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Original Article

COMPARATIVE EVALUATION OF THE EFFECT OF SODIUM THIOSULFATE VERSUS PROANTHOCYANIDIN AS FINAL IRRIGANTS ON THE BOND STRENGTH OF EPOXY RESIN SEALER TO ROOT DENTIN TREATED WITH SODIUM HYPOCHLORITE (A COMPARATIVE IN-VITRO STUDY)

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Abstract:

Aim: Comparing the effect of Proanthocyanidin and Sodium thiosulfate solutions as final irrigants on the push-out bond strength of epoxy-resin sealer to sodium-hypochlorite-treated root dentin and studying the physicochemical properties of both solutions for potential routine use.

Subjects and methods: Thirty-three single-rooted extracted teeth were decoronated and mechanically prepared using rotary NiTi instruments and irrigated with 2.5% sodium hypochlorite. Samples were randomly allocated into three groups according to the final irrigant: group1(control), Saline; group2, 6.5% proanthocyanidin, and group3, 5% sodium thiosulfate. All samples were obturated using gutta-percha and AH-plus epoxy-resin sealer and then cross-sectioned and subjected to push-out bond strength test at different root levels. For physicochemical evaluation, a sample of each experimental irrigant was tested for surface tension using the du-Noüy-ring method via force-tensiometer. Viscosity was tested using a rotational digital viscometer and pH was measured using a benchtop pH-meter.

Statistical analysis was performed using one-way ANOVA test followed by Tukey's post-hoc test for intergroup comparisons. Repeated measures ANOVA followed by Bonferroni post-hoc test were used for intragroup comparisons. The significance level was set at $p \leq 0.05$.

Results: Proanthocyanidin-treated group showed -significantly- the highest mean push-out bond strength values (~1.58 MPa) compared to other groups. Proanthocyanidin solution showed significantly lower mean surface tension (46.71 mN/m) and viscosity (37.43 KU). Sodium thiosulfate showed a slightly alkaline pH (~8.2), while proanthocyanidin showed a slightly acidic pH (~5.8).

Conclusions: Proanthocyanidin solution can improve the bond strength of epoxy-resin sealer to sodium-hypochlorite-treated dentin. It also showed favorable physicochemical properties that make it promising for routine endodontic use.

Keywords: Proanthocyanidin; push-out bond strength; collagen cross-linking; anti-oxidant; reducing agen

Introduction

Sodium hypochlorite (NaOCl) is the most popular root canal irrigant, due to its powerful antibacterial action, dissolution of necrotic pulp tissue and dissolution of the organic part of the smear layer. Many of the actions of NaOCl are attributed to its powerful oxidizing effect [1,2]. Bonding of epoxy-resin sealer to root dentin is thought to occur by the reaction of its open epoxide rings with the exposed amino-groups in the dentinal collagen forming covalent bonds [3]. Unfortunately, bonding of resin sealer is negatively affected by NaOCl as it causes degradation of dentinal collagen in the surface dentin to which the sealer bonds, in addition to production of Oxygen which hinders resin polymerization and tubular infiltration of the sealer [4–7]. Several studies have shown that using antioxidants or neutralizing agents on dentin could reverse the adverse effects of NaOCl and improve the adhesion quality of resin to NaOCl-treated-root-dentin [8]. Examples of those antioxidants include Proanthocyanidin and Sodium thiosulfate [9,10].

Proanthocyanidin (PA) is a naturally-occurring plant metabolite found mainly in grape-seed among other sources. It has been demonstrated to improve resin-dentin bonding due to its combined collagen cross–linking and anti-collagenolytic effects that prevent degradation of dentinal collagen within the hybrid layer [11]. In addition, it has a powerful antioxidant action that was found to counteract the oxidizing effect of sodium hypochlorite [12].

Sodium thiosulfate (ST) is an inorganic compound that was introduced as a neutralizing agent for NaOCl in microbiological studies [13,14]. When used as an endodontic irrigant, ST was shown to neutralize NaOCl by a chemical action, via the antioxidant effect of ST which eliminates the residual free radicals formed on the dentin surface after NaOCl irrigation, and a physical action through the washing out of the solution. This promotes the complete polymerization of resin material [10]. Therefore, ST can be used to enhance dentin-resin bonding after NaOCl treatment [15,16]

Before new irrigants are introduced into clinical practice, it's important to study their physicochemical properties, such as surface tension, viscosity and pH as these can strongly affect their action and effectiveness. Surface tension and viscosity are the flow properties which determine the irrigant's fluid dynamics and in turn its performance and efficiency in the clinical situation. Surface tension forces tend to inhibit the spread of a liquid over surfaces as well as limit its penetrability into capillary tubes such as the dentinal tubules [17]. Similarly, viscosity resists the flow of the irrigant inside the root canal and thus affects its accessibility into the anatomical complexities of the root canal system [18,19]. Hence, low surface tension and low viscosity are desirable properties that can enhance irrigant's efficiency by improving the solution's wettability, contact to dentinal walls and penetrability into main and lateral canals and dentinal tubules [18,20–22]. pH can influence many of the properties of the root canal irrigant. such as antibacterial efficacy and shelf life [23–26], as well as cytotoxicity [27], NaOCl tissue dissolving ability [28], and EDTA smear layer removal and chelating ability [29].

Research has shown that both PA and ST could improve the bonding between epoxy-resin sealer and NaOCl-treated root dentin [10,30]. However, no studies have compared both solutions to each other regarding their effects on the push-out bond strength of epoxy-resin sealer, nor regarding their physicochemical properties. So, this study was performed to compare and evaluate the effect of PA and ST on the push-out bond strength between epoxy-resin sealer and NaOCl-treated root dentin, in addition to studying the surface tension, viscosity and pH of both PA and ST solutions.

Subjects and methods

Preparation of final irrigating solutions:

- PA solution: 6.5 grams of PA-rich grape-seed extract powder was collected from the capsules (Gervital, MEPACO-MEDIFOOD, Sharkeya, Egypt) and dissolved in 100 ml of distilled water to make a solution of 6.5% concentration.
- ST solution: 5 grams of ST crystals (El-Nasr Pharmaceutical chemicals, Al Salam First, Cairo, Egypt) were dissolved in 100 ml of distilled water to make 5% ST solution.

Sample size calculation:

Sample size was calculated using the PS software (version 3.1.2). It was found that 11 teeth per group was an appropriate sample size for the study with a total sample size 33 teeth (three groups). The power was 80% and α error probability = 0.05. The magnitude of the effect to be detected was estimated as mean and standard deviation of the variable of interest and obtained from the scientific literature [31].

Preparation of samples:

Thirty-three freshly-extracted human single-rooted mandibular premolars were decoronated at the cemento-enamel junction using a low-speed diamond disc to obtain a standardized root length of approximately 15 mm. Root canals were negotiated with K-file size #10 (Mani Inc., Kiyohara Industrial Park, Utsunomiya, Tochigi, Japan) to establish apical patency and then the working-length was measured. The root canals were instrumented with a crown-down technique using ProTaper Next rotary Ni-Ti system (Dentsply Maillefer, Ballaigues, Switzerland), starting with X1(#17/.04), X2(#25/.06), X3(#30/.07) and finally X4(#40/.06), at 300-RPM-rotational-speed and 200-gcm-torque for all files. The irrigation protocol was as follows:

- During instrumentation, the canals were irrigated with 3 ml of 2.6% NaOCl in-between instrumentation cycles, using a disposable plastic syringe with a 30-G side-vented needle to reach 1–2 mm from the working-length.
- After instrumentation, the canals were flushed with 5 ml of 2.6% NaOCl followed by 5 ml of 17% ethylenediaminetetraacetic acid (EDTA) solution then finally 5 ml of 2.6 % NaOCl solution.
- A 5-ml-saline-flush was used between different irrigating solutions to prevent any possible interactions.

The samples were then randomly distributed into three groups (n = 11) according to the final irrigating solution as follows: Group I (control): normal saline solution; Group II, 6.5% PA solution; Group III, 5% ST solution. Each group received a 5-ml-final flush of the corresponding irrigant for 5 minutes.

All samples were dried with paper points and obturated using single-cone technique with X4 master gutta-percha cone and AH-plus epoxy-resin sealer (Dentsply DeTrey GmbH, Konstanz, Germany). Excess gutta-percha was removed using a hot condenser and the warm coronal gutta-percha mass was vertically condensed with a hand-plugger, then the root canal entrance was sealed with a temporary filling material. Obturation quality was verified radiographically. All samples were kept on gauze pads at 37ºC and 100% relative humidity for one week to allow proper setting of the sealer.

Push-out bond strength test* [32–34]*:

- The teeth were embedded in acrylic resin blocks, and then sectioned horizontally using Isomet-4000 high-speed, water-cooled linear precision saw (Buehler USA, Lake Bluff, Illinois, USA) at 2500 RPM, to obtain slices of 2 mm thickness from the coronal, middle and apical root thirds of each specimen at lengths 3 mm, 8 mm and 12 mm, respectively.
- Each root section was subjected to compressive loading via the universal testing-machine (Instron, Coronation Road, High Wycombe, Buckinghamshire, UK) at a crosshead speed of 0.5 mm/min using a stainless-steel cylindrical plunger of diameter 0.5, 0.7 and 1.0 mm for apical, middle and coronal sections, respectively. The plunger tip only contacted the filling material without touching the canal walls.
- The force was applied until bond failure occurred, which was manifested by extrusion of the obturation material and a sudden drop along the load-deflection curve.
- To calculate the push-out bond strength in Megapascals (MPa), the load at failure recorded in Newtons (N) was divided by the adhesion surface area, which was calculated through the following formula: $(\pi r_1 + \pi r_2) \times \sqrt{[(r_1 - r_2)^2 + h^2]}$ [33,34].
  [where $\pi = 3.14$ (constant), r_1 was the larger (coronal) radius, r_2 was the smaller (apical) radius (Figure 1), and h was the section thickness, (r_1 and r_2 were measured via stereomicroscope and h was measured via a digital caliper)].

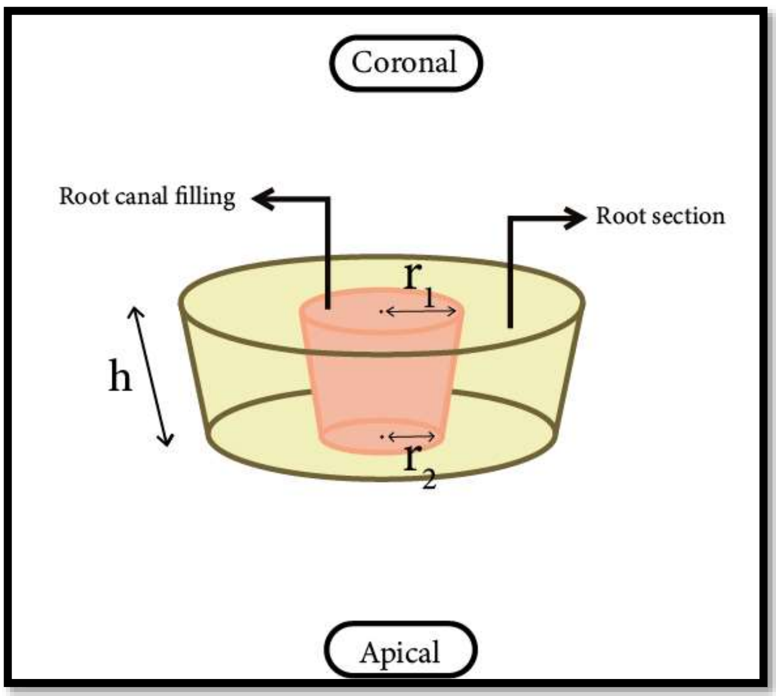

Figure 1: Diagram showing measurements for calculation of adhesion surface area.

Measurement of physicochemical properties:

Surface tension:

A freshly prepared sample of each experimental solution was tested for surface tension using du-Noüy-ring method via Krüss-K6 force tensiometer (Krüss GmbH, Hamburg, Germany), at a constant room temperature of 25°C.The principle of the device depends on the proportion between surface tension and the detachment force of the device’s platinum-ring from the solution surface. Before measurements, zero calibration was obtained using distilled water. For each tested sample, 50 mL of the solution was placed in a 50-mm-diameter shallow glass dish. The dish was elevated by a precision screw until contact with the platinum ring was maintained. The platinum ring was centralized into the middle of the container to avoid edge effects. The sample was allowed to equilibrate for 90 seconds. The ring was then raised by a torsion mechanism until detached from the solution. The tension readings at the instant of surface detachment were recorded. Measurements were repeated seven times for each tested solution and mean values were recorded [20].

Viscosity:

Viscosity of the tested solutions was measured using Sheen rotational digital Kreb's Viscometer (TCQ Sheen industrial Physics Inks and coatings, Molbaan 19, Netherlands) at temperature 25°C. According to manufacturer's instructions, a freshly prepared sample of each solution was poured into a 250-ml-container that was placed on the magnetic base of the apparatus. The paddle of the apparatus was rotated at a rotational speed of 200 RPM. Ten seconds after reaching steady rotation, it was automatically stopped, and the viscosity value was given. Ten measurements were taken for each sample to calculate the mean value [35].

pH:

Measurement of pH for both tested solutions was carried out using a digital benchtop pH-meter (Mettler Toledo, Ohio, USA) at 25°C. Before measurement, the device was calibrated using standard buffer solutions with known pH values of (pH 7.0) and (pH 4.0) respectively. The glass electrode of the device was rinsed with distilled water before and after calibration. Each sample was poured into a 50-ml-beaker. The glass electrode of the device was immersed into the solution. It was then allowed to equilibrate for one minute until a stable reading was given on the digital screen. The measurement was repeated three times for each sample and a mean value was calculated and recorded [25,36].

Addressing potential sources of Bias:

Selection bias was avoided by randomization and allocation concealment. Detection and reporting biases were avoided by blinding of the outcome assessor and statistician, in addition to the use of objective testing methods and the accurate recording and reporting of all collected data.

Statistical analysis:

Numerical data were presented as mean and standard deviation (SD) values. They were explored for normality by checking the data distribution and using Shapiro-Wilk test. Data showed parametric distribution so one-way ANOVA followed by Tukey's post-hoc test was used for intergroup comparisons. Repeated measures ANOVA followed by Bonferroni post-hoc test was used for intragroup comparisons. The significance level was set at $p \leq 0.05$.

Results

1. Push-out bond strength (MPa):

<u>**Effect of irrigation protocol:**</u>

- **Overall:** (Table: 1, Figure: 2)
  There was a significant difference between different groups ($p=0.004$). The highest value was found in PA, followed by

ST, while the lowest value was found in saline. PA had a significantly higher value than other groups (p<0.001), with no significant difference between ST and Saline groups.

- **Coronal and middle thirds:** (Table: 2, Figure: 3)

There was a significant difference between different groups (p<0.05). PA showed the highest values (p<0.001), while no significant difference was found between ST and saline groups.

- **Apical third:** (Table: 2, Figure: 3)

There was no statistically significant difference between the three groups (p=0.08).

<u>Effect of root section:</u> (Table: 2, Figure: 4)

There was a significant difference between values measured at different sections (p<0.001). The highest values were measured at the coronal section, followed by the middle section, while the lowest values were found at the apical section. The values measured at the coronal section were significantly higher than those measured at other sections (p<0.001), with no significant difference between middle and apical sections.

2. Physicochemical properties

Regarding Surface tension (Table: 3, Figure: 5) and viscosity (Table: 4, Figure: 6), ST solution showed significantly higher mean values than PA solution (p<0.001).

Regarding the pH, ST had a significantly higher value than PA (p<0.001), with ST solution being slightly alkaline, and PA solution being slightly acidic (Table: 5, Figure: 7).

Table (1): Mean and Standard deviation (SD) values of overall push-out bond strength (MPa) for different irrigation protocols:

Overall push-out bond strength (MPa) (mean±SD)			p-value
Saline	**PA**	**ST**	
0.98 ± 0.41^{B}	1.58 ± 0.65^{A}	1.09 ± 0.44^{B}	0.004*

Different superscript letters indicate a statistically significant difference. *; significant ($p \leq 0.05$

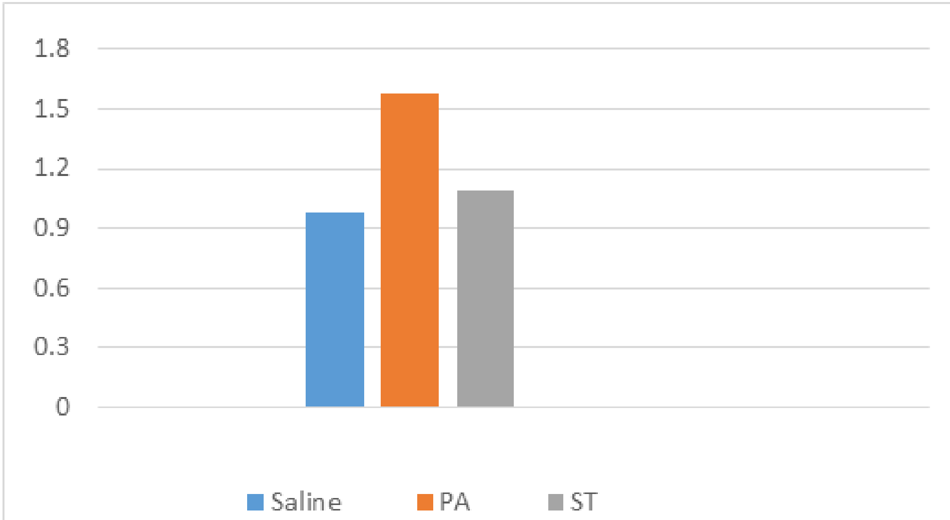

Figure 2: Average overall push-out bond strength (MPa) for different irrigation protocols.

Table (2): Mean and Standard deviation (SD) values of push-out bond strength (MPa) for different irrigation protocols and root sections:

Root section	Push-out bond strength (MPa) (mean±SD)			p-value
	Saline	PA	ST	
Coronal	1.60 ± 0.37^{Ba}	2.86 ± 0.77^{Aa}	1.85 ± 0.56^{Ba}	<0.001*
Middle	0.69 ± 0.18^{Bb}	1.07 ± 0.38^{Ab}	0.72 ± 0.10^{Bb}	0.002*
Apical	0.65 ± 0.15^{Ab}	0.80 ± 0.17^{Ab}	0.70 ± 0.14^{Ab}	0.080ns
p-value	<0.001*	<0.001*	<0.001*	

Different upper and lowercase superscript letters indicate a statistically significant difference within the same horizontal row and vertical columns respectively *; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

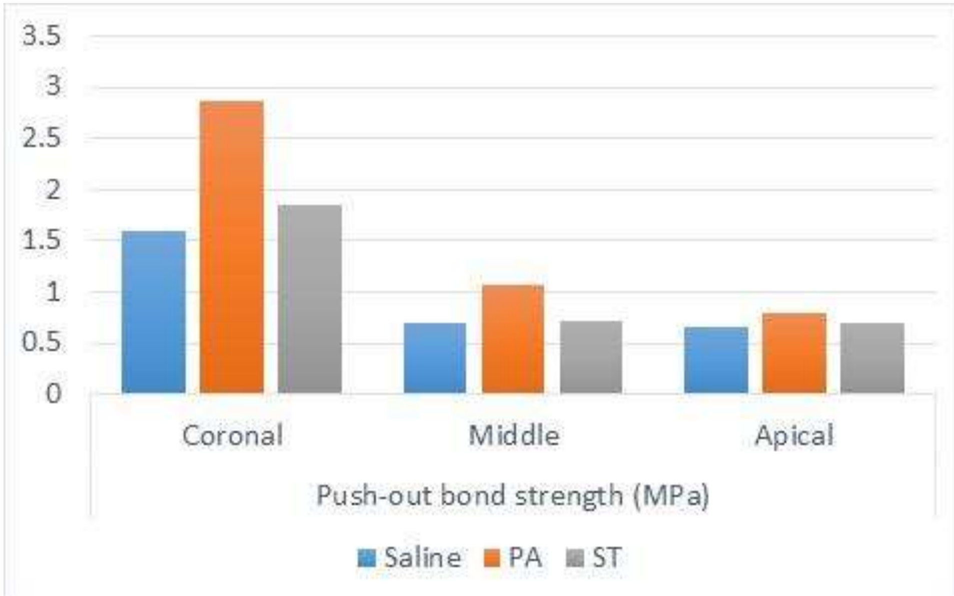

Figure 3: Average push-out bond strength (MPa) for different irrigation protocols.

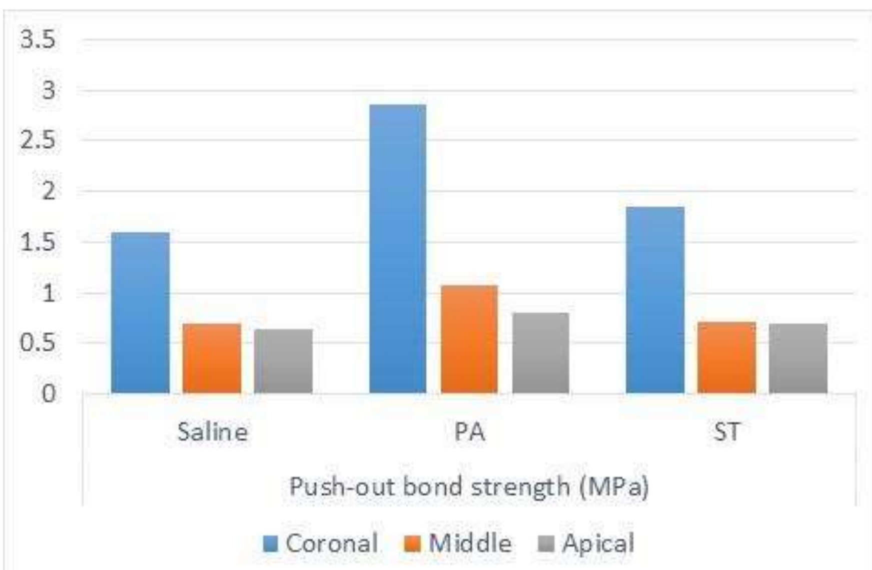

Figure 4: Average push-out bond strength (MPa) for different root sections.

Table (3): Mean and Standard deviation (SD) values of surface tension (mN/m) for different irrigation protocols:

Surface tension (mN/m) (mean±SD)		p-value
PA	**ST**	
46.71±0.11	58.51±0.52	**<0.001***

*; significant ($p \leq 0.05$) ns; non-significant (p>0.05)

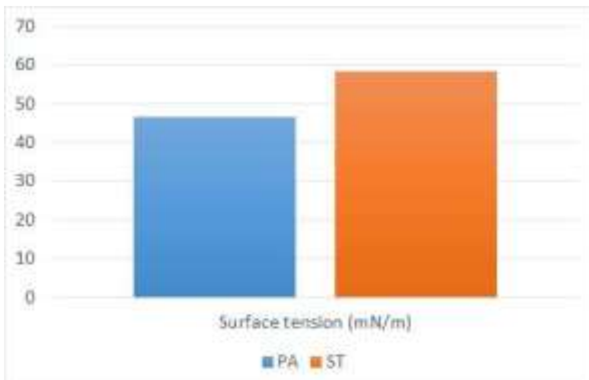

Figure 5: Average surface tension (mN/m) for different irrigation protocols.

Table (4): Mean and Standard deviation (SD) values of viscosity (Krebs unit) for different irrigation protocols:

Viscosity (Krebs unit) (mean±SD)		p-value
PA	**ST**	
37.43±0.51	41.34±0.41	**<0.001***

*; significant ($p \leq 0.05$) ns; non-significant (p>0.05)

Figure 6: Average viscosity (Krebs unit) for different irrigation protocols.

Table (5): Mean and Standard deviation (SD) values of pH for different irrigation protocols:

pH (mean±SD)		p-value
PA	ST	
5.77±0.06	8.17±0.15	**<0.001***

*; significant ($p \leq 0.05$), ns; non-significant (p>0.05)

Figure 7: Average pH for different irrigation protocols.

Discussion

For bonding between the root canal wall and epoxy-resin sealers, dentinal collagen is the prime substrate. Epoxy-resin sealer was shown to chemically bond with dentin via formation of the hybrid layer, by covalent bonding between the exposed amino-groups of collagen and the open epoxide ring of the sealer [3,37]. Although NaOCl is considered a gold standard root canal irrigant, it was shown to cause degradation of the dentinal collagen by its strong deproteinizing and oxidizing effects, and hence compromising bonding and sealing [7].

Therefore, the aim of this study was to evaluate and compare the effects of collagen cross-linking and reducing agents when used as final rinses on the bond strength of epoxy-resin sealer to root dentin, in addition to studying their physicochemical properties for potential routine use in endodontic practice.

Results of the present study demonstrated that the lowest mean values of push-out bond strength belonged to the control group. The use of NaOCl during instrumentation was shown to cause degradation of dentinal collagen [7] and therefore hinder the bonding with epoxy-resin which is mainly dependent on dentinal collagen [3]. Due to its high alkalinity, NaOCl can destroy the highly cross-linked, apatite-encapsulated collagen molecules when it infiltrates the water compartments of mineralized collagen [7]. The breakdown products of NaOCl were shown to break the pyridinoline cross-links present in type I collagen [38]. Moreover, those breakdown products were shown to severely fragment long peptide chains and chlorinate protein terminal groups [7,39]. Another possible reason for the hinderance of bonding caused by NaOCl is the generation of oxygen bubbles at the resin–dentin interface which may interfere with resin infiltration into the dentinal tubules and inter-tubular dentin [5].

The highest mean value of push-out bond strength was found in the PA-group with a statistically significant difference from other groups for the coronal and middle thirds. These results are in accordance with several previous studies [12,30,40,41]. This could be attributed to the dual mechanism of PA action which depends on both collagen cross-linking and antioxidant, free-radical-scavenging actions [12,42–46]. PA was shown to have excellent antioxidant and free radical scavenging power via donating hydrogen atoms from their multiple donor sites to bind the unstable free radical molecules [12,42,45]. Moreover, it's capable of recycling other antioxidants by removing their bounded free radicals and thus freeing them up to bind more free radicals. The presence of gallic acid in the structure of PA further strengthens its radical scavenging activity [42]. The collagen cross-linking action of PA could be attributed to the high affinity of proline-rich proteins of collagen to PA which enables the formation of strong hydrogen bonds between them creating additional inter- and intra-molecular cross-links [43,44,46]. Moreover, PA increases the resistance of collagen to degradation by masking the cleavage sites on the collagen molecule [47,48], in addition to inhibiting the enzymatic activity of matrix metallo-proteinases (MMPs) [49]. Furthermore, the slightly acidic pH of PA solution (~5.8) could be another reason for the improved bond strength of epoxy-resin sealer to root dentin [41].

ST is a powerful inorganic antioxidant that has shown the potential to neutralize the oxidizing effect of NaOCl and hence restore the compromised bond strength to NaOCl-treated dentin [16]. It neutralizes unpaired electrons and thus eliminates the residual free radicals produced by NaOCl on the dentin surface which hinders adequate bonding with resin [10].

The mean push-out bond strength of the ST-group was found to be statistically less than that of PA-group, with no statistically significant difference from the control group. A possible explanation could be that intact collagen is required for chemical bonding to occur with epoxy-resin sealer. Since collagen is adversely affected by NaOCl as explained before, bonding was compromised. Although ST has been proven as a potent antioxidant [10,16,50], it does not have a collagen cross-linking effect. This could be the reason why ST was unable to restore the compromised bonding between dentin and epoxy-resin in the current study. This finding is in accordance with the findings of [51], where irrigation with ST and sodium ascorbate improved bonding of methacrylate-resin sealer but showed no effect on the bonding of epoxy-resin sealer.

Regarding the effect of root thirds, the highest mean values of push-out bond strength of all groups were observed in the coronal third with a statistically significant difference from the middle and apical thirds. This difference has been reported in the literature [5,37]. The presence of such a difference could be due to differences in the number of dentinal tubules and the tubular diameters among the root thirds. The number and diameter of dentinal tubules is greater in the coronal third and decreases from coronal to apical direction. Therefore, more resin infiltration and resin tag formation can occur in the coronal third and thus, the bond strength is higher [52]. Regarding the comparison between middle and apical thirds, no significant difference was found. This could be due to the circular cross section of the root canal at the apical third, which results in a higher resistance to dislodgment during the push-out test [53].

The measured bond strength values in the current study ranged from 0.65 to 2.8 MPa. These values are in accordance with the values reported by [30] and [41]. [40] and [9] also reported close values, taking into consideration the difference in methodology and irrigation protocols. However, according to [54], the typical values of dentin bond strength reported for adhesive resins such as Epiphany (Pentron Clinical Technologies, Wallingford, CT) were 20 to 30 MPa, while the values reported for epoxy resin such as AH-plus (which was used in the current study) were 6 MPa or less. The variation between those values and the values reported by our study and the aforementioned studies [9,30,40,41] could be attributed to the difference in the method of bond strength measurement, where push-out method was used in those studies, in contrast to the studies reported by [54] which used tensile and shear methods [55,56]. In addition, the difference in irrigation protocols utilized before obturation could have also resulted in such variation.

Measurements of surface tension and viscosity showed statistically lower mean values of both parameters for PA solution. This suggests better flow characteristics of PA and thus better penetrability into the root canal system and dentinal tubules as stated in the previous literature [18–20,57–59]. However, further studies are needed to investigate the actual penetration depth and dentin wettability of PA and ST in order to confirm this assumption.

Results of pH measurement showed a slightly acidic pH (~5.8) for PA solution. This slightly acidic pH provides chemical stability for the solution [60] as well as improved antimicrobial effects [61]. Moreover, it may enhance the bonding of epoxy-resin sealer. This is thought to occur by causing slight demineralization of the surface dentin, exposing intact collagen with which epoxy-resin bonds [30,60].

As for ST, the pH was found to be slightly alkaline (~8.2). It was shown that the use of an alkaline irrigant of pH 8 or more after irrigation with a bactericidal irrigant such as NaOCl could kill the residual surviving bacteria in the root canal [62]. This could be a possible benefit from the alkaline pH of ST solution. However, the applicability of such a hypothesis on ST requires further analysis. The literature has not yet investigated the effect of this pH neither on the properties of ST solution nor on its clinical performance as an endodontic irrigant. Therefore, further research is still warranted in that context.

Limitations of the current study include inability to generalize the results of this sample to all tooth types, as the sample used in this study consisted of mandibular premolars, which differ in root canal anatomy and morphology from other tooth types. Therefore, Further studies on different samples with different morphologies are required. Additionally, the finidings of the current study cannot be confirmed by clinical studies due to the nature of the used testing method which is not applicable in-vivo. The results provided by the current study offer only quantitative data about bonding of the sealer. Additional qualitative data by failure mode analysis and scanning electron microscopy examination could have complemented those results and provided a better understanding. Finally, a potential imprecision of the push-out test is the creation of non-uniform stress distribution [63]. This limitation was prevented in this study by using 2-mm-thick slices as recommended by [64].

Conclusions

Within the limitations of this study, it could be concluded that:

- The use of 6.5% PA solution as final irrigation can improve the bond strength of epoxy resin sealer to root dentin treated with sodium hypochlorite.
- The use of 5% ST solution as a final irrigant does not have a significant effect on the push-out bond strength of epoxy-resin sealer.
- PA solution has favorable physicochemical properties which suggest adequate flow and penetrability into the root canal system, as well as efficient performance as a root canal irrigant.

Conflict of interests

No conflict of interests.

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This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethics:

This study protocol was approved by the ethical committee of the faculty of dentistry- Cairo university on: 26-1-2021. Approval number: 23-1-21.

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