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Epigallocatechin Gallate Modified Resin Infiltrate: In Vitro Evaluation Of Its Physical Properties.

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ABSTRACT

The purpose of the present study was to assess the impact of Epigallocatechin-3-gallate (EGCG) integration on the physical characteristics of resin infiltrant. Thirty specimens of Icon resin infiltrant were prepared with varying concentrations of EGCG and divided into three groups (n=10): 0 wt. % (control group with no EGCG added), 0.5 wt. % EGCG, and 1.0 wt. % EGCG. Random allocation was employed to assign the specimens to the respective test groups. To assess water sorption and solubility, the defined specifications outlined in ISO 4049 were followed. Furthermore, the color stability of the specimens was evaluated using a spectrophotometer to measure the CIE Lab* parameters. By calculating the color change, the study targeted to quantify the degree of color stability across the group samples. Regarding water sorption, solubility, and color stability evaluation, significant differences were clearly noticed between the tested groups. Incorporation of EGCG into the resin infiltrant improves its physical properties revealing promising results. This suggested that the integration of EGCG into resin infiltrant might be an effective strategy to be applied clinically. However, clinical trials would be needed to verify true outcomes.

Keywords: Epigallocatechin Gallate. Resin Infiltrant. Water Sorption. Colour Stability

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INTRODUCTION

Dental caries is a dynamic process caused by an imbalance in the dental surface's demineralization and remineralization in response to the concomitant changes in pH on tooth surface [1]. Incipient lesion is the first stage of caries process limited to enamel. It is characterized by a nearly intact surface with porous subsurface which could be reversed, stopped, or progressed to cavitation [2]. Remineralization as a non-invasive treatment, is increasingly being used as a primary tool of minimal intervention dentistry (MID) in caries management [3]. According to The International Caries Detection and Assessment System (ICDAS), topical fluoride application is the recommended non-invasive protocol for controlling early caries [4]. However, remineralization caused by topical fluoride application necessitates repeated treatment sessions and controlled long-term recall visits, which needs durable motivation and patient cooperation which is frequently perceived to be challenging [5].

The penetration of resin into the subsurface microporosities of early carious lesions has been proposed as a new microinvasive strategy [6]. Resin infiltration for treating incipient carious lesions is considered as a linkage between caries prevention and restoration as it could change the optic characteristics of the lesion and mask the white spots [7].

By blocking the micropores that serve as diffusion pathways for the acids, resin infiltration stops the progression of lesions by perfusing the subsurface porosities with resin through capillary action. As an alternative of being limited to the lesion surface, this technique intended to form an impermeable internal barrier within the lesion [8]. However, the inherent wet nature of oral cavity and the acids released by plaque bacteria cause resin dissolution with subsequent discoloration [9]. Numerous studies have been conducted in an effort to enhance the resin's therapeutic and physical qualities by adding bioactive fillers [10,11].

Epigallocatechin-3-gallate (EGCG) is the principal bioactive ingredient of green tea, having multiple biological effects particularly inhibition of Streptococcus mutans bacteria [12]. By smearing enamel with gel containing EGCG, an organic layer being resistant to acids was formed [13]. Furthermore, adding EGCG to adhesive resins enhanced their anti-caries qualities [14]. Additionally, EGCG decreased the adhesive's solubility while having no negative effects on other physicochemical properties [15,16].

Recently, there has been a shift towards development of restorative materials with bio-active functions and considerable durability. Accordingly, EGCG presents promising bioactive properties with predictable improvement of resin infiltration. However up till now, there has been no available data on incorporation of EGCG with resin infiltration technique. Therefore, the objective of the present study was to evaluate the effect of Epigallocatechin-3-gallate (EGCG) incorporation on the physical properties of resin infiltrant. The null hypothesis was that the incorporation of epigallocatechin-3-gallate (EGCG) with resin infiltration would have a positive impact on its physical characteristics.

METHODS AND MATERIALS

The study comprised a total sample size of 30 specimens, divided into three groups. To ensure accurate sample size determination, an online, freely available sample size calculator (http://www.sample-size.net/means-effect-size/) was utilized. The calculations aimed to achieve a statistical power of 80% and set the significance level at alpha = 0.05, resulting in a requirement of ten specimens per group.

Specimen Preparation

EGCG from Sigma-Aldrich (St Louis, Missouri, USA) was incorporated into the resin infiltrant (Icon, DMG, Hamburg, Germany) at various weight concentrations using an electric digital scale (AG245 Metter, Switzerland). The groups were designed as follows:

- Group I (control): Icon without EGCG.
- Group II: 0.5 wt% EGCG-Icon.
- Group III: 1.0 wt% EGCG-Icon.

The ingredients were thoroughly mixed using a DAC 150 Speed Mixer (Flacktek, Landrum, SC, USA) at 1300 rpm for 1 minute. Light curing was achieved by a blue-light emitting diode device (Ivoclar Vivadent, Austria) with a light irradiance of 1150 mW/cm2, as confirmed by a power-meter (Ophir Optronics Inc., Danvers, USA). The curing process was carried out on both sides of the specimens, with the tip of curing light being within 1 mm distance.

Water sorption and solubility

To measure the resin's water sorption (Wsp) and solubility (Wsl), it was placed inside two transparent strips of a Teflon mold that had a diameter of 8.66 mm and a thickness of 0.60 mm. These dimensions adhered to the ISO 4049 specification. Ten specimens were prepared for the test. The light-cured samples were first stored for 24 hours in a dry, dark environment before being moved to desiccators that were kept at 37± 1°C. The samples were taken out of the first desiccator after 24 hours, put in a second one for 2 hours at 25± 1°C, and weighed on an electric digital scale. This process was recurring till a persistent mass (m1) was attained with a maximum variation of ± 0.0001 g. Readings were taken 60 days (m2) after the dried-up specimens were submerged in deionized water at 37± 1°C. After this reading, the samples underwent a conditioning procedure in accordance with the cycle described for m1 to ensure a steady dry mass (m3). The percentages of water sorption (Wsp) and solubility (Wsl) were calculated using the following formulas:

$$Wsp = 100 \times ((m2 - m1)/m1)$$
$$Wsl = 100 \times ((m1 - m3)/m1)$$

Color Stability

Specimens for color stability test were prepared using the similar procedure used for the other tests. Following light activation, the specimens were kept in a dark and dry environment at 37°C for 24 hours. Color measurements were taken at baseline 24 hours after curing and at the end of 60 days bathing in 37°C distilled water, with weekly changes to the water medium. A spectrophotometer (CM2600d; Konica Minolta, Ramsey, NJ, USA) was handled to verify the CIE Lab* parameters. The backgrounds that were white and black were used to calibrate the spectrophotometer. The CIELab system comprises three axes: L* (lightness, ranging from 0 = black to 100 = white), a* (ranging from -a = green to +a = red), and b* (ranging from -b = blue to +b = yellow). All values were based on a D65 illuminant and 10° observer geometry. The readings were taken three times at the center of each specimen's top surface. The following formula was used to calculate color changes (ΔE) [17]:

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

where ΔL = L (60 days) − L (baseline),
Δa = a (60 days) − a (baseline),
and Δb = b (60 days) − b (baseline). The "baseline" values were recorded after 24 hours of light curing procedure.

Statistical analysis

By looking at the distribution and running normalcy tests, such as the Shapiro-Wilk and Kolmogorov-Smirnov tests, the normalcy of the numerical data was evaluated. The data were reported as the mean, standard deviation (SD), and 95% confidence intervals. Parametric data were analyzed using one-way analysis of variance (ANOVA) to compare the groups. Tukey's post hoc test was used for pair-wise comparisons.

A significant level of $P \leq 0.05$ was established. All statistical analyses were accomplished using IBM SPSS Statistics Version 20 for Windows (IBM Corporation, NY, USA), an established statistical software package widely used in medical research.

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RESULTS

Water solubility

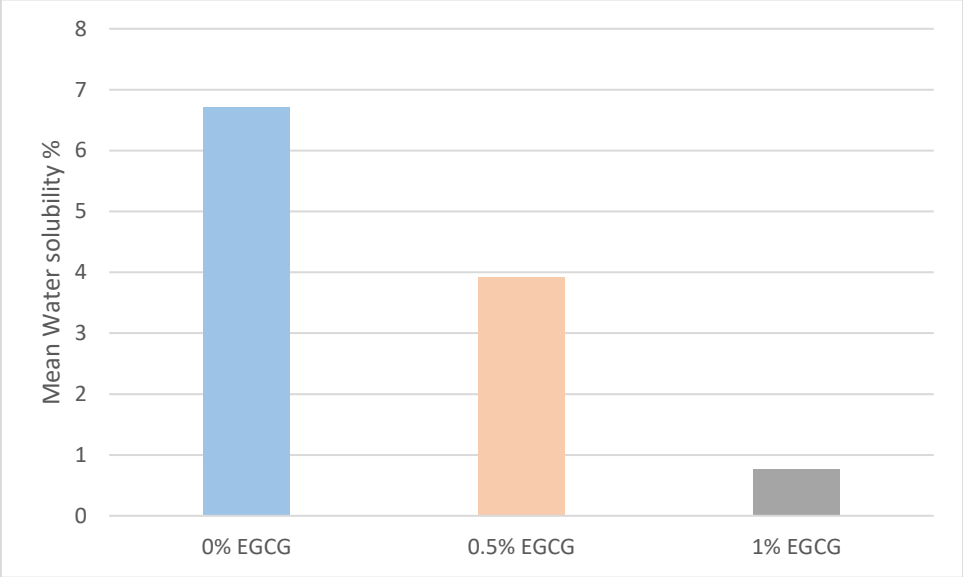
A significant difference was noticed amongst the tested groups (P-value < 0.001) when comparing water solubility (table 1 and figure 1). The control group had the maximum mean solubility, according to a statistical analysis utilizing pairwise comparisons between the s The 0.5% EGCG group demonstrated a significant lower mean value, whereas the 1% EGCG group exhibited the lowest mean water solubility, also statistically significant.

Table 1: Comparison of the water solubility (%) between the three groups using descriptive statistics and the results of a one-way analysis of variance (ANOVA) test.

	0% EGCG (control)	0.5% EGCG	1% EGCG	*P*-value	*Effect size (Eta Squared)*
Mean (SD)	6.71(0.35) ^{A}	3.92 (0.37) ^{B}	0.76 (0.09) ^{C}	<0.001*	0.987
95% CI	6.01 – 7.41	3.18 – 4.66	0.58 – 0.94		

**: Significant at P ≤ 0.05, Different superscripts are statistically significantly different*

Figure 1: Mean water solubility % of the tested groups.



Water sorption

A significant difference was found among the groups (P-value < 0.001, Effect size = 0.996) when comparing water sorption (table 2 and figure 2). The control group had the highest mean sorption, which was statistically significant, according to pairwise comparisons between the groups. The 0.5% EGCG group showed a significantly lower mean value, while the 1% EGCG group demonstrated the lowest mean water sorption, also statistically significant.

Table 2: Comparison of the water sorption (%) between the three groups using descriptive statistics and one-way analysis of variance (ANOVA) test.

	0% EGCG (control)	0.5% EGCG	1% EGCG	*P*-value	*Effect size (Eta Squared)*
Mean (SD)	28.41 (0.55) ^{A}	15.1 (0.62) ^{B}	10.91 (0.35) ^{C}	<0.001*	0.996
95% CI	27.31 – 29.51	13.86 – 16.34	10.21 – 11.16		

**: Distinct superscripts exhibit statistically significant differences, with significance set at P ≤ 0.05.*

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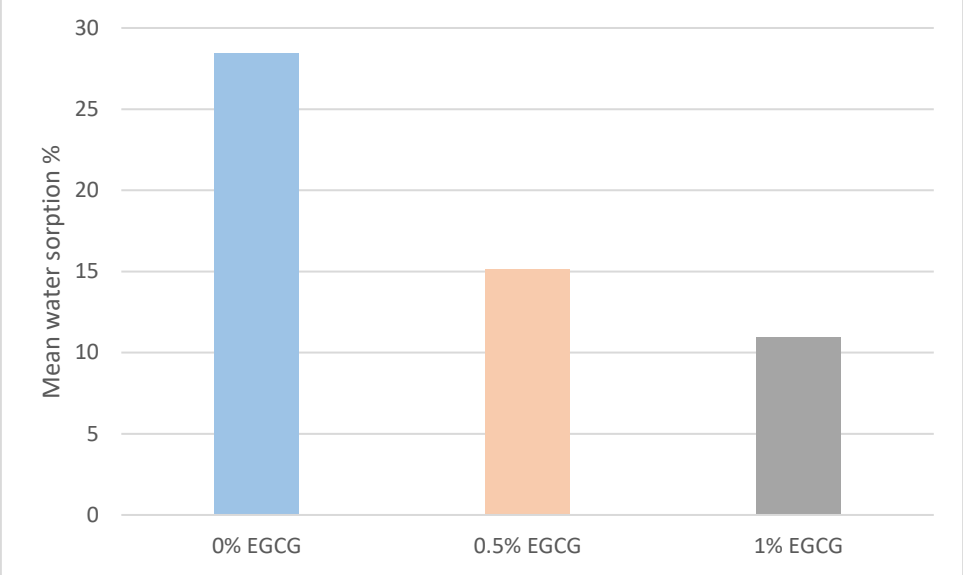
Figure 2: Mean water sorption %of the tested groups.



Color parameters

Regarding ΔL, a statistically significant difference was observed among the groups (P-value = 0.001, Effect size = 0.405) (table 3). Pair-wise comparisons revealed that the control group exhibited the highest mean ΔL, which was statistically significant. The 0.5% EGCG group presented no statistically significant difference compared to the 1% EGCG group (p-value 0.839).

Concerning ΔA, a statistically significant difference was found between the groups (P-value 0.003, Effect size = 0.347). Pair-wise comparisons showed that the control group had the highest mean ΔA, which was statistically significant. The 0.5% EGCG group exhibited no statistically significant difference compared to the 1% EGCG group (p-value 0.963).

Regarding ΔB, a statistically significant difference was observed between the groups (P-value <0.001, Effect size = 0.916). Pair-wise comparisons revealed that the control group displayed the highest mean ΔB, which was statistically significant. The 0.5% EGCG group showed no statistically significant difference compared to the 1% EGCG group (p-value 0.934).

Regarding ΔE, a statistically significant difference was found between the groups (P-value <0.001, Effect size = 0.923). Pair-wise comparisons showed that the control group exhibited the highest median ΔE, which was statistically significant. The 0.5% EGCG group exhibited no statistically significant difference compared to the 1% EGCG group (p-value 0.816).

Table 3: Results of the Kruskal-Wallis's test used to compare color parameters among the tested groups.

	0% EGCG (Control)	0.5% EGCG	1% EGCG	*P*-value	*Effect size (Eta Squared)*
ΔL	2.27 (-1.01 – 5.55) ^{A}	0.81 (0.51 – 1.11) ^{C}	0.57 (0.36 – 0.78) ^{B}	0.001*	0.405
Δa	-0.74 (-2.24 - 0.76) ^{A}	-0.07 (-0.22 – 0.08) ^{B}	-0.12 (-0.326 - 0.086) ^{C}	0.003*	0.347
Δb	-7.44 (-11.02 – -3.86)	-0.33 (-1.17 – 0.51)	-0.16 (-0.84 - 0.52)	<0.001*	0.916
ΔE	8.0 (4.46 – 11.54) ^{A}	0.97 (0.71 – 1.22) ^{B}	0.69 (0.47 – 0.91) ^{C}	<0.001*	0.923

**: Distinct superscripts within the same row exhibit statistically significant differences, with significance set at P ≤ 0.05.*

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DISCUSSION

Resin infiltration technique utilizing hydrophilic, light-polymerized flowable resins that can permeate the incipient lesions' subsurface tiny-pores and block the infiltration of cariogenic microorganisms and their byproducts aiming to arrest the progression of the lesion [18]. A significant increase in the micro-hardness of initial enamel carious lesions was noticed after resin infiltration that can also camouflage aesthetically disfiguring white lesions [19].

The effectiveness of the Icon resin infiltration treatment for non-cavitated caries lesions has been shown in numerous studies [20]. Any restorative intervention must be resistant to solubility and maintain color stability over time [21]. Because of its higher lesion penetration coefficient, the TEGDMA (Triethylene glycol dimethacrylate) monomer is the principal component of the resin infiltrant [22]. Nonetheless, TEGDMA has high water sorption rate, resulting in resin discoloration [23]. This could explain the Icon resin infiltration material's proclivity for water sorption and solubility in addition to of being filler-free.

The application of EGCG incorporated materials provides a new strategy in the field of dentistry. The therapeutic effect of EGCG has been widely investigated in restorative dentistry [24]. According to Fonseca et al., adding ECGC at a concentration of 0.5 wt% could modify the tested adhesive resins to be less soluble. They attributed these results to the ability of EGCG to lower the adhesive's hydrophilicity [25].

In the present study, the 0.5 wt% and 1.0 wt% EGCG dilutions were used, according to Agee et al. both concentrations were recommended for indorsing chains cross-linking of Bis-GMA and inhibiting water sorption due to their lower hydrophilicity [26]. In this study, compared to the non-modified specimens, merging of EGCG at concentrations of 0.5% and 1% caused significant decrease of water sorption and solubility in the tested resin. It might be explained by EGCG's capacity to lessen the resin's hydrophilicity and potentially interact with the hydroxyl groups of monomers through hydrogen bonding [27]. As a result of the difficulty in producing a uniform combination of the branched catechin molecule (EGCG) and the polymer network, Neri et al. (2014) [15] proved that the solubility of EGCG-modified adhesive resin decreased when compared to the control group. A reduction in solubility could be attributed to the release of the components being more challenging due to the strong potential bonds between the monomers in the adhesive and the hydroxyl radical in EGCG.

The methodology used in this study aligns with previous research that employed spectrophotometry in addition to the CIE Lab* coordinate system as it is broadly employed in dentistry. The CIE Lab* system offers advantages such as repeatability, sensitivity, and objectivity [28]. It was selected in this study to evaluate color change (ΔE) due to its suitability for detecting small color variations [29]. Perceptible color differences range between ΔE values of 1 and 3, while ΔE values more than 3.3 are regarded as a type of clinical failure [30,31]. A 60-day exposure period with specimens immersed in distilled water regarded as satisfactory in assessing staining ability over time [32].

In the present study, all tested materials exhibited color changes after the 60-day immersion period. Some differences were observed among the materials. Icon showed a significant variation in the Δa parameter (green-red component), while the 0.5% EGCG group exhibited the highest ΔL values (lightness). When judging the total color variations (ΔE), Icon displayed the greatest values (ΔE 8), that could be regarded as not accepted clinically. This may be attributed to the discoloration of the resin because of gradual degradation of the resin matrix and TEGDMA water sorption [33].

Therefore, in this study, Icon resin infiltrant, exhibited greater discoloration compared to the other groups. This finding is consistent with recent studies that have reported significant color alterations with Icon resin infiltration after staining processes [34,35]. Though, the outcomes showed that there was no significant difference between the two concentrations used for the EGCG-Icon group. However, EGCG has improved the color stability of resin infiltrant in both concentrations. Therefore, the null hypothesis tested, that the incorporation of epigallocatechin-3-gallate (EGCG) into resin infiltration would have a positive impact on its physical properties was accepted.

CONCLUSION

According to the findings of the current study, given its limitations, incorporation of bioactive particles of Epigallocatechin-3-gallate (EGCG) into the resin infiltrant improved its color stability and its resistance to water solubility.

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Data availability

Data sets associated with this article will be provided by the corresponding author upon request.

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