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Push-out Bond Strength of Resin-based Sealer After Using of Nanochitosan as a Final Irrigant: An In-vitro Study

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ABSTRACT

Background: The study aimed to assess the push-out bond strength (PBS) of resin-based sealers following three different endodontic irrigation protocols: group I (2.5% Sodium hypochlorite (NaOCl)+ saline irrigation) as a negative control, group II (2.5% NaOCl + 17% Ethylenediaminetetraacetic acid (EDTA) irrigation) as positive control, and group III (2.5% NaOCl+0.2% chitosan nanoparticles(CNPs). **Materials and Methods:** Twenty-four extracted teeth with a single root were prepared using rotary NiTi files set to size 35/.04 and standardized to a root length of 12 mm. Based on the irrigation protocol used, samples were randomly divided into three groups, with eight roots per group. Using the cold lateral compaction technique, gutta-percha and ADSEAL resin-based sealant were inserted into the canals. Universal testing machine was used to evaluate the push-out bond strength of 2-mm-thick specimens taken from the coronal third. The data was examined with the use of Bonferroni's post-hoc test ($\alpha=0.05$) and one-way ANOVA.
Results: Highest mean push-out bond strength was observed in the CNPs group (4.74 ± 0.478 MPa), followed by the EDTA group (4.59 ± 1.551 MPa), ANOVA showed a significant difference among groups ($p=0.015$). Both CNPs and EDTA groups exhibited significantly higher bond strength than the control ($p<0.05$), with no significant difference between CNPs and EDTA. **Conclusion:** Root canal dentine adhered better to resin-based sealers when 0.2% CNPs were added.

Keywords: Chitosan nanoparticles, Final irrigant, bond strength, Resin-based sealer, EDTA.

1. Introduction

The key to a successful endodontic treatment is a thorough cleaning and shape of the root canals, followed by a fluid-tight closure. While mechanically instrumenting the root canals, a smear layer of inorganic and organic materials is generated on the canal walls. This smear layer reduces the quality of obturation and makes it harder for the sealant to penetrate the dentinal tubules. For better sealer adaptation and to remove the inorganic smear layer, supplemental chelating agents are recommended as final irrigants. (Antunes *et al*., 2020; Buldur and Kapdan, 2017).

Sodium hypochlorite (NaOCl) with its potent antibacterial capabilities and ability to dissolves organic tissue, is still the most used endodontic irrigant. Nevertheless, its impact on the inorganic part of the smear layer is restricted. (Topbas and Adiguzel, 2017, Bukhari and Babaeer, 2019). EDTA is widely utilized as chelating agent in conjucation with NaOCl. While EDTA is effective, prolonged use can lead to dentin erosion, decrease microhardness, and negatively affect the bond strength of resin-

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based sealers. Additionally, its, extrusion into periapical tissues may cause irritation. (Alkhudhairy and AlRefeai, 2024; Bhatia *et al*., 2025).

Because of these restrictions, looking for safer, more biocompatible alternatives is essential. Endodontists are showing a lot of interest in chitosan since it is a naturally occurring cationic polysaccharide that is biocompatible, biodegradable, non-toxic, antibacterial, and has the capacity to chelate (Hasanin *et al*., 2018; Ferreira-Reguera *et al*., 2025). Deshpande *et al.* (2025) and Huang *et al.* (2023) found that chitosan nanoparticles (CNPs) are a potential final irrigant because of their increased surface area, higher penetration into dentinal tubules, and improved interaction with root canal dentin.

Root canal sealer's adhesive bond strength may be affected by endodontic irrigants because of its ability to alter dentin's chemical composition and structural integrity. Few research have examined how chitosan and CNPs affect the PBS of sealers to dentinal walls, despite many investigations into their antibacterial properties. Because of this, the current research set out to determine how chitosan nanoparticles endodontic irrigant affected the strength of the sealer's push-out binding to root canal dentin.

2. Materials and Methods

2.1. Ethical consideration.

The present study's research design and objective were approved by the research ethics committee of the faculty of dentistry at Minia University (119).

2.2. Sample size calculation

Using a two-sided hypothesis test and an actual power (1-β error) of 0.8 (80%) and a significance threshold (α error) of 0.05 (5%), the sample size was calculated using the G power statistical power analysis tool (version 3.1.9.4). A total of twenty-four subjects, with eight in each group, will be sufficient to detect a large effect size (f) of 0.73, according to Anju1*et al.* (2022). There were a total of eight samples separated into three groups based on the irrigation protocol: group I, which served as a negative control without acids or chelating agents, group II, which was a positive control, and group III, (2.5% NaOCl + 0.2% CNPs irrigation).

2.3. Samples preparation.

Preserved in a 0.1% thymol solution, we gathered single-rooted lower premolar teeth that were devoid of flaws, fissures, and prior root canal operations in accordance with the protocols established by the Research Ethics Committee. A diamond disc was used with water chilling to remove the dental crowns at the cementoenamel junction. The disc was positioned perpendicular to the tooth's long axis and the root length was 12 mm. The WL were determined by subtracting 1 mm from the length at which the No. 10 K-file (Mani, Japan) could be seen clearly via the apical foramen, when done visually. To mimic real-life circumstances, pink wax was applied to the root apices (Associated Dental Products Ltd, Swindone, UK) (Alhashimy and Aljadwaa, 2025).

Rotary endodontic files (Ramo medical co. Ltd, Changzhou, China) were used to shape the canals using the crown down approach until the working length was achieved and the file size reached 35 taper 4. Every time a file was changed during preparation, 2 mL of NaOCl was irrigated into the root canals for 1 minute. Two millimeters from the working length, an irrigating needle was inserted into a disposable syringe. Five milliliters of distilled water (DW) was used to flush the canals between solutions after mechanical cleaning in order to prevent chemical reactions. The next step was to administer the last irrigant to each group in a standardized amount of 5 mL for a duration of 3 minutes. As a last step, the canals were washed DW to flush out any remaining irrigant, and then they were dried with paper points. Using the cold lateral compaction method and gutta percha (Meta BioMed, Korea), the roots that were prepared were obturated. Radiographic imaging confirmed the presence of the hermetic occlusion (Ratih *et al*., 2020).

2.4. Nanochitosan preparation.

Previous research (loutfy *et al*., 2016; Hasanin *et al*., 2018; Loutfy *et al*., 2020; and Habib *et al*.,.2021) detailed the steps used to create chitosan nanoparticles (CNPs) by an ionotropic gelation process. Mixed with 1% acetic acid (pH=4), chitosan powder (middle molecular weight, DD>85%) was agitated continuously for six hours to produce a homogeneous solution. Saturated with sodium tripolyphosphate (TPP), the mixture was then gradually added while stirring continuously. The production of nanoparticles was detected when the formerly clear fluid became hazy. Washing the mixture with deionized water (DH2O) many times after centrifugation helped eliminate any unreacted components. The next step was to produce a homogenous combination of 100 ml of DDH2O and 0.2 gram of chitosan nanoparticles powder by stirring and sonication. This yielded 0.2% CNPs.

2.5. Characterizations nano-chitosan

2.5.1. Transmission Electron Microscope (TEM):

Specifically, high-resolution transmission electron microscopy (HR-TEM) was used. The produced chitosan appears in the TEM picture as having an average size of 30±5 nanometers, indicating that it is in the nanoscale. (figure 1).

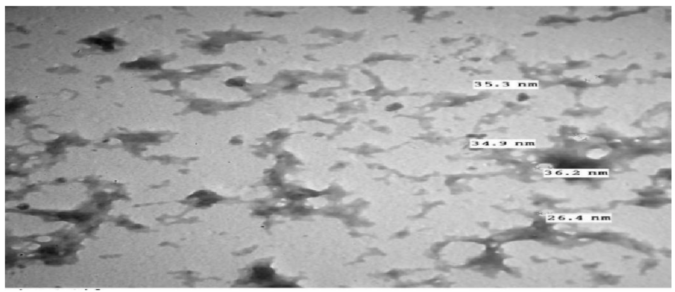

Fig. 1: TEM image shows average size of nanoparticles

2.5.2. Scanning Electron Microscope (SEM)

SEM is a powerful tool for studying nanoparticle shape and profile. SEM image of chitosan nanoparticles show that they have a spheroidal shape. (figure 2).

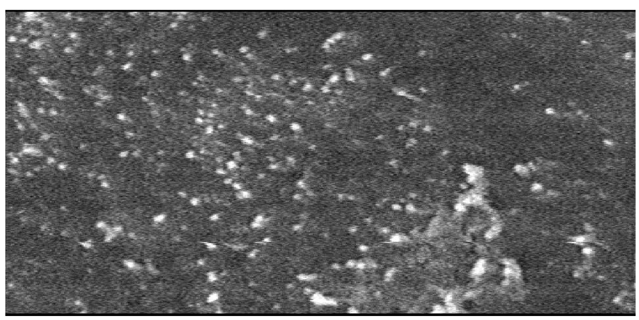

Fig. 2: SEM image shows spheroidal shape of chitosan nanoparticles.

2.6. Push-out bond strength test

According to Veeramachaneni *et al.* (2022), the test was conducted. With the resin-based sealer ADSEAL (Meta Biomed, Cheongju-si, Korea) fully set. The prepared roots were embedded using a cylindrical Teflon mold and self-curing acrylic resin. After that, sections were cut from the cervical third of the sample using water that was continuously cooled to a thickness of 2±0.05 mm. A digital caliper was used to verify the thickness of the sample. To facilitate fixation on the universal testing machine (Instron 3366,USA.), the sliced samples were centered within a copper ring mold measuring 16±0.05 mm in diameter and 3±0.05 mm in height. Lastly, the surface that is opposite the loading plunger was marked on the apical side of every slice. A stainless-steel plunger tip cylindrical in shape measuring 0.8±0.05 mm was positioned in the middle of the core material to exert an apical-coronal dislodging force at a crosshead speed of 2 mm/min until debonding occurred. A formula was used to quantify the dislodging force in Newtons and convert the maximal value to mega-pascals (Mpa):

$$\textbf{Bond strength} = F/A\ (\boldsymbol{\pi h\ (r_1 + r_2)})$$

F = maximum force (N) (recorded from the universal testing machine)

A = lateral area $= \boldsymbol{\pi h\ (r_1 + r_2)}$ π = Constant 3.14
r_1 = apical radius r_2 = coronal radius h = thickness of the sample in millimeters

2.7. Statistical analysis.

To ensure that the data distribution was normal, the Kolmogorov-Smirnov and Shapiro-Wilk tests were used. Since the data were found to have a normal distribution, the results were presented as the mean and standard deviation. Analysis of variance (ANOVA) and Bonferroni's post hoc test were used for the intergroup comparisons. A significance threshold of $p \leq 0.05$ was used for the test.

3. Results

Group III (NaOCl + CNPs) has the highest mean value of PBS (4.74 Mpa±0.478), followed by Group II (NaOCl + EDTA) (4.59Mpa±1.551). Group I (NaOCl + saline) has the lowest mean (3.02 Mpa±0.529). ANOVA test revealed a significant different between groups (p=0.015). Post hoc showed no significant difference between groups II and III, which were both significant higher compared to Group I (Table 1, Figurre3)

Table 1: Descriptive statistics analysis for Push-out bond strength (Mpa) and comparison between groups (ANOVA test).

Group	Mean	Std. Dev.	Min	Max	P value
Group I (NaOCl+saline)	3.02^{b}	0.529	2.33	3.77	
Group II (NaOCl+EDTA)	4.59^{a}	1.551	3.29	7.20	**0.015***
Group III (NaOCl+CNPs)	4.74^{a}	0.478	4.25	5.58	

Significance level $p \leq 0.05$, *significant
Post hoc test: values with the same superscript letter are not significantly different

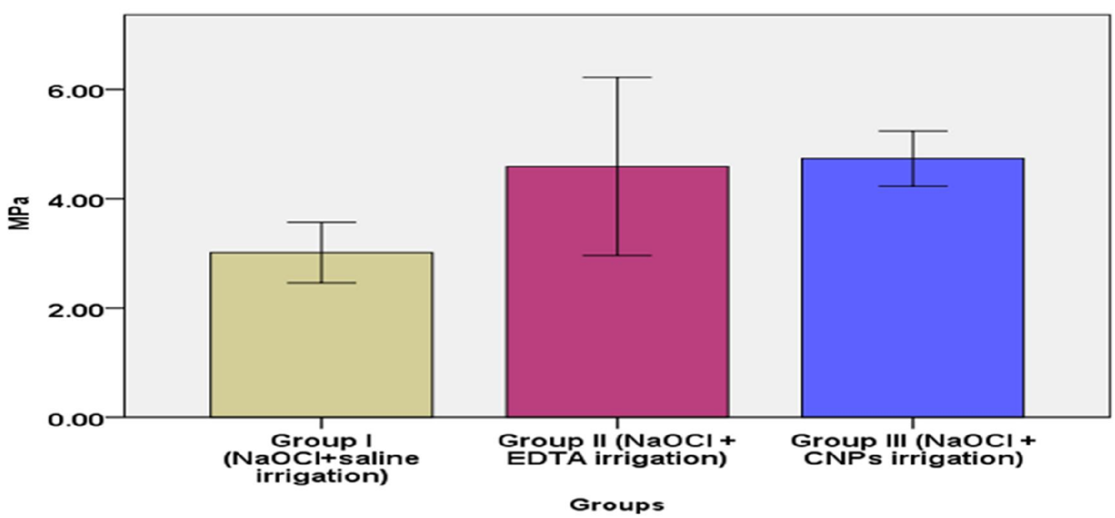

Fig. 3: A bar graph showing the mean push-out bond strength (MPa) for each group.

4. Discussion

The main goals of endodontic irrigation are to flush out bacteria and pulp tissue remains, especially in hard-to-reach places like the apical third where mechanical instruments cannot reach. The binding strength of the sealer to dentin directly affects sealing ability and resistance to leakage, thus it's important to choose a sealer that adheres well to both the dentin wall and the obturating substance for optimal obturation. Endodontic sealers' binding strength to dentin is an important quality since it reduces the likelihood that the root canal filling material will come loose during masticatory force. (Buldur and Kapdan 2017; Gomes *et al*., 2023).

This in vitro research minimized anatomical heterogeneity by standardizing single-rooted human teeth with straight canals to a root length of 12 mm. Pink wax was used to plug the apical foramen of each tooth in order to mimic the anatomical circumstances. The resin-based sealer's adherence was evaluated using the PBS test because it allows for the determination of bond strengths even at low values and offers a valid shear stress evaluation at the dentin-sealer interface (Alhashimy and Aljadwaa 2025).

Previous research (Ratih *et al*., 2020) indicated that regardless of the irrigant utilized, a 3-minute application time resulted in considerably greater bond strength and better apical sealing compared to a 1-minute application time. Therefore, this duration was chosen for simple and standardized comparison of the final irrigant.

The results demonstrated that final irrigation with NaOCl followed by chitosan nanoparticles (CNPs) yielded the highest push-out bond strength (4.74 ± 0.478 MPa), after that (NaOCl + EDTA; 4.59 ± 1.551 MPa). Both were significantly superior to the control group (NaOCl + saline; 3.02 ± 0.529 MPa).

These results are in line with a number of earlier investigations that reported comparable bond strengths between 0.2% chitosan nanoparticles and 17% EDTA, including (Bayram *et al.,* 2017; Mazzi-Chaves *et al*.,2019; Antunes *et al.,* 2019; Ratih *et al.,* 2020; Mohamed *et al.,* 2021 and Salem *et al.,* 2023). Additionally, the enhanced bonding observed in the study of Youssef *et al.* (2023), who reported that the 0.2% CNPs group had significantly stronger bonds than the EDTA group.

Wetting ability, sealer surface tension, dentin structure intermolecular surface energy, and smear layer presence or absence are among the factors that might affect adhesive characteristics (Bayram *et al.,* 2017). Prior research indicated that by using 0.2% nanochitosan, the smear layer may be effectively eliminated, leading to enhanced mechanical interlocking and deeper penetration of the sealer. Thus, the primary explanation for the enhanced performance is that the smear layer is effectively removed by both EDTA and CNPs, allowing the sealer to penetrate dentinal tubules more deeply and improving mechanical interlocking. The hydrophilic nature and quantity of hydroxyl and amino groups in chitosan nanoparticles provide them significant chelating capacity and great wetting capabilities. These qualities allow them to promote close contact and ionic interactions with dentin (Monika *et al.,* 2025).

5. Conclusion

The in vitro study's drawbacks were that the resin-based sealer had a PBS of 0.2% after a final rinse with NaOCl that was statistically similar to 17% EDTA and much better than the saline control, as well as the highest PBS to root dentin surface. These results suggest that CNPs, probably as a result of their chelating capacity, hydrophilicity, and nanoscale characteristics, efficiently eliminate the smear layer, allowing for improved sealer penetration and mechanical interlocking with dentin. As a safer and more effective final irrigant, 0.2% chitosan nanoparticles show great potential in contemporary endodontic therapy.

The focus of future research should be on doing in-vivo investigations that include clinically relevant aspects. Further investigation is required to evaluate the efficacy of 0.2% chitosan nanoparticles and 17% EDTA as final irrigants in removing the smear layer.

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