EFFICACY OF CHITOSAN AS A POST SPACE IRRIGANT ON BOND STRENGTH OF FIBER POSTS LUTED WITH SELF-ADHESIVE CEMENTS: AN IN-VITRO STUDY

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ABSTRACT

Aim: To evaluate the efficacy of chitosan as a post- space irrigant on bond strength of glass fiber posts luted with self-adhesive resin cements.

Methodology: thirty single rooted human mandibular premolars with similar dimensions were decoronated to a standard root length of 15mm, endodontically treated and subjected to post preparation to a 10mm length. Specimens were randomly divided in to 3 groups according to the post space irrigant (PSI) to be used in to group (Saline, EDTA, Chitosan) n=10 each. Following irrigation procedure glass fiber posts (GFP) (Glassix radiopaque, H Nordin, Chailly/Montyreux, Switzerlandand) were cemented using self-adhesive resin cement (RelyX U100). Specimens were sectioned perpendicular to the long axis into 1mm thick slices. Three slices (one representative of each root third) from each specimen were subjected to push- out test and bond strength in MPa was statistically analyzed.

Results: the bond strength was significantly higher in both EDTA and Chitosan groups than saline group P < 0.001. Bond strength was significantly affected by the region of the root canal P< 0.001.

Conclusion: PSI affects the bond strength of GFP luted with self-adhesive resin cements. Chitosan can serve as a safer alternative for EDTA as a chelating agent. Chitosan as a PSI has a positive influence on the bond strength of GFP luted with self-adhesive resin cements.

KEYWORDS: Chitosan, Glass fiber post, Self-adhesive cement, Post space irrigant, Push out bond strength.

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INTRODUCTION

Success of endodontically treated teeth is not only determined by the debridement, and a fluid tight obturation of the root canal persi. It's more a multidisciplinary systematic process including accurate diagnosis, endodontic treatment and finally placement of a suitable coronal restoration[1]. Coronal leakage is considered as a silent killer, which tends to sneak up un-noticed for months causing extreme damage before its presence is acknowledged. [2]

For decades the focus of endodontic research has been on the apical seal of the root canal treatment. [3,4] No doubt; it is well proven that the apical leakage lead to endodontic failure but the fact that a failure rate of 20- 30 % is found in teeth with well obturated teeth. Which has led to a significant increase in research focus on the coronal seal of endodontically treated teeth [5, 6, 7, 8] .

In a retrospective study by Ray and trope [9] a clinical assessment of the incidence of apical pathosis after endodontic treatment. They found that the quality of coronal restoration is significantly more important than the quality of the obturation in regards to the periapical health   Glass fiber posts are one of the many treatment modalities indicated for restoring endodontically treated teeth that need retention for a core build-up. [10, 11]

Its modulus of elasticity is comparable to that of dentin which is a favorable characteristics allowing uniform, balanced stress distribution of the masticatory forces along the root canal walls [12, 13] . At the same time the evolution of adhesive luting cements have also contributed to the high success rate of these types of posts [14, 15] and reduction in the risk of vertical root fracture 16 Furthermore; fiber posts are known to enhance esthetics and provide better light transmission to the apical region ensuring proper polymerization even in the deep areas of the root canal.[17] However; the inherent draw back in post application is the need to prepare post space which eventually causes the deposition of a thick, heavy secondary smear layer on the root canal walls with remnants of gutta percha and sealer along with the dentin shatters [18, 19, 20] Which of course jeopardizes the proper adhesion of the fiber post to dentin and may finally lead to leakage and secondary apical periodontitis [21, 22]

Presence of this smear layer not only leads to leakage around the post but is considered a source of nutrition for microorganisms [23]

Pereira etal [24] studied the mode of failure of fiber posts via SEM and concluded that the main weak point in fiber post adhesion is the cement / dentin interface regardless of the type of luting cement or cementation protocol used. Most studies have shown comparable results where the prevalent mode of failure is between the cement and the dentin surface [25]

Self-adhesive resin cements have been developed recently to simplify and overcome the sensitivity of the multiple step techniques. However; regarding the root canal system especially after post-space preparation the permeation ability through this thick smear layer and creation of a true hybrid layer is questionable [26]

From the previous context it is of paramount importance to plan the type of irrigant to be used after post space preparation not only to remove the smear layer but one which does not interfere with the setting reaction of the luting cement

So far ethylene diamine tetra-acetic acid EDTA is considered the most commonly used irrigant for smear layer removal [27, 28] . However; in advertent erosion of periradicular dentin is observed when exposed to EDTA for more than 3 minutes [29, 30, 31]

Moreover; application of EDTA to dentin is known to alter the physical and chemical properties of dentin causing increased adherence of enterococcus faecalis which is microorganism strongly linked to post-treatment infections [32, 33, 34] Together with its limited antibacterial activity and the raised environmental concerns [35]

From the above mentioned; many attempts have been made to find a suitable alternative to overcome these draw backs [36] . Chitosan, a recently introduced compound which is a natural polysaccharide produced from de acetylation of chitin (obtained from shells of crabs and shrimp) [37]

Many medical fields have favored the use of such compound due to its abundant presence in nature and its more environmentally friendly characteristic 38   In addition to biocompatibility, biodegradability, and antibacterial activity which are few of its outstanding properties that have encouraged dental researchers to further integrate this compound into more fields of dentistry [37, 38, 39]

Silva etal [40] found that chitosan produces smear layer removal comparable to that of EDTA with very little erosion than EDTA. In addition to Zhou etal 27 whom compared MTAD to chitosan in removing the smear layer . they found chitosan more efficient than MTAD especially in the apical third of the root canal . Even when using low concentrations of chitosan was capable of efficiently removing smear and providing a clean dentin surface [40, 41]

It is worth mentioning that chitosan in contrast to EDTA; has a well -documented strong antibacterial activity against oral flora and is able to inhibit bacterial growth and suppress bacterial enzymes [42] From the previous chitosan appears to be a justified candidate as an alternative for EDTA.

Few studies have investigated effect of irrigation solution after post space preparation on the bond strength of fiber posts. Most researches are directed to investigate the effect of chemical remnants of irrigation solutions during the chemo-mechanical preparation of the root canal which is actually diminished during the post space preparation; that removes a considerable amount of the dentin upon which they are attached to. [43] The null hypotheses is that : 1- Chitosan as PSI does not influence bond strength of glass fiber posts luted with self-adhesive resin cements 2- Bond strength at different root regions exhibits similar bond strength values.

AIM OF THE STUDY

Evaluate the efficacy of chitosan as a post- space irrigant on bond strength of glass fiber posts luted with self-adhesive resin cements.

MATERIALS AND METHODS

1. Specimen selection and preparation:

Thirty intact human, single rooted mandibular premolars recently extracted for orthodontic reasons were used in this study. Only teeth with completely formed apices and no signs of external resorption or cracks were included, radiographs were taken in buccolingual and mesiodistal directions to confirm single root canal. The external surfaces of all samples were thoroughly cleaned of all soft and hard tissue debris after being immersed for 10 minutes in 5.25 % sodium hypochlorite.

All teeth included in the present Study were with in comparable measurements and a statistical confirmation was done to eliminate any samples that were out of similar range of measurement. [44] Selected samples were stored in 0.1% thymol solution to prevent them from becoming brittle or drying out. [45] Teeth were stored in distilled water with 0.1% thymol (Caelo, Hilden, Germany) for no longer than a month. One trained operator performed all root canal treatment, post space preparation and post cementation procedures for standardization purpose

All samples were decoronated to establish a standardized root length of 15mm using a double coated diamond disc (Diatech, Coltene, Switzerland) mounted on a straight hand piece and under copious water coolant.

2. Root canal treatment

Working length was confirmed by passing a standard type K- file # 10 (Dentsply, Maillefer: Ballaigues, Switzerland) in to the root canal till visible at the apex then retracted 1mm coronally. Working length was set at 14mm.

All specimens were instrumented using Protaper rotary files (Dentsply, Maillefer: Ballaigues, Switzerland) mounted on gear reduction, torque controlled endo motor (X-smart, Dentsply, Sirona, Switzerland). Canals were instrumented up to F4 and 1% NaOCl (Egyptian company for household bleach-Egypt) was used in between files and finally flushed with 5ml of saline.

Root canals were dried using Protaper paper points (Dentsply, Maillefer: Ballaigues, Switzerland), then obturated in a single cone technique using the corresponding gutta percha point F4 (Dentsply, Maillefer: Ballaigues, Switzerland) and AH-Plus (Dentsply, Maillefer).

The excess gutta-percha was cut off using a red hot plugger 1mm below the coronal end of specimens and sealed with flowable composite (Tetric EVO Flow, Ivoclar, Vivadent, Germany) and stored at 37 C^0 in 100% humidity for 7 days to ensure complete setting.

3. Post space preparation

Composite seal was removed with a round carbide bur (BR-31, DIA-BURS, Mani, Japan) from all specimens then Post space preparation was performed to a standardized length of 10 mm using the corresponding drill of the glass fiber post (GFP) system (Glassix radiopaque, H Nordin, Chailly/ Monteux, Switzerland) mounted on a low speed hand piece. [46]

An apical seal of 4 mm of the root canal filling was maintained undisturbed in each specimen. Periapical radiograph was taken to confirm complete removal of gutta percha in the coronal 10mm and a 4mm apical seal. Specimens were maintained moist by placing them in gauze sponge soaked in saline throughout the endodontic treatment and post- space preparation processes

The GFP were tried passively in the post space to ensure their fit down into the whole prepared depth.

4. Grouping of the specimens and irrigation procedure:

Each specimen was placed separately into an Eppendorf vial filled with silicon based impression material (Dentsply, Switzerland) up to 2mm apical to the cervical margin which allowed simulation of the clinical simulation during polymerization so that only the cervical area of the root was exposed to the light emitted from the curing unit (preventing any light curing improvement via light diffusion through the root dentin) [43]

Specimens were then assigned randomly in to three equal groups 10 specimens each according to the PSI to be used in each group

Group (saline): n=10 post space was irrigated with 5ml of saline for 3 min

Group (EDTA): n=10 post space was irrigated with 5ml of 17% EDTA for 3 min

Group (chitosan): n=10 post space was irrigated with 5ml of 0.2% chitosan for 3 min.

Chitosan preparation: 0.2 % chitosan was prepared by dilution of 0.2 gram chitosan (Nano Tech, dream land, Egypt) with a 90% deacetylation in 100 ml of 1% acetic acid then stirred in a magnetic stirrer for 2 hours till a homogenous crystalline solution was obtained with a PH of 3.2. Each specimen was irrigated with 5 ml of the assigned PSI for 3 min delivered with a plastic disposable syringe of 27 gauge needle placed to the full length of the post space , moved in an in and out movement. All post spaces were dried using a standard paper point # 80(Dentsply, Maillefer).

5. Post cementation procedure

The GFP (Glassix radiopaque, H Nordin, Chailly/Montyreux, Switzerlandand) were cleaned with 70% ethyl alcohol followed by application of a thin coat of silane coupling agent (Monobond Plus, Ivoclar Vivadent)) using a microbrush and left for 1 min then dried with air jet.

Self-adhesive resin cement (Rely XU100 3M, ESPE) was mixed according to manufacturer's instructions and delivered into the post space using a lentulo spiral (Dentsply, Maillefer) mounted on a low speed hand piece. Respectively the GFP were coated with cement and immediately seated in place and held under finger pressure for 2 min.

Excess resin cement was removed using a microbrush then the posts were light cured for 40sec from the buccal then 40 more seconds from the lingual side with the curing unit tip 45 degree to the long axis of the post (with 600 mW/cm²). A 3mm thick composite resin core (nano hybrid universal A3 shade 3M ESOE Z250XT, Seefeld, Germany) was placed to seal the coronal portion then Specimens were removed from the Eppendorf vial and then stored in 100% humidity at 37˚C for 7 days. [24]

6. Push-out bond strength test:

All specimens were genteelly notched with a high speed carbide bur on the buccal surface then mounted into self-cured acrylic (cold cure acrylic resin, Acrostone, Egypt) in a standard cylindrical plastic ring in an upright position using a centralizing device.

Nine horizontal sections perpendicular to the long axis of the specimen from the cervical margin down were done to obtain a slice thickness if 1mm each using a high- concentration double coated diamond disc (Diatech, Coltene, Switzerland) mounted on a straight hand piece and under copious water coolant.

The first 3 slices (1, 2, 3) were termed coronal, the second 3 slices (4, 5, 6) termed middle and finally the third 3 slices (7, 8, 9) were termed apical. Once the slices of each specimen were obtained the diameter of post from the coronal and apical surfaces for slices (2, 5, 8) - as representatives of each region- were recorded via a digital caliper. Push out test was performed for each selected slice.

Push out test was performed with a universal testing machine (Lloyd LRX, Lloyd Instruments, Fareham Hants, UK) at 0.5 mm/ min attached to a 500N load cell. **Fig (1)**

The slices were placed in an orientation such that the force was applied in an apico- coronal direction (till it provoked failure) by means of metal piston only contacting the post till dislodgment which was recorded in newtons (N). Bond strength value was obtained in MPa via dividing the force (provoking failure) in N by the bonding area
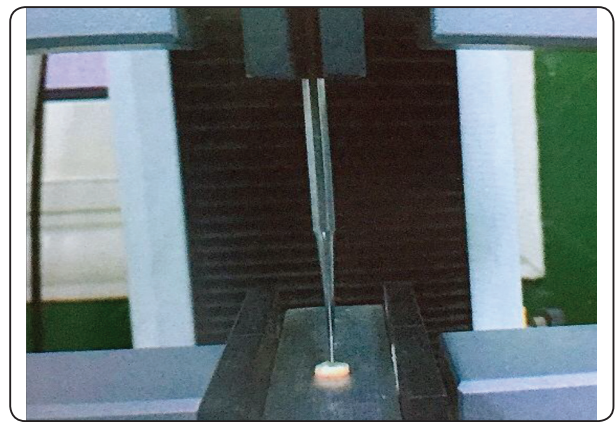

Fig. (1) Push out test (specimen under the piston of the universal testing machine)

Taking into consideration the conical shape of the post by using the following formula:

Area= π (R+ r) [(h²) + (R+ r)²] 0.5

$\pi = 3.4$

R- Radius in the coronal surface in mm

r- radius in the apical surface in mm

h- Slice thickness in mm

RESULTS

The descriptive analysis, in terms of means and standard deviation, of the push out bond strength values of fiber glass posts to intraradicular dentin subjected to the different tested (PSI) in different sites: **Tab (1), Fig (2)**

The descriptive analysis, in terms of means and standard deviation, of the push out bond strength values of fiber glass posts to intraradicular dentin in different sites: **Tab 2, Fig 3**

TABLE (1): Push out bond strength between different irrigation types in different sites

Site	Saline	EDTA	Chitosan	P value
	N=10	N=10	N=10	
Coronal third	(4.03-5.56)[a]	(17.01-19.3)[b]	(16.88-19.92)[b]	<0.001*
	4.8±0.48	18.14±0.85	18.31±0.87	
Middle third	(2.45-4.02)[a]	(14.01-16.09)[b]	(14.56-16.74)[c]	<0.001*
	3.33±0.57	15.13±0.7	15.76±0.71	
Apical third	(1.36-2.97)[a]	(9.56-12.98)[b]	(10.66-13.65)[b]	<0.001*
	2.2±0.5	11.54±0.98	12.27±0.96	

One-Way ANOVA test for quantitative data between the three groups followed by post Hoc analysis between each two groups

Superscripts with different small letters indicate significance difference between each two groups

*: Significant level at P value < 0.05

TABLE (2): Push out bond strength between different sites in different irrigation types

Irrigation type	Coronal third	Middle third	Apical third	P Value
	N=10	N=10	N=10	
Saline	(4.03-5.56)[c]	(2.45-4.02)[b]	(1.36-2.97)[a]	<0.001*
	4.8±0.48	3.33±0.57	2.2±0.5	
EDTA	(17.01-19.3)[c]	(14.01-16.09)[b]	(9.56-12.98)[a]	<0.001*
	18.14±0.85	15.13±0.7	11.54±0.98	
Chitosan	(16.88-19.92)[c]	(14.56-16.74)[b]	(10.66-13.65)[a]	<0.001*
	18.31±0.87	15.76±0.71	12.27±0.96	

One-Way ANOVA test for quantitative data between the three groups followed by post Hoc analysis between each two groups
Superscripts with different small letters indicate significance difference between each two groups
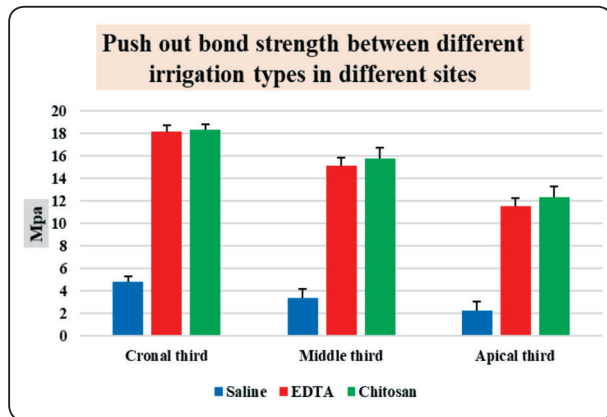**: Significant level at P value < 0.05*

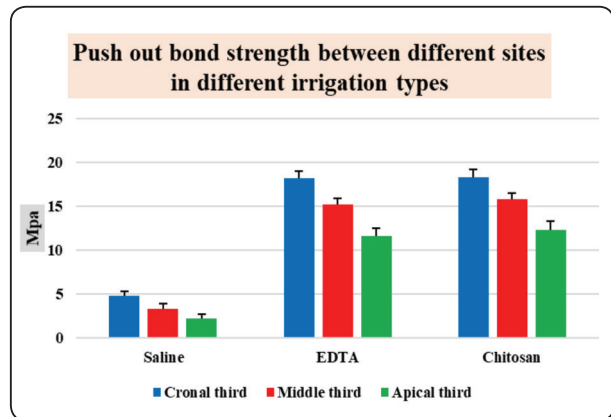Fig. (2) Push- out bond strength between different irrigation types in different sites

Fig. (3) Push out bond strength between different sites in different PSI types

One-Way ANOVA test for quantitative data between the different irrigation types followed by post Hoc analysis between each two groups shown in Tab (3).

TABLE (3): Push out bond strength between different irrigation types regardless the site

	Saline	EDTA	Chitosan	P value
	N=30	N=30	N=30	
Push out bond strength	(1.36-5.56)[a] 3.44±1.19	(9.56-19.3)[b] 14.94±2.86	(10.66-19.92)[b] 15.44±2.65	<0.001*

One-Way ANOVA test for quantitative data between the three groups followed by post Hoc analysis between each two groups

Superscripts with different small letters indicate significance difference between each two groups

*: Significant level at P value < 0.05

One-Way ANOVA test for quantitative data between the different root thirds followed by post Hoc analysis between each two groups Tab (4).

TABLE (4): Push out bond strength between different sites regardless the irrigation type

	Coronal third	Middle third	Apical third	P value
	N=30	N=30	N=30	
Push out bond strength	(4.03-19.92)[b] 13.75±6.48	(2.45-16.74) 11.41±5.85	(1.36-13.65)[a] 8.67±4.73	0.004*

One-Way ANOVA test for quantitative data between the three groups followed by post Hoc analysis between each two groups

Superscripts with different small letters indicate significance difference between each two groups

*: Significant level at P value < 0.05

DISCUSSION

Relation between the quality of final coronal restoration and the overall prognosis of an ETT has been stressed on recently due high failure percentage in well obturated teeth with bad quality coronal restoration [1, 5-9]

With the good success rate of glass fiber posts and the new advents in adhesive dentistry more practitioners are encouraged to use these systems , owing to their comparable modulus of elasticity to dentin a more favorable balanced distribution of force along the root canal walls [12, 13]. Many studies have investigated the bond strength of GFP [47-51]. Mostly comparing the different adhesive protocols used to our knowledge none have investigated the effect of chitosan on the bond strength of GFP.

Self-adhesive resin cements (Rely X U100) have good bond strength according to Bitter etal [52] and Onay etal [53] . Chemically interacting with the hydroxyapatite of dentin [54] they are moist- tolerant due to the phosphoric acid ester that requires wet dentin to develop this chemical reaction [55] . Moreover it is a dual cured resin improves its polymerization even in deep areas of the root canal where the light transmission is decreased

Even though self-adhesive systems don't mainly depend on hybrid layer formation to establish a good bond strength, adaptation to the dentin wall

increases the bond strength significantly as reported by Goracci etal [49], Monticelli etal [56].

This cement was chosen due to the rising popularity of these simply applied cements between practitioners that favor a one-step cementation system to the more complex and sensitive multistep systems. [43] However; the heavy and thick smear layer forming over the post space prepared surface is likely to jeopardize the bond strength of self-adhesive resin cements.[18-20] This justifies the investigation of effect of different PSI on the optimizing of the bond strength. EDTA has long been used as a chelating agent well documented for its ability to remove smear layer. [27, 28]

While providing the desired aim of removing the smear layer it falls short of antibacterial property and stabilizing the dentin structure in addition to the environmental pollution issue and the unfavorable alteration in collagen which favors attachment of E faecalis – microorganism strongly linked to endodontic treatment failure- this has provoked the search for new biocompatible and environmentally friendly alternative chelating agent. [25, 32-34]

Chitosan – a partially deacetylated chitin- which is known for its antibacterial, biocompatibility, biodegradability and bio adhesive properties seams as suitable alternative [41] Specially that it has been reported to provide comparable smear layer removal to that of 17% EDTA and 10% acetic acid with significantly less erosion of the peri-tubular dentin. [57]

Many researchers have encouraged the use of chitosan due to the fact that it has superior antibacterial properties and high inhibiting capabilities against recolonization of bacteria over the dentin surface. [58, 59]

Moreover; it has been shown in previous studies that chitosan improves the mechanical and biological characteristics of dentin collagen which strongly relates to bond strength [34, 58, 60, 61]

Regarding the present study human teeth were used this is because human teeth simulate- the bonding properties, modulus of elasticity, thermal conductivity and strength- of clinical situation. [62]

Teeth were decoronated to a standard root length of 15 mm this was to eliminate any coronal anatomy or access cavity variables and ensure no factorial variables were included into the study. 1% NaOCl was used in between file during endodontic treatment which is of low concentration that is reported not to interfere with resin polymerization and most probably the affected dentin surface is removed during post space preparation [63]. AH-plus root canal sealer selected to obturate the specimens which is an epoxy resin based sealer known to bond efficiently to dentin and to other composite resins and possesses no interference with polymerization of self-adhesive resin cements. [64, 65]

Efficacy of irrigation solution is determined by time, concentration, volume. The PSI protocols for this study were set at 3 minutes and a volume of 5ml which is well documented for both EDTA and chitosan to provide efficient smear layer removal while decreasing risk of erosion. [66]

GFP were subjected to salinization process; step that has been documented to improve the chemical and mechanical retention of the GFP and resin cement. [49]

Bond strength was evaluated via push out test using a universal testing machine ; a method well documented to provide reliable results [67] and applies more homogenous forces with less variability with in results and is more recommended for GFP. [68]

Regarding the push out bond strength of different PSI tested as expected the lowest bond strength was recorded with saline along the different root sites. Which comes in accordance with many previous studies. [27, 31] and was attributed to the thick heavy layer of smear formed during the post space preparation procedure and the in ability of saline to remove such a layer.

On the other hand, both EDTA and chitosan recorded significantly higher bond strength in comparison with saline group in all three regions of the root. Regarding EDTA group high bond strength which was significantly higher than saline group (p< 0.001) in agreement with previous studies.[69-71]

Due to the well documented fact that 17 %EDTA is a powerful chelating agent capable of removing the smear layer and produces a clean dentin surface that is free for adaptation and the chemical reaction of the resin to dentin to form the ionic bonding which may explain the high bond strength. [54, 72, 73, 74]

Chitosan; showed comparable results with EDTA recording high bond strength regardless of the site indicating the positive effect of application of 0.2% chitosan for 3min to dentine surface on the bond strength of fiber posts cemented with self-adhesive resins this comes in accordance with previously recorded results. [40, 41, 57]

Chitosan 0.2% is reported to produce cleaner dentinal walls and efficiently more smear layer removal than did (15% EDTA, 17% EDTA and 10% citric acid) with less erosion of the intraradicular dentin [57] . When self-adhesive cement comes into contact with clean dentin surface thought it's not likely to form dentin tags along the whole dentine wall but it's capable of chemically interacting with calcium ions of the hydroxyapatite via the carboxylic groups from the polyalkenoic acid producing an ionic bond which attributes to the bond strength. [54]

The absorbed part of chitosan or remnants that are attached to the intraradicular dentin actually has been reported to improve the mechanical and biological properties of collagen and improves the bond strength and durability. [59, 60, 75]

In addition to the antibacterial activity of chitosan that inhibits bacterial enzymes responsible for the degradation of dentin collagen. This leads to a more strong and stable dentin/ cement bond which also contribute to the bond strength. [58, 59]

Regarding the bond strength of different sites '; a significant difference was fond among the root thirds regardless of the type of PSI used. the apical third showed significantly lower bond strength for all PSI   at (2.2, 11.54, 12.27) for saline , EDTA and chitosan respectively in comparison with the middle (3.33, 15.13, 15.76) and coronal (4.8, 18.14, 18.31) P< 0.001. This was in agreement with results of previous studies[24,27]. Whom attributed the difference in bond strength to the larger diameters found in the coronal and middle thirds which provide more irrigant solution volume and contact resulting in effective smear layer removal while less volume reaches the apical third. Moreover; the smear layer which is characterized as more compacted into the dentinal tubules and densely attached to the dentin wall in the apical third while on the other hand in the coronal and middle thirds it is loose. [24, 27]

It is noteworthy that the structure of dentine itself differs apically due to the decrease in the number of the dentinal tubules and the presence of a more sclerotic dentin nature which also explains the decrease in bond strength apically than coronally. [27] Though the bond strength in (EDTA and chitosan) groups decreased within their own groups they still were significantly higher than the saline group which is probably due to the irritants' capability to remove the smear layer [27, 28, 40]

In contrast with the results of the present study many studies have found no statistically significant difference in bond strength among root regions[10, 43, 76-78]. This difference in the results may be due to the different methodologies used.

CONCLUSION

Within the limitations of the present study the following can be concluded:

1. PSI affects the bond strength of GFP luted with self-adhesive resin cements
2. Chitosan can serve as a safer alternative for EDTA as a chelating agent
3. Chitosan as a PSI has a positive influence on the bond strength of GFP luted with self-adhesive resin cements

Further studies are required to evaluate the bond strength of GFP luted with self-adhesive cements after thermocycling and in vivo to establish the durability of such protocols.

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