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Effect of gallic acid as a final irrigant on push-out bond strength of an epoxy resin sealer to root canal dentin

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ABSTRACT

Introduction: Ethylenediaminetetraacetic acid (EDTA), a widely used chelating agent, compromises the bond strength of resin-based sealers when used as a final irrigating solution. Hence, the push-out bond strength of AH Plus, a resin-based sealer, was analyzed when gallic acid, "a chelator" was used as a last irrigating solution.
Methods: Forty human single-rooted teeth were accessed and randomly assigned into 4 groups (n = 10): EA (17% EDTA), MA (7% maleic acid), GA (10% gallic acid), and SA (saline). After irrigation, the root canals were coated with AH Plus sealer and obturated using gutta-percha. The root canals were sectioned horizontally into coronal, middle, and apical thirds for evaluating the push-out bond strength. A universal testing machine with a compressive loading of 1 mm/min was employed to test the samples.
Results: An increased push-out bond strength was noted with GA in coronal one-third compared to EA and SA but not significant compared to MA ($P > 0.05$). Similar observations were seen in the middle one-third; however, it was statistically significant compared to all other groups ($P < 0.05$). Also, the difference between the groups in the apical one-third was not significant. SA showed the lowest push-out bond strength than experimental groups, which was significant in all three sections ($P < 0.05$).
Conclusion: The final rinse of 10% gallic acid increased the push-out bond strength of AH Plus to the root dentin in all thirds of the root canal. Hence, gallic acid 10% might be an effective alternative solution in place of synthetic chelators.

Implication for health policy/practice/research/medical education:
Gallic Acid can be a preferred final irrigating solution during chemo-mechanical preparation when employing the resin-based AH Plus sealer. The higher bond strength of the sealer to root dentin signifies improved sealing ability, which results in less microleakage.

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Introduction

The favorable result of root canal treatment is determined by complete debridement, shaping, and disinfection of the root canal system (1). Thorough removal of the smear layer, which contains bacteria and dentin debris, is essential for a favorable prognosis in root canal treatment. Various procedures have been widely studied to effectively remove the smear layer. Hence, an appropriate condition of root dentin for adhesive materials needs to be determined considering the outcome of endodontic treatment on dentin bonding. Chelating agents have been recommended to enhance smear layer removal, resulting in increased contact area and better diffusion of sealers (2). Clearing away the smear layer improves the adaptation of obturating materials to the root canal walls, improves the bond strength of the resin sealer mass, and reduces coronal and apical microleakage (3,4).

Ethylenediaminetetraacetic acid (EDTA) and maleic acid synthetic chelators have been the irrigating solution of choice as the last rinse agents for the elimination of the smear layer (5). Despite their effectiveness, they have been found to erode and reduce the microhardness of the dentin

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surface and possess inherent toxicity (6). Moreover, EDTA has been reported to cause irreversible decalcification of the periapical and adversely affect the neuroimmunology regulatory mechanism (7). Hence, there is a need for biocompatible materials. In this context, there has been a growing interest in phytotherapy due to its potential therapeutic applications. This resurgence of interest in plant-derived drugs is mainly because of their safety and dependability compared to synthetic drugs (8,9).

Gallic acid (3,4,5-trihydroxybenzoic acid) is a common organic acid commonly found in plants. It can be found in several food sources, including almonds, tea, grapes, berries, and pomegranate, either in its free form or as a derivative. Edible fruits have attracted substantial interest as they contain bioactive phytochemicals such as gallic acid. Amla (*Emblica officinalis*) fruit contains gallic acid as a major constituent, apart from nicotinic acid, carotene, vitamin C, tannins, and riboflavin (10).

In pharmaceutical industries, the commonly used phytochemical is gallic acid because of its beneficial properties. The mechanism and its effectiveness have been supported by several *in vitro* and *in vivo* studies and also in cell culture, preclinical, and clinical trials. It acts as a free radical scavenger playing a significant role in anticancer activity. Additionally, its antifungal and viral properties and its ability to protect cells against oxidative damage, due to its antioxidant nature, make it a promising material. It has astringent properties and is used for treating internal hemorrhage (11).

It has been reported that the maximum elimination of the smear layer in root canal dentin was by amla extract containing gallic acid compared to other herbal extract solutions. This was correlated to its strong chelation properties due to its pH of 2.5 (12).

Careful literature research revealed that there was limited information regarding the dislodgement resistance of AH Plus sealer following the use of gallic acid. Push-out tests have been used in endodontic research to assess the adhesion of sealers to canal surfaces and core material and to evaluate their sealing ability (4). Hence, the goal of the present investigation was to assess the efficacy of different endodontic irrigating solutions as a final rinse on the push-out bond strength of AH Plus sealer an epoxy resin-based sealer. We hypothesized that there is no effect of various endodontic irrigants on the push-out bond strength of AH Plus resin-based sealer.

Materials and Methods

Sample size calculation

The sample size for this *in vitro* investigation was determined based on the push-out bond strength of Biodentine to root canal dentin treated with various irrigating solutions. Hence, 10 for each group was estimated as the sample size, with a power estimate of 80% and confidence interval of 95%.

Specimen preparation

For this study, ethical clearance was acquired from the institutional review board (SRMU/M&HS/SRMDC/2021/S/022) for the use of human teeth, which were extracted. Forty single-rooted human teeth in total were chosen, their outermost soft tissues were eliminated and kept at 4 °C in a 0.1% thymol solution (Thermo Fisher Scientific, Chennai, India) until used. The teeth with single straight canals, mature apex, and without any defects or calcifications, were taken following confirmation with the buccal and mesial radiographs.

Preparation of experimental stock solution: 10% gallic acid

By dissolving 10 g of accurately weighed gallic acid powder (Sigma-Aldrich, Bangalore, Karnataka, India) in 100 mL distilled water, a stock solution of gallic acid was obtained. The gallic acid solution was sonicated for 30 min and filtered using Whatman filter papers (No. 41). The filtrate was concentrated under a vacuum and the resulting extract was purified.

Preparation of samples

A total of 40 freshly extracted human single-straight rooted anterior teeth with a completely formed root apex were used. First, the superficial soft tissues were removed and stored at 4 °C in 0.1% thymol solution until use. With the help of a diamond disc, all the teeth were sectioned at the cementoenamel junction to acquire 12 mm of standardized root length. The working length was determined by placing a No.10 K file (Mani Inc, Japan) into the root canal such that it went beyond the apical foramen (observed under magnifying loupes). Following this, 1 mm was subtracted. The root apices were sealed with sticky wax to reproduce a closed-end system, thereby preventing the flow of irrigation solution through the apical foramen and enabling the reverse flow of the irrigating solutions.

Cleaning and shaping of the root canals were done using rotary endodontic instruments up to size F3 ProTaper. In all groups, irrigation protocols were carried out by positioning a 27-gauge side-vented needle (SS White, Germany) short of the working length by 1 mm for 1 minutes of time in between each instrument change. The apical foramen of each root canal was kept patent by surpassing a 10-size K file beyond the root apex. The prepared root sections were then randomly assigned into four groups (n = 10) and irrigated with the following solution for 10 minutes.

Final irrigation regimens

Group EA: 17% EDTA (5 mL)
Group MA: 7% maleic acid (5 mL)
Group GA: 10% gallic acid (5 mL)
Group SA: saline

All the prepared root canals were dried with paper points (FKG Dentaire SA, Switzerland) following the respective final irrigation regimen.

AH Plus resin sealer was manipulated based on the manufacturer's protocol and coated against the walls of the root canals using a lentulo spiral, following which the canals were obturated using gutta-percha by lateral condensation technique. The quality of the obturation was confirmed with radiographs. All teeth were incubated at 37 °C after storing in phosphate-buffered saline (pH=7.4) until further evaluation.

Push-out bond strength

The root canals were sectioned horizontally in coronal, middle, and apical one-thirds by a hard tissue microtome (Hoverlabs, Ambala Cantt, Haryana, India) under continuous water cooling. The universal testing machine (TSI; Tec-Sol, Chennai, Tamil Nadu, India) was used to measure the push-out bond strength. Stainless steel plungers measuring 0.6 mm were used by directing the force apicocoronally at a crosshead speed of 1 mm/min, ensuring that they were only made in touch with the filler substance. The following equation was carried out to analyze the adhesion surface area.

Adhesion surface area $(mm^2) = D1 + D2/2 \times \pi \times H$,

where D1 and D2 depict the largest and smallest canal diameter, respectively; π and H represent the constant 3.14 and thickness of the root slice, respectively. The push-out bond strengths were estimated in Megapascal using the following formula:

Push-out bond strength (MPa) = Force (N) / Adhesion surface area (mm^2).

Statistical analysis

The obtained data were statistically analyzed using SPSS software, version 19 (IBM SPSS Statistics, Somers, NY). One-way ANOVA and Tukey test were used to evaluate the data. *P* values of <0.05 were considered statistically significant (95% confidence level).

Results

Table 1 presents the mean bond strength (MPa) of AH Plus with various irrigating solutions and their standard deviations. The results of one-way ANOVA indicated that the push-out bond strength was influenced significantly by the choice of irrigation protocol ($P < 0.05$). Statistically significant difference among the groups tested was noted. GA (10% gallic acid) was observed with the highest push-out bond strength in all three sections compared to all other groups (EA, MA, and SA). In the coronal third, significantly higher bond strength was seen in GA (7.73 ± 1.34 MPa) compared to the EA ($P = 0.025$) and SA ($P = 0.001$) groups but was not significantly different compared to the MA group ($P = 0.928$). In the middle third, the push-out bond strength of GA (7.70 ± 0.96 MPa) was significantly higher compared to the EA ($P = 0.000$), MA ($P = 0.018$), and SA ($P = 0.001$) groups. In the apical third, no significant difference was observed in GA, MA, and EA groups but the mean bond strength of GA (7.74 ± 0.61 MPa) was significantly different compared to SA ($P = 0.001$). Group SA (saline control) had the least bond strength compared to EA, MA, and GA groups in all three sections (3.79 ± 0.72, 2.46 ± 0.22, 5.77 ± 1.46 MPa for coronal, middle, and apical one-thirds respectively) ($P = 0.001$).

Table 1. Mean (± SD) push-out bond strength (in MPa) of AH Plus to root dentin following the use of 17% EA, 7% MA, 10% GA, and SA as a final irrigating solution

Root section	Groups	Bond strength (MPa) Mean ± SD
Coronal (n = 10)	17% EA	5.97 ± 1.59[b]
	7% MA	7.37 ± 1.25[a]
	10% GA	7.73 ± 1.34[a]
	SA	3.79 ± 0.72[c]
Middle (n = 10)	17% EA	5.75 ± 1.00[a]
	7% MA	6.34± 1.20[a]
	10% GA	7.70 ± 0.96[a]
	SA	2.46 ± 0.22[b]
Apical (n = 10)	17% EA	7.65± 0.89[a]
	7% MA	6.95± 0.52[a]
	10% GA	7.74± 0.61[a]
	SA	5.77± 1.46[b]

SD, standard deviation; EA, ethylene diamine tetra acidic acid; MA, maleic acid, GA, gallic acid; SA, saline.
In every section of the root, a statistically significant difference is depicted using different lower-case letters ($P < 0.05$).

Discussion

The current *in vitro* study found that the final rinse using various chelating chemicals had different effects on the push-out bond strength of AH Plus. Hence, the null hypothesis of this study was rejected. Although it does not appear that the bond strength of the root canal sealer and clinical success are directly correlated, it is probable that an obturating material with a lesser bond strength reflects the survival of the endodontically treated tooth. Pane et al reported that push-out bond tests measure the bond strength of the obturating materials to the root dentin (13). The shear stress produced by this test at the dentin-sealer interface is equivalent to that found in practical clinical conditions. In our experiment, 1.5 mm dentin discs were used, although various dimensions of dentin discs are recommended for push-out tests. However, using thick discs appears to augment the area of friction, causing a misrepresentation of the bond strength (14).

We observed an increased push-out bond strength of AH Plus sealer in all three sections when the canals were irrigated with GA compared to EA and SA as a final rinse. These findings are in concurrence with a study by Christopher et al, who documented that the penetration of Resilon and RealSeal SE was optimized following the use of gallic acid (15). The improved performance of gallic acid could be attributed to the presence of three hydroxyl groups making it an active phenolic acid with high radical scavenging capacity. The residual oxygen could be removed by the redox potential of gallic acid following the use of sodium hypochlorite, thus enhancing the dentin tubular penetration of the resin sealer and increasing the bond strength (16). In addition, gallic acid has been specified as a chelator because of its therapeutic effects on heavy metal detoxification (17). The polyphenols (ortho-dihydroxy), i.e., molecules bearing galloyl or catechol groups in gallic acid, are claimed to be responsible for their chelating activity, in addition to their lower pH (18). Hence, the highest push-out bond strength obtained by gallic acid in this study could be because of smear layer removal due to its chelating tendency. Similar findings of smear layer removal with the use of gallic acid comparable to EDTA have been reported in a study conducted by Bhargava et al (16).

In the coronal and middle thirds, EA had lower push-out bond strength compared to MA; however, there was no statistical significance. These results are consistent with earlier literature evidence (19,20). One possible explanation for this is the pH variation during the decalcification process. Although EDTA is a chelating agent, it is effective even at neutral pH, negating the demand for a high hydrogen ion concentration to achieve "decalcification". However, during decalcification, the interchange of calcium with hydrogen in dentin results in decreased pH over time, thus reducing its efficacy. On the other hand, GA and MA, having an acidic pH, exert continued superior demineralizing efficacies within a shortened time period. This could explain the higher push-out bond strength obtained by GA and MA in this study. These results are similar to the findings of Ballal et al (21).

The push-out bond strength of MA was equivalent to that of GA, although not statistically significant. This is in conjunction with the findings of Christopher et al who reported improved penetration of resin-based obturating materials, which indirectly reflects the improved bonding between resin-obturating materials and root dentin (15). In addition, to achieve optimal wettability, it is important that the surface tension of a liquid contacting substrate is as low as possible (22). The lower surface tension of 7% maleic acid (0.06345 N/m) resulted in higher push-out bond strength when compared to 17% EDTA (0.0783 N/m). In this study, a concentration of 7% MA was chosen, as higher concentrations may cause damage to the intertubular dentin (23). Moreover, when EDTA is used as a final rinse, it exposes a fine layer of demineralized collagen fibrils on the treated surface, which have low surface free energy (24). These two factors could have caused poor wetting and spreading of the sealers reflecting on the decreased push-out bond strength obtained in the current study. This is in accordance with the findings of Ballal et al (25,26). SA, which was used as a control, showed the lowest bond strength.

In the apical one-third, no significant difference in push-out bond strength was noted among GA, MA, and EA except SA, which served as the control. Also, the push-out bond strength value in the apical one-third was lower than the coronal and middle one-thirds for all the irrigating solutions used. This could be due to the high number of open tubules and the vapor lock effect in the apical region, which could have prevented the penetration of the irrigating solution (27).

Conclusion

The adhesive ability of the sealer is highly influenced by the type of dentin pretreatment. The findings of our investigation showed that final irrigation with 10% gallic acid during chemo-mechanical preparation was favorable for an endodontic procedure as it resulted in a higher push-out bond strength of AH Plus, a resin-based sealer to the root canal dentin in all thirds of the root canal. The bond strength results were significantly higher compared to the widely used 17% EDTA solution. The result produced by maleic acid was comparable to gallic acid. Gallic acid, being a chelating agent, seems to be an ideal irrigating solution for resin-based sealers. However, further studies are needed to extrapolate its effect on the mechanical properties of dentin and in canals with curvatures and ramifications.

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Authors' contributions

SK, SM, and SN provided the study design. KS, SK, and SN performed experiments. SK and SN analyzed the data, KS prepared the first draft. SK, SM, and SN edited and completed the manuscript. All authors read and approved the final version of the manuscript for publication.

Conflict of interests

There are no conflicts of interest.

Ethical considerations

The study's ethical approval was obtained from the institutional review board and ethical committee (SRMU/M&HS/SRMDC/2021/S/022).

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