmbH and Co. KG, Bad Sackingen, Germany) with regards to the manufacturer's instructions. Measured CIE L^* , a^* , and b^* at each point were compared to that of the control specimen and the color difference (ΔE) was calculated as follows;^[20]

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

where; L^* is the color value (lightness), a^* and b^* represent chromaticity.

Statistical analysis

Collected data were statistically analyzed using analysis of variance (ANOVA) and Tukey *post hoc* tests for pairwise comparison at $P \leq 0.05$.

Results

Figure 1 presents the FTIR spectra of the GIC set specimen, GS oil, GS powder, and the developed formulations by blending the GS oil and EEGS with GIC. On addition of 5% GS oil to GIC, a decrease in the absorbance pattern at peak 3452 cm^{-1} was very prominent with non obvious shift and this change was also observed with 5% EEGS-modified GIC. Furthermore, a shift of the peak at 627 cm^{-1} was noted with the modification of GIC with 5% GS oil to 602 cm^{-1} and the same change was noticed with 5% EEGS-modified GIC. On modification with 3% EEGS, multiple peaks appeared at wavelengths $3500\text{--}3750 \text{ cm}^{-1}$, with the most prominent peaks at 3542 , 3652 , and 3731 cm^{-1} . The absorbance pattern at peak 3452 cm^{-1} decreased with both ratios of EEGS (3 and 5%).

The results for compressive strength, shear bond strength, surface roughness, water sorption and solubility are

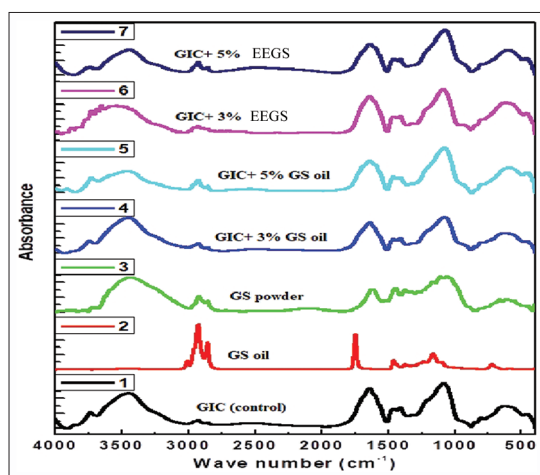


Figure 1: FTIR spectra of unmodified glass ionomer cement (GIC), grape seed (GS) oil, GS powder, and the GS-modified GIC. EEGS (ethanolic extract of grape seed)

illustrated in Table 1. Their graphical presentations are shown in Figure 2.

Regarding compressive strength results (MPa) of the studied groups, GIC modified by 3% GS oil exhibited the highest mean value ($136.56 \pm 2.1 \text{ MPa}$) while that modified by 5% EEGS had the lowest value (29.97 ± 1.35). ANOVA analysis indicated a significant difference among the tested groups ($P \leq 0.05$). Tukey test revealed that each group was significantly different from all the other groups ($P \leq 0.05$).

According to shear bond strength results, the highest mean value ($5.18 \pm 0.62 \text{ MPa}$) belongs to GIC modified by 3% GS oil while GIC modified by 3% EEGS exhibited the lowest value ($2.63 \pm 0.09 \text{ MPa}$). A significant difference was detected among the studied groups ($P \leq 0.05$). Tukey test demonstrated non significant difference between the control and GS oil-modified GIC groups (II and III) and between EEGS-modified GIC Groups (IV and V). On the other hand, the control and GS oil-modified GIC groups were significantly different from EEGS-modified GIC groups ($P \leq 0.05$).

As for surface roughness results, GIC modified by 3% GS oil exhibited the lowest value ($0.62 \pm 0.06 \mu\text{m}$), while 5% EEGS-modified cement had the highest value ($0.91 \pm 0.07 \mu\text{m}$). ANOVA indicated significance among groups ($P \leq 0.05$). *Post hoc* test identified that Groups IV and V were significantly different from both Groups I and III and Group II. Conversely, non significant difference was detected between Group IV and V and between Groups I and III at $P \leq 0.05$.

Water sorption results ($\mu\text{g}/\text{mm}^3$) of the studied groups showed that GIC modified by 3% GS oil exhibited the lowest mean value ($166.97 \pm 8.40 \mu\text{g}/\text{mm}^3$) while that modified by 3% EEGS had the highest value ($238.02 \pm 12.80 \mu\text{g}/\text{mm}^3$). ANOVA analysis showed a significant difference among groups ($P \leq 0.05$). *Post hoc* test revealed non significant difference between EEGS-modified GIC groups, and those groups were significantly different from Groups I (control), II and III. Moreover, Group I, II, and III were significantly different from each other ($P \leq 0.05$).

Water solubility results indicated that Group II had the lowest value ($50.28 \pm 9.82 \mu\text{g}/\text{mm}^3$) while Group IV had the highest value ($64.22 \pm 6.59 \mu\text{g}/\text{mm}^3$). ANOVA analysis showed a significant difference among groups ($P \leq 0.05$). *Post hoc* test verified non significant difference between Groups; I and III, and V and also between Groups I, IV, and V. Alternatively, significance was detected between Groups; I and II, and Groups; II and IV ($P \leq 0.05$).

Color difference results are shown in Table 2. Both oil-modified groups exhibited slight color change. GIC modified by 3% EEGS exhibited a highly appreciable color change meanwhile, the cement modified by 5% EEGS changed to another color. ANOVA indicated significance among groups ($P \leq 0.05$). Tukey test revealed

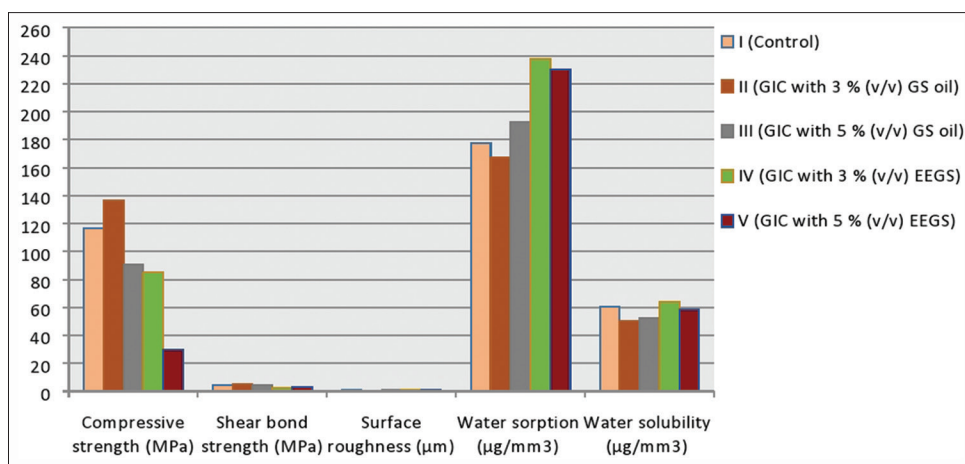


Figure 2: Graphical presentation of the compressive strength, shear bond strength, surface roughness and water sorption and solubility results. (GIC: Glass ionomer cement; GS: Grape seed; EEGS: Ethanolic extract of grape seed)

Table 1: Means, standard deviations and Tukey's analysis of the physical properties of the studied groups

Group	Compressive strength (MPa)	Shear bond strength (MPa)	Surface roughness (µm)	Water sorption (µg/mm ³)	Water solubility (µg/mm ³)
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
I (control)	116.42 ^b ±3.02	4.4 ^a ±0.37	0.76 ^b ±0.12	177.76 ^c ±5.2	60.70 ^{ab} ±3.92
II (GIC with 3% [v/v] GS oil)	136.56 ^a ±2.1	5.18 ^a ±0.62	0.62 ^c ±0.06	166.97 ^d ±8.40	50.28 ^a ±9.82
III (GIC with 5% [v/v] GS oil)	90.88 ^c ±4.34	4.26 ^a ±1.4	0.74 ^b ±0.03	192.57 ^b ±2.18	52.54 ^{bc} ±7.52
IV (GIC with 3% [v/v] EEGS)	84.90 ^d ±7.38	2.63 ^b ±0.09	0.82 ^a ±0.05	238.02 ^a ±12.80	64.22 ^a ±6.59
V (GIC with 5% [v/v] EEGS)	29.97 ^e ±1.35	3.13 ^b ±0.42	0.91 ^a ±0.07	230.21 ^a ±6.58	58.42 ^{abc} ±2.98
<i>P</i>	0.0001	0.0001	0.0001	0.0001	0.0194

*Means with the same superscript letter in each column are not significantly different at $P \leq 0.05$. GIC: Glass ionomer cement; GS: Grape seed; EEGS: Ethanolic extract of grape seed; SD: Standard deviation

Table 2: Means, standard deviations and Tukey's analysis of the color difference of the studied groups

Groups	Color difference (ΔE), Mean±SD
II (GIC with 3% [v/v] GS oil)	1.95 ^c ±0.15
III (GIC with 5% [v/v] GS oil)	2.76 ^c ±0.23
IV (GIC with 3% [v/v] EEGS)	8.03 ^b ±0.50
V (GIC with 5% [v/v] EEGS)	14.62 ^a ±1.35
<i>P</i>	0.0001

*Means with the same superscript letter are not significantly different at $P \leq 0.05$. GIC: Glass ionomer cement; GS: Grape seed; EEGS: Ethanolic extract of grape seed; SD: Standard deviation

that both oil-modified GIC groups were significantly different from Group IV and V. As well Group IV was significantly different from Group V, while no significance detected between Group II and III ($P \leq 0.05$). A graphical presentation of the color difference results is shown in Figure 3.

Discussion

The spectrum analysis of the GS oil, EEGS and the GIC confirmed their chemical structure and the structural changes on setting of GIC. For GIC, Disappearance of the bands around 1250 and 1710 cm^{-1} is an indicator to

aluminum polyacrylate formation.^[21] The bands observed around 1464, 1640 proved the completion of the reaction between the particles of the GIC. Finally, the two peaks at wavelengths around 627 and 3452 cm^{-1} are related to hydro-structures in the GIC.^[22]

GS oil showed sharp peak at 1746 cm^{-1} . This peak is belonging to the carbonyl (C = O) stretching vibration.^[23] The spectral band at 3009 cm^{-1} is referring to the C-H stretching vibration of the double bond (=CH) groups. Asymmetric and symmetric stretching vibrations of CH_2 groups are settled at 2926 and 2855 cm^{-1} , respectively. The peak at 1744 cm^{-1} is attributed to the absorption of the C = O bonds of the ester groups and the presence of the fatty acids and their glycerides, as well as pectins and lignins. The bands around 1653 cm^{-1} indicated the stretching of COO – and aromatic C = C groups, and bending vibrations of OH groups.^[24] The CH_3 bending at 1376 cm^{-1} , the scissoring at 1318 cm^{-1} , and the C-O stretching at $\sim 1035 \text{ cm}^{-1}$ are related to polysaccharide structures.^[25]

For the EEGS spectrum, the absorption bands at 3433 cm^{-1} representing O-H stretching vibrations are characteristic to alcohol presence. The bands at 3009 cm^{-1} are due to the stretching vibration of C-H groups indicative to alkene

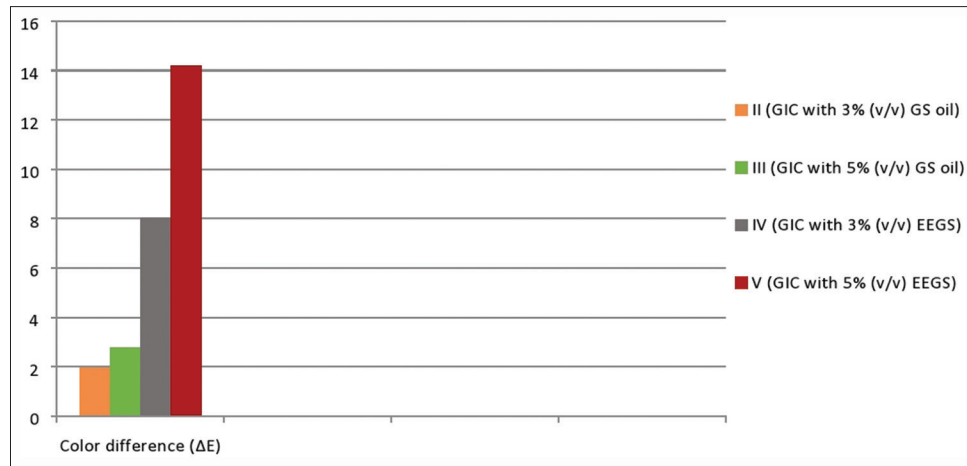


Figure 3: Graphical presentation of the color difference results. (GIC: Glass ionomer cement; GS: Grape seed; EEGS: Ethanolic extract of grape seed)

presence. The bands at 2926 and 2857 cm^{-1} are due to alkanes stretching vibration. The strong bands at 1620 cm^{-1} represent the bending vibrations of $\text{C}=\text{O}$ indicative of esters. The $\text{C}=\text{C}$ groups display strong bands at 1528 and 1442 cm^{-1} . The band at 1377 cm^{-1} represents alkyl group. The bands at 1283 , 1163 , and 1111 cm^{-1} characterize alcohol $\text{C}-\text{O}$.^[26]

The null hypothesis was rejected because both GS formulations (oil and ethanolic extract) in 3 and 5% modified the physical properties of the conventional GIC. For both Compressive and shear bond strengths, the findings of this study reported statistically significant differences between GIC modified by GS oil and that modified by EEGS. The incorporation of modifiers; whatever in solution or oil form, adversely affected the physical properties of the parent material. This might be related to the ability of these materials to interfere with the acid base reaction and prevent some carboxylic groups from participating in the reaction. They are supposed to hinder the acid attack to the glass powder and the leaching of ions from the glass. However, this effect seems to be more pronounced with higher concentrations (5%).^[27]

On the other hand, the results reflected the ability of 3% GS oil to enhance the compressive strength and shear bond strength due to another contributing factor. The oil itself may act as an adhesive penetrating the pores within the formed matrix and thus promoting cohesion and developing a stronger matrix.^[27]

As well, the sufficient number of the unreacted carboxylic groups may contribute to appropriate bonding with the tooth structure.^[28] The compressive strength results are in harmony with the study by Palmer *et al.* whereby the compressive strength decreased with increasing the additives ratio to the parent cement.^[29] Similarly, consistent with Xie *et al.* who incorporated quaternary ammonium compound in GIC as an antibacterial agent.^[30]

For surface roughness several factors are known to influence the surface roughness. Among these factors, the

particle size and distribution of the additive within the parent material. Furthermore, the hand mixing of the GIC used in the study is an important factor to be considered since it increases the chance of developing pores within the mixed cement.^[31] The ability of the oil form of the GS to seal the pores within the matrix producing more cohesive structure may explain the lower surface roughness values of the oil modified GIC groups rather than the control and the EEGS-modified ones which possess lack of sealing capacity.^[32] This finding is consistent with the compressive strength results and validation.

Water sorption and solubility of the EEGS-modified GIC in both ratios exhibited higher values than the oil modified cement did. The GS oil contains lesser amount of polyphenols characterized by its hydrophilic nature when compared to the EEGS. This elucidates the higher sorption ability of EEGS-modified cement.^[33] In addition, the sealing propensity of the GS oil renders more cohesive structure resistant to water solubility.^[32]

The color of GS oil ranges from yellow to yellowish green while the EEGS exhibited dark brown color. The color of the different forms clarifies the ΔE values of the different studied groups. The higher the concentration of the additive form of GS, the higher the color change detected. The minimal color changes related to the oil-modified groups ($\Delta E < 3.3$) considered as perceptible by skilled operators but clinically agreeable. Conversely, values higher than 3.3 (those detected by the two concentrations of EEGS-modified cement), regarded as noticeable by nonskilled persons and are, hence, clinically unpleasant.^[34]

Conclusions

Although different formulations of GS (oil and ethanolic extract) in different ratios (3 and 5% v/v) induced changes in most physical properties of conventional GIC, 3% (v/v) GS oil may have the potential to enhance the majority of its properties without compromising its adhesion to the tooth structure as well as its esthetic. Furthermore, it may

offer an approach to diminish its sensitivity to moisture by decreasing water sorption and solubility vulnerability.

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Conflicts of interest

There are no conflicts of interest.

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