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*Research Article*

# The Influence of Nano Chitosan as Final Irrigation on Push-Out Bond Strength of Different Endodontic Sealers

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## Abstract

**Aims:** The present study aimed to evaluate the influence of Chitosan nanoparticle irrigants at two concentrations (0.2% and 0.5%) and compare them with other irrigants on the push-out bond strength of obturation endodontic sealers.

**Materials and Methods:** Fifty-four human lower premolar teeth with single, straight roots were extracted and utilized. Teeth were prepared to a 12 mm length. Biomechanical preparation of the canal was carried out through the ProTaper universal system. Six Groups were developed depending on the final irrigation. GI: 0.2% Chitosan nanoparticles (CNPs), GII:0.5% Chitosan nanoparticles (CNPs), GIII: 17% Ethylenediaminetetraacetic acid (EDTA), GIV: 3% Sodium hypochlorite (NaOCl), GV: 2% Chlorohexidine (CHX), and GVI: Distilled water as the control group. Afterward, the groups were subdivided into 3 subgroups (n=3) based on the sealer used: (A) AH Plus sealer, (B) MTA Fillapex sealer, (C) EndoFill sealer. F3 gutta-percha cone and a single cone procedure were used to fill all samples with sealer. From each sample, three slices, 2 mm thick, were cut at 3, 7, and 11 mm from the apex. They were labeled coronal, middle, and apical in that order. A universal testing machine was used to analyze push-out bond strength (POBS), and a stereomicroscope with a 40X magnification was utilized to examine the mode of failure. Then, the data were collected and analyzed statistically.

**Results**: The highest mean Push-out bond strength (POBS) at the apical third is Chitosan nanoparticles (CNP), followed by Ethylenediaminetetraacetic acid (EDTA), then Sodium hypochlorite (NaOCl), 0.5% Chitosan nanoparticles (CNP) significantly differs from 0.2% Chitosan nanoparticles (CNP). Chlorohexidine (CHX) increased Push-out bond strength POBS of AH Plus sealer and MTA Fillapex at coronal and middle, respectively. The lowest mean Push-out bond strength (POBS) is Sodium hypochlorite (NaOCl) and distilled water

**Conclusion**: Chitosan nanoparticles (CNPs) are a new final washing improve bond strength of resin-based and MTA-based sealers better than 17% Ethylenediaminetetraacetic acid (EDTA). And 2% Chlorohexidine (CHX) is as effective as chitosan nanoparticles (CNPs) in improving bond strength of resin-based AH Plus, MTA Fillapex and EndoFill sealers.

**Keywords**: Chitosan nanoparticles (CNPs), EDTA, POBS, Sealer.

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**INTRODUCTION**

The quality of obturation is improved by removing the smear layer and by the root canal sealer's adherence to the dentinal tubules. This layer can hinder the adaptation of endodontic materials to dentinal walls, weaken the binding strength of endodontic sealer, and prevent intracanal medications and disinfectants from entering the dentinal tubules. This is avoided by using a variety of irrigants, with the final solution being especially crucial for smear layer removal and disinfection. (1)

Sodium hypochlorite (NaOCl) is frequently selected among irrigation solutions because of its many advantageous qualities, including its ability to effectively remove remaining pulp tissue and the organic component of the smear layer produced during biomechanical preparation, as well as its antibacterial effect. NaOCl has several drawbacks, including toxicity, dentin degradation due to collagen breakdown, and inability to remove inorganic components of the smear layer so advised to use the chelating agent EDTA. It has been shown that NaOCl prevents AH Plus sealers from polymerizing when used as a last wash. (2)

A biocompatible disinfectant with a wide range of antibacterial action is chlorhexidine (CHX). CHX can enhance root canal sealing by decreasing the activity of collagen-disruptive matrix metalloproteinases in radicular dentin. (3) EDTA, the most used chelating agent, is utilized as a last rinse to eliminate the smear layer that develops during canal shaping. Soluble calcium chelates are created when EDTA joins the calcium ions $Ca2+$ in the dentin. (4)

Nowadays, nanotechnology is widely used. Nanotechnology was employed in the dental industry as nano dentistry. (5) Materials with constituent sizes ranging from 0 to 100 nm are referred to as nanomaterials. Atoms, grains, fibers, films, and nano-holes are examples of nanomaterials. These nanomaterials have better physical, chemical, and biological properties. (6)

Chitosan (CH), a naturally occurring polymer that is generated from chitin, is present in the shells of crustaceans, such as crabs and shrimp. (7) Chitosan is thought of as a chelating agent because of its capacity to bond with proteins, lipids, cholesterol, and metal ions. Because it is biodegradable into harmless residues and possesses mucoadhesive, analgesic, hemostatic, antibacterial, and antifungal activities, it is superior to EDTA. Chitosan is safe profile, biodegradability, and biocompatibility, with fungi- and bacteriostatic properties. (8) Numerous techniques, such as ionotropic gelation, micro-emulsion, emulsification solvent diffusion, and emulsion-based solvent evaporation, can be used to create chitosan nanoparticles. (9)

The final root filling's quality is also affected by the physicochemical characteristics of root canal sealers, which also have biological significance. (10) Sealers are mainly used to fuse core material (gutta-percha) to the root canal wall to generate monoblocks. (11) Epoxy resin sealers, such as AH Plus, are steadily outperforming other sealers because of their advantageous physical characteristics, decreased solubility, suitable biological achievement, superior sealing capability, and increased micro retention to radicular dentin. (12)

Biocompatible bioceramic sealants have been inserted in the market because they do not shrink upon setting and are thought to reduce the inflammatory response in the event of an overfill. Recently released, MTA Fillapex is a calcium silicate-based bioceramic sealer that combines the biological qualities of MTA with the physicochemical characteristics of a resin-based root canal sealer. Moreover, it can sustain a comparatively steady calcium release for up to 14 days and exhibit a high pH value for a long-lasting antimicrobial impact. MTA Fillapex exhibits a high flow rate (27 mm) and a minimal film thickness, facilitating its penetration into lateral and accessory canals, MTA Fillapex provides exceptional sealing efficacy that remains unaffected by thermal conditions. However, this sealer has disadvantages as lack of solubility and staining of the tooth. (13)

The push-out bond strength (POBS) test is a credible technique for determining a material's binding strength. (14)

The null hypothesis states that there was no difference in the effect of the last washing by CNPs solution and other solutions on POBS of endodontic obturation materials.

The purpose of this study is to evaluate the influence of Chitosan nanoparticle irrigant with two different concentrations (0.2%, 0.5%) and compare them with other irrigations on push-out bond strength of AH Plus, MTA Fillapex, and EndoFill sealers.

## MATERIALS AND METHODS

The University Ethics and Research Committee reviewed and approved this study in compliance with the code (**UoM.Dent.23/73)**. Fifty-four human lower mandibular premolar tooth samples were taken from unidentified individuals between the ages of 15-35 at an orthodontic center in Mosul. Inclusion criteria: single-rooted, single canal, closed apex without caries or calcification, no external or internal resorption, and root canal treatment. Exclusion criteria: Teeth with fractures or cracks, immature open apices, dilatation, or anatomical or morphological abnormalities were excluded. Angulated radiographs were taken in the buccolingual and mesiodistal directions to identify teeth having a single canal. Furthermore, any tooth with cracks or root abnormalities was excluded using a 40X stereomicroscope. A schematic diagram of the experimental work and sample distribution is summarized in Figure 1.

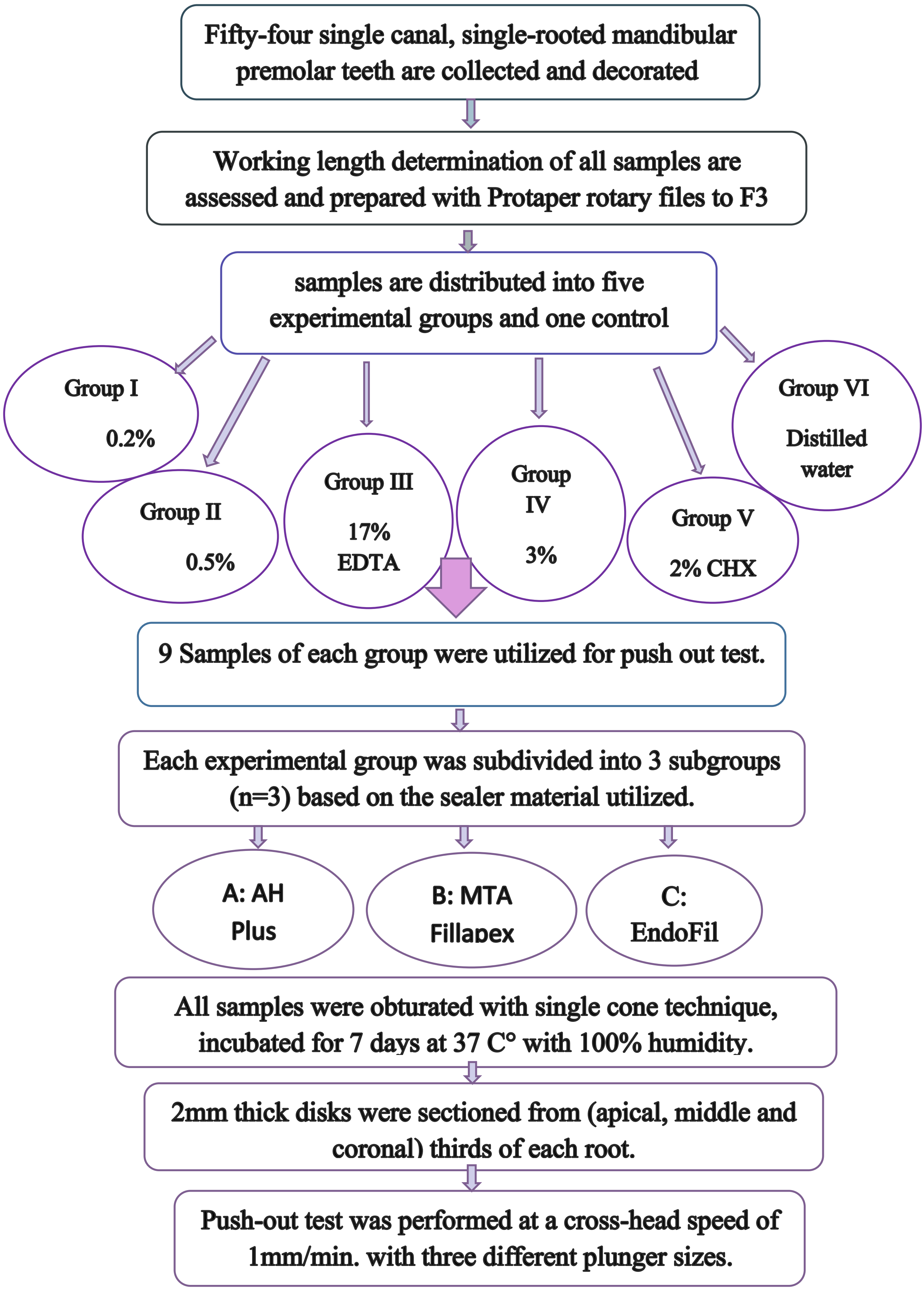


**Figure. 1:** Schematic diagram of the experimental work and sample distribution

To standardize a working length of 12 mm, the crown of every premolar was trimmed using a diamond disc 0.2mm (NEXUS MEDODENT, Korea) transverse to the long axis of the tooth with plenty of water as cooling. All the canals were accessed, and a barbed broach was utilized for pulp extirpation. A size 10 K-file was used to verify apical patency. Subsequently, a K-file of size 15 was put into the canal; it was carefully pushed back and forth in a reciprocating movement until the tip of the file was visible at the apical foramen. An endodontic ruler was used to measure this distance. The visually assessed canal length was subtracted by 1 mm to determine the corrected working length (WL).

A system of closed canals was established. Each root of a decoronated tooth had its exterior surface covered by tray adhesive bond, and the root's apex was coated with wax, which was left to solidify before being reinserted in its stimulated socket in a polyvinyl chloride (PVC) tube filled with polysiloxane elastomer impression material (Zetaplus, Zhermack, Badia Polesine, Italy) to be placed in the surveyor for easy canal preparation and obturation. All canals were shaped and prepared with Nickel-Titanium ProTaper universal rotary system files from size Six to F3 in a crown-down manner connected to an Eighteenth endo-motor with a torque of 3 Newton/centimeter and a velocity of 250 revolutions per minute, following the manufacturer's instructions. Between each file, the canals were cleaned with 2 mL of 2.5% NaOCl for 2 minutes, and apical patency was checked. A gauge 30 double-sided vent syringe was utilized for the irrigation of the canals. At the end of instrumentation, 5 mL of distilled water was flushed into the canals to get rid of any remnants of NaOCl.

**Preparation of Irrigating Solution**

**A. 0.2% chitosan nanoparticle solution**

The preparation of 0.2% nano chitosan solution was prepared by dissolving 2 gm of nano chitosan powder (78 nm) (Shaanxi Sangherb Bio-Tech Inc CO., Ltd, China) was weighed in a high-precision portable balance (Mettler Toledo PL-S Classic Light, UK) in V:100 ml of 1% acetic acid. A magnetic stirring machine (DAIHAN LABTECH CO., LTD) was employed to stir the mixture for 2 hours at room temperature (±23℃) till a crystalline, homogeneous solution (PH 3.8). (15)

**B. 0.5% Chitosan nanoparticles solution**

To prepare 0.5% chitosan nanoparticles. About 1 ml of glacial acetic acid was added to 100 ml of distilled water to obtain 1% acetic acid. Then 5 gm of nano Chitosan powder (78 nm) (Shaanxi Sangherb Bio-Tech Inc CO., Ltd, China) was weighed in high precision balance and added to the solution above of 1% acetic acid. The mixture was agitated using a magnetic stirrer to produce a viscous solution (PH 4.1) for 2 hours at room temperature (±23℃). (16)

**Sample Grouping**

Six groups were developed based on final irrigation; each group contained 9 samples and was irrigated finally as the following:

**Group I**: Samples were lastly washed with 3ml of 0.2% CNP.

**Group II**: Samples were lastly washed with 3ml of 0.5% CNP.

**Group III**: Samples were lastly washed with 3ml of 7% EDTA

**Group IV**: Samples were lastly washed with 3ml of 3% NaOCl.

**Group V**: Samples were lastly washed with 3ml of 2% CHX.

**Group VI**: Samples were lastly washed with 3ml of distilled water.

Using a 3-mL syringe and a 30-G double-side-vented needle, 1 mL of final irrigant was poured into the root canal for 3 min. In the last 20 s, the remaining 2 mL of irrigant was expressed into the root canal by inserting the needle 1 mm short of the working length and moving in an up-and-down motion with an amplitude of 1–2 mm. Finally, as a standard for all groups, 10 mL of distilled water was used for the last flush to get rid of any irrigation residue. Then, dry all canals with absorbent paper points size F3. Based on the type of sealer used to obturate the canal, each group was randomly split into three subgroups (n=3).

**Group A:** Canals were obturated with AH Plus sealer (Dentsply, Germany), which was mixed in uniform volume of both pastes on a mixing pad with a plastic spatula in accordance with the manufacturer's instructions to obtain a homogeneous consistency of the sealer that was delivered into canals using a 30-lentulo spiral.

**Group B**: Canals were obturated with MTA Fillapex sealer **(A**ngelus /Brazil), which was mixed using a self-mixing tip attached to a syringe and was delivered into root canals in the same manner as the previous sealer.

**Group C:** Canals were obturated with EndoFill sealer (PD, Switzerland), which was mixed according to the manufacturer's directions of powder and liquid to form a paste consistency and was delivered into root canals in the same manner as the previous sealer.

The single-cone obturation approach was used to produce root canal obturation in each group. Up to working length, a standardized gutta-percha cone that had the same size as the master apical file was placed into the root canal. To achieve tugback to a predefined working length, an F3 Pro-Taper master cone was first inserted within each canal. Lentulo spiral fixed on a contra-angle, low- speed handpiece. Two-thirds of the lentulo were covered with sealer and inserted into the canal at a speed of 300 rpm in a clockwise direction, 1 mm short of the working length. Lentulo spiral standardization was applied three times in all canals for a duration of 50 seconds each. With the handpiece rotating continuously, the lentulo was gradually extracted from the canal. In order to achieve maximal sealer distribution inside the canal, the tip of the F3 master cone was also dipped in a thin layer of its respective sealer and introduced into the root canal slowly, moving up and down the pump as it did so, until it reached the necessary working length.

Extra-coronal gutta-percha was removed using heated plastic tools, and vertical compression was performed with a plunger 1 mm below the orifice level, and then the coronal access of all root canals was closed with a flowable composite barrier. The samples were then maintained moist by being wrapped in saline-soaked gauze and put in an airtight container to ensure that the sealer set completely. To fully set, they were incubated for 7 days at 37 degrees Celsius and 100% humidity.

**Sectioning Procedure**

Each sample was embedded in chemically cured acrylic resin block

Each sample was embedded in a chemically cured acrylic resin block

Each root was centrally and vertically embedded in a clear cold-cured acrylic resin custom-made mold block with 15mm in length and 10mm in width. The acrylic molds were marked with a waterproof marker to serve as a guide for sectioning, and each specimen's apical surface was marked as well.

Each root block was fixed with a vice bench to facilitate handling and sectioning that was carried out in horizontal plane perpendicular to the long axis of the root with a diamond disc (0.2 mm thickness) at low speed on straight handpiece with constant fresh cooling water into three equal sections (2mm thickness) where the most apical 3 mm was discarded and the remaining 2 mm apically represented the apical section, then a slice of 2 mm height was cut at 7 and 11 mm from the apex to represent the middle and cervical sections, correspondingly. The precise height of the slices was checked using a digital caliper. Each section was viewed under stereomicroscope (Optika, Italy) at 20 X magnification from apical and coronal surfaces to check there were no voids in sealer. Only a circle-shaped root section with a uniform sealer layer was chosen in order to guarantee a uniform distribution of force during push-out testing and, consequently, precise measurements. Before putting the samples through the push-out test, the diameter of the canal in both the apical and coronal aspects was measured using a 40x magnification stereomicroscope (Optika, Italy). The sections were then maintained at 100% humidity until the test.

A cylindrical plunger installed on a universal testing machine was used to apply a compressive force on each slice's apical aspect to prevent any obstacles during the push-out testing due to the tapering of the root canal. To calculate the punch pin size, the obturated region of the slice at each level was measured from the apical surface. To load the root filling of each part, three distinct plunger sizes 0.7, 0.5, and 0.3 mm that corresponded to the coronal, middle, and apical sections, respectively, were employed. The tip of the plunger was modified to conceal root filling material without coming into contact with the canal wall. Every section was set up on a metal base with a hole in the center and an apical surface facing the plunger that was smaller in diameter. (17) To enable the filling material to readily fall through the hole, the center of the tested specimen was positioned over it once the bond between the test material and the dentin wall had been broken. Until the first dislodgment of the obturating material and a sharp drop in load deflection, a cross-head speed of 1 mm/min was used. The greatest force used for debonding was recorded in Newtons (N). The greatest debonding force, measured in newtons (N), divided by the surface area adhesion (A) (mm2) yielded the sealer's bond strength, which was measured in megapascals (MPa. (18)

**Bond Strength (MPa)=debonding force (N) /Surface area ($mm^2$)(A)**

$$\mathbf{A(mm^2) = \pi(r_1 + r_2)\sqrt{[(r_1 - r_2)^2 + h^2]}}$$

**π= 3.14, h= the thickness of section in mm, $r_1$= is the coronal radius, $r_2$= is the apical radius.**

**Failure mode assessment**

Following the push-out test, a stereomicroscope (Optika, Italy) with a 40X magnification was used to investigate the failure mode. Three types of failures were identified: type I (adhesive failure at the sealer-dentin interface, no material remains on the canal wall), type II (cohesive failure within the obturating material, with material present on the entire canal wall), or type III (mixed failure in both dentin and sealer, with material present on the canal wall as patches).

**Statistical Analysis**

All collected data were subjected to statistical analysis with descriptive statistics (Mean, standard deviation, standard error, minimum, and maximum) by the Statistical Package for the Social Sciences (SPSS) software, version 24, for Windows (IBM, Armonk, New York). According to the test of normality (Shapiro-Wilk test), which states that the measurements do not follow a normal distribution, which prompted us to use nonparametric tests to find differences between the groups at different thirds. Kruskal-Wallis was used. The level of significance was set as $p \leq 0.05$

## RESULTS

The descriptive statistics (mean, standard deviation) of push-out bond strength values for each sealer subgroup (A, B, C) in different root thirds (apical, middle, and cervical) that were irrigated with six groups are shown in Tables 1, 2, and 3 and Figure 2. GI showed higher mean bond strength values (11.79 ± 9.27) of the A group, (12.43 ± 11.74) of the B group, and (9.8 ± 13.82) of the C group at the apical level than at the middle and cervical levels, although there was no significant difference. GII showed higher mean values at the apical level than the middle and cervical levels of A, B, and C (13.79 ± 8.26), (9.41 ± 4.96), and (9.41 ± 4.96), respectively, although there was no significant difference. GIII showed a higher mean value at the apical level than the middle and coronal levels of A (5.54 ± 4.13), B (5.72 ± 2.57), and C (4.76 ± 2.96). The chelating agents 0.2 and 0.5 CNPs have a positive effect on push-out bond strength of all sealers compared to 17% EDTA. GV showed higher mean push-out bond strength values (9.63 ± 14.03) at the cervical of Group A, at the middle third (9 ± 12.23) of Group B, and apical third (9.34 ± 11.89) of Group C. CHX improved dislodgement resistance of AH Plus and MTA Fillapex sealer. GIV higher mean (5.42 ± 4.95) at the apical than middle and cervical levels of A. NaOCl has a negative effect on the push-out of AH Plus.

**Table 1:** Descriptive statistics (mean and standard deviation) for three tested sealers in six groups at apical third

| Groups | AH Plus Mean (MPa) ± SD. | MTA Fillapex Mean (MPa) ± SD. | EndoFill Mean (MPa) ± SD. |
|---|---|---|---|
| **Group I** | 11.79 ± 9.27 | 12.43 ± 11.74 | 9.8 ± 13.82 |
| **Group II** | 13.79 ± 8.26 | 9.41 ± 4.96 | 9.41 ± 4.96 |
| **Group III** | 5.54 ± 4.13 | 5.72 ± 2.57 | 4.76 ± 2.96 |
| **Group IV** | 5.42 ± 4.95 | 3.87 ± 3.58 | 4.31 ± 2.9 |
| **Group V** | 3.18 ± 4.7 | 3.95 ± 3.41 | 9.34 ± 11.89 |
| **Group VI** | 5.83 ± 2.7 | 6.07 ± 3.12 | 6.56 ± 2.67 |
| ***P*-value** | 0.584 | 0.798 | 0.77 |

SD: Standard deviation, MPa: Megapascal

**Table 2:** Descriptive statistics (mean and standard deviation) for three tested sealers in six groups at the middle third

| Groups | AH Plus Mean (MPa) ± SD. | MTA Fillapex Mean (MPa) ± SD. | EndoFill Mean (MPa) ± SD |
|---|---|---|---|
| **Group I** | 11.62 ± 11.34 | 7.33 ± 3.45 | 9.32 ± 3.93 |
| **Group II** | 13.67 ± 4.89 | 7.33 ± 3.45 | 9.17 ± 11 |
| **Group III** | 4.6 ± 3.77 | 4.65 ± 4.98 | 4.57 ± 5.09 |
| **Group IV** | 3.85 ± 3.54 | 1.13 ± 1.22 | 1.11 ± 1.24 |
| **Group V** | 1.43 ± 1.86 | 9 ± 12.233 | 4.99 ± 1.8 |
| **Group VI** | 2.08 ± 0.87 | 3.48 ± 3.22 | 3.2 ± 3.53 |
| ***p*-value** | 0.544 | 0.415 | 0.485 |

SD: Standard deviation, MPa: Megapascal

**Table 3:** Descriptive statistics (mean and standard deviation) for three tested sealers in six groups at cervical third

| Groups | AH Plus Mean (MPa) ± SD. | MTA Fillapex Mean (MPa) ± SD. | EndoFill Mean (MPa) ± SD. |
|---|---|---|---|
| **Group I** | 3.39 ± 4.3 | 3.14 ± 3.89 | 6.86 ± 6.44 |
| **Group II** | 1.08 ± 1.02 | 3.14 ± 3.89 | 2.31 ± 2.07 |
| **Group III** | 3.52 ± 4.92 | 2.04 ± 1.49 | 2.16 ± 1.37 |
| **Group IV** | 2.94 ± 4.32 | 2.47 ± 1.93 | 2.53 ± 1.85 |
| **Group V** | 9.63 ± 14.03 | 1.81 ± 1.48 | 2.06 ± 1.08 |
| **Group VI** | 2.73 ± 1.48 | 2.25 ± 1.31 | 1.2 ± 1.2 |
| ***p*-value** | 0.91 | 0.997 | 0.865 |

SD: Standard deviation, MPa: Megapascal

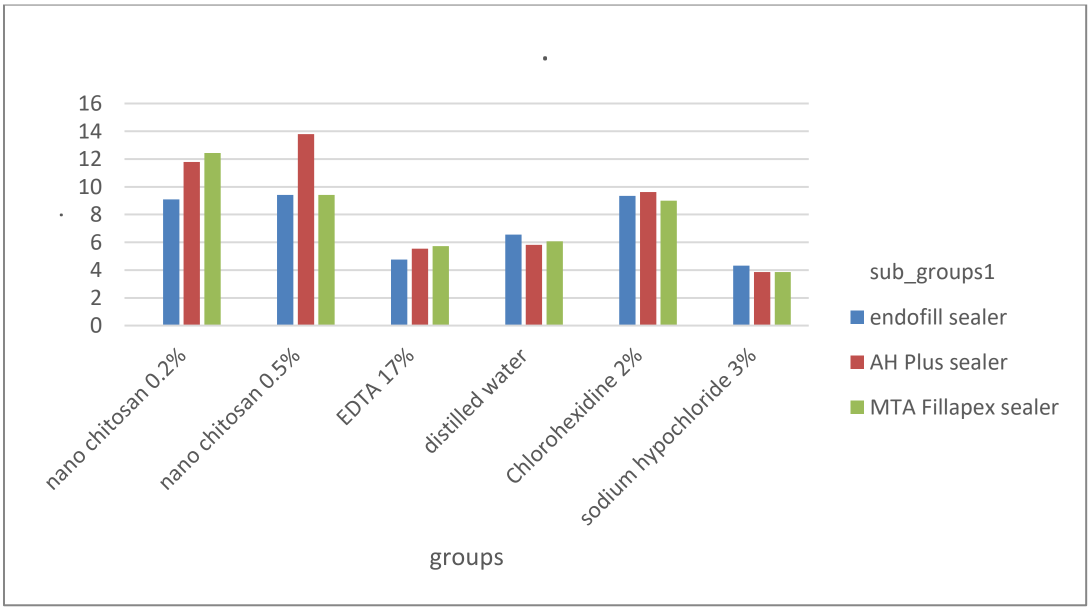


**Figure. 2:** Histogram of the mean POBS for each sealer: A: AH Plus, B: MTA Fillapex, C: EndoFill with irrigation groups.

The highest mean values were shown at the apical level and the lowest at the cervical level. However, the difference between the mean values of all thirds was not significant. Regardless of the root canal sealer used, the mean values were statistically significantly higher within group 0.5% CNPs ($p<0.05$) when the groups were compared based solely on the irrigation solutions. Other irrigation solution groups did not show any statistically significant differences (Table 4).

**Table 4:** Comparisons of POBS means for different root sections in each experimental group alone.

| Groups | Root levels | Mean ± SD | p- value |
|---|---|---|---|
| | Apical | 3.24 ± 2.98 | |
| G I | Middle | 2.88 ± 2.59 | 0.67 |
| | Coronal | 1.75± 2.89 | |
| | Apical | 4.67 ± 1.81 | |
| G II | Middle | 2.19 ± 1.02 | 0.004 (**) |
| | Coronal | 1.95 ± 1.93 | |
| | Apical | 4.35 ± 2.08 | |
| G III | Middle | 2.44 ± 1.76 | 0.071 |
| | Coronal | 1.64 ± 1.25 | |
| | Apical | 2.62 ± 1.86 | |
| G IV | Middle | 1.8 ± 1.77 | 0.49 |
| | Coronal | 1.6 ± 1.41 | |
| | Apical | 3.59 ± 2.16 | |
| G V | Middle | 2.62 ± 2.07 | 0.42 |
| | Coronal | 1.53 ± 1.16 | |
| | Apical | 3.16 ± 0.55 | |
| G VI | Middle | 2.43 ± 2.15 | 0.3 |
| | Coronal | 1.72 ± 1.38 | |

**Failure mode analysis**

The three modes of failure are identified under a stereomicroscope (Original magnification X40) and illustrated in Figure 3. The failure mode percentages are reported in Table 5 for each experimental group at each section.

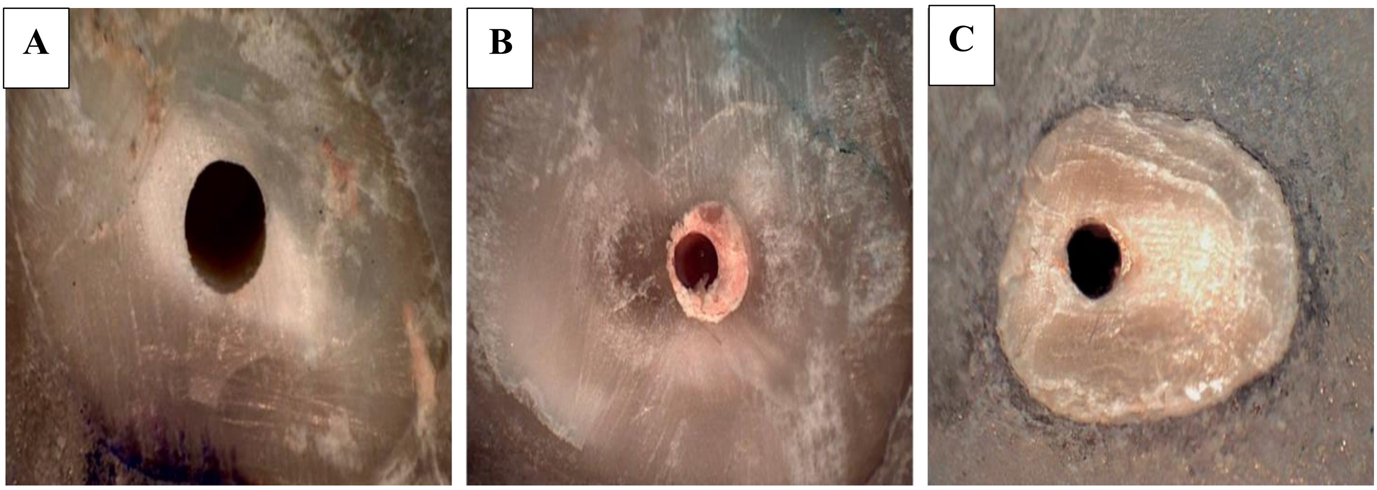


**Fig. 3:** Stereomicroscope view mode of failures (Original magnification X40) it: (A) Adhesive failure, (B) Cohesive failure, (C) Mixed failure

**Table 5:** Percentages of failure modes for the different groups at different root sections

| Grs | Root leve | AH Plus | | | MTA Fillapex | | | EndoFill | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Adhesive | Cohesive | Mixed | Adhesive | Cohesive | Mixed | Adhesive | Cohesive | Mixed |
| GI | Apical | 33.3% | | 66.66% | | | 100% | 100% | | |
| | Middle | 66.66% | | 33.3% | 100% | | | 66.6% | | 33.3% |
| | Coronal | 33.33% | 33.33% | 33.33% | 100% | | | 100% | | |
| G II | Apical | | | 100% | | | 100% | 100% | | |
| | Middle | 33.3% | | 66.6% | 100% | | | 33.33% | | 66.66% |
| | Coronal | 33.33% | | 66.66% | | | 100% | 100% | | |
| G III | Apical | | | 100% | | | 100% | 33.3% | | 66.66% |
| | Middle | 66.66% | | 33.33% | | | 100% | 100% | | |
| | Coronal | | 66.6% | 33.33% | | 66.66% | 33.33% | | | 100% |
| G IV | Apical | | | 100% | | | 100% | 100% | | |
| | Middle | 100% | | | | 66.66% | 33.33% | | | 100% |
| | Coronal | 33.33% | | 66.66% | 33.3% | 66.66% | | 100% | | |
| G V | Apical | | | 100% | | | 100% | 33.33% | | 66.6% |
| | Middle | | | 100% | 100% | | | 100% | | |
| | Coronal | | 100% | | 100% | | | | | 100% |
| G VI | Apical | 66.6% | | 33.3% | 66.6% | | 33.33% | 33.3% | | 66.6% |
| | Middle | 66.6% | 33.3% | | 100% | | | 100% | | |
| | Coronal | 66.66% | | 33.3% | 33.33% | | 66.6% | 100% | | |

## DISCUSSION

The process of adhesion of the sealer to the dentin root and the core material (gutta-percha) is a complex process depending on the type of sealer and the conditions of the dentin. The endodontic sealer's adhesive capability is gauged by its bond strength to dentin, often known as its dislodgement resistance. The success of endodontic therapy will be increased if there is a strong bond interaction between the sealer and the core material as well as between the sealer and the radicular dentine(19).

Chitosan nanoparticles provide several benefits in endodontics, including the ability to eliminate the smear layer, chelating capabilities when employed as an irrigation agent, and the capacity to erode dentine but not intertubular dentine. Because chitosan has deeper absorption and penetrates dentinal tubules and intertubular dentin, this study used it in the form of nanoparticles to eliminate the smear layer and boost sealer infiltration. (20) The mechanism of chelating action is unclear, but it is believed to occur through absorption, ion exchange, and chelation properties. There are two theories explaining the chelating process of chitosan, namely: (I) Chemical chain bridge model, two or more amino groups from one chain of chitosan will bind to the same metal ion (II) the hook or free-arm model", which mentions that only one amino group of the material structure is involved in the binding, which is the metal ion attached to the amino group. (21) Chitosan polymer is made up of a chain of chitin dimers. This chitin dimer has two nitrogen atoms with free electron pairs that allow the metal to engage with the chelating chemical. In an acidic environment, amino groups exist in the protonated bipolymer, resulting in a full positive charge ($-NH_3^+$). This structure enhances binding with other molecules, leading to the occurrence of adsorption. (22)

Ratih et al. (23) concluded that a 3 min exposure time produced greater apical sealing capability and stronger connection than 1 min, independent final irrigation utilized. Thus, the application time of 3 min is chosen when using chitosan nanoparticles for lastly irrigation solution. For greater sealing capability and binding strength, the application of irrigations for a longer time is needed, which is most efficient in clearing the apical third canal from the smear layer.

The bonding strength of AH Plus to root dentin was increased as the smear layer was eliminated but an adverse impact on the calcium silicate base sealer as it facilitates exposure of the collagen network and improves AH Plus sealer penetration into the dentinal tubules. MTA Fillapex had higher bonding strength when the smear layer was maintained than when it was not. This relates to the fact that the smear layer's moisture has a beneficial effect on MTA Fillapex's adherence to the canal wall because MTA Fillapex can release calcium and hydroxyl ions that form hydroxyapatites through a process known as biomineralization when they come into contact with fluids containing phosphate. By depositing these apatites in the collagen fibrils, they encourage the formation of an interfacial layer known as a mineral infiltration zone and tag-like structures between MTA and radicular dentin. (24,25)

Chelating drugs alter the ratio of calcium to phosphate in the tooth structure; in addition, removing the smear layer that is rich with calcium and phosphate may adversely affect the adherence of bioceramic sealers, which primarily rely on the calcium ions found in dentine for the biomineralization process. Compared to EDTA, CNPs showed less adverse effects on the binding strength and adhesion of the bioceramic sealer because chitosan causes less erosion and changes the surface structure and ca\p level. (26,24)

In contrast to this study, which found that 0.2% and 0.5% CNPs have a stronger bond strength than 17% EDTA, other investigations found that 0.2% chitosan provided the same bond strength as 17% EDTA. (27)

CNPs improve bonding strength of AH Plus sealer. The effect of CNP in clearing smear layer may be due to chelating capability and hydrophilicity nature of CNP that made it is intimate contact to radicular dentin, quickly adsorb into canal wall and insert deeply in dentinal tubules. Additionally, the cationic nature of CNP makes it easier for chelating chemicals and calcium ions found in the dentinal wall to interact. When the amino group in chitosan is protonated, other molecules move away to adsorb into the root canal dentin, allowing the substance to enter the dentinal tubules more deeply. Because the dentinal tubules open after instrumentation and irrigation, the root canal wall has an uneven surface and AH Plus sealer is hydrophobic so the hydrophilic nature of chitosan can improve the wettability of sealer to this wall. Additionally, the smear layer can be eliminated by utilizing nanosized chitosan particles to speed up the irrigation solution's entry into the dentinal tubules. This will enable the endodontic material and root canal wall to bond with enough strength. (28,23)

According to the current investigation, CNPs enhance the MTA Fillapex sealer's resistance to dislocation. This is consistent with a prior study that found that final irrigation of 0.2% chitosan increases the dislocation resistance of an MTA resin hybrid root canal sealer. To improve the stability of MTA bonds, chitosan is utilized for extrafibrillar dentin demineralization. This tactic has been suggested to stop water penetrating hybrid layers and lessen endogenous collagen breakdown caused by matrix metalloproteinases (MMP). Recent data indicates that the chelating ability of chitosan, not the solvent's function, is responsible for demineralizing extrafibrillar dentin, despite a body of research suggesting that the acetic acid solvent is the reason for chitosan's effectiveness in calcium ion dissolution. (29,30)

Kaki and Genç Şen, (31) concluded that 17% EDTA improves the bond strength of AH Plus and chitosan can't improve the push-out bonding strength of it, this is in disagreement with our study that revealed group III's mean push-out bond strength (17% EDTA) was less than that of groups I and II (0.2% and 0.5% CNP) and reduce the bonding strength of AH Plus. This might be attributed to the fact that EDTA has lower viscosity compared to chitosan and its limited ability to penetrate sealer. This result was consistent with a prior study that found that AH Plus sealer penetration was minimal following a final rinse with 17% EDTA. (32)

In our study, EDTA reduces the bonding strength of MTA Fillapex. This is in accordance with a prior study that stated that EDTA reduces the bond strength of MTA sealer. This could be explained by the fact that although MTA is being hydrated, calcium ions are still being chelated by residual EDTA in the root canal dentin, which prevents the hydrated products from precipitating. (33)

Due to the sealers' incapacity to penetrate the dentinal tubules, GVI (distilled water) showed lower mean bond strength values, and all sealers showed lower bond strength values when irrigated with distilled water. Distilled water did not remove the smear layer, which has an undetermined thickness and volume because it contains a lot of water. (*34*)

In this study, the use of NaOCl as a final irrigant considerably reduces the dislocation resilience of AH Plus and MTA Fillapex to root canal dentin. This could be explained by the fact that NaOCl decomposes into intermediate radicals made from proteins and chloramines when it comes into contact with organic components in the root canal. This leads to damage to the collagen link, which is important for the chemical bond of AH Plus with dentin's amino groups. Another plausible, NaOCl inhibits the polymerization of AH Plus due to oxygen bubbles formed after its application. NaOCl reduces the dislocation resilience of MTA Fillapex because it decreases the calcium and phosphorus levels in tooth structure, which the adhesion of MTA Fillapex to dentine depends on calcium and phosphorus for the biomineralization process (*35*,*36*).

In the present study, 2% CHX showed increased bond strength of AH Plus at the coronal section (9.63 ± 14.03) and MTA Fillapex at the middle section (9 ±12.233). This may have explained the adsorption of CHX by dentin, favoring resin infiltration into dentinal tubules. Additionally, CHX makes endodontic sealers more wettable on dentin. This is because CHX contains a surface surfactant, which raises surface energy and encourages a higher wetting ability to dentin. Furthermore, this material improves the durability of the resin and dentin link by having a broad-spectrum matrix metalloproteinase (MMP) inhibitory activity (*37*,*38*).

The results of the study showed that the EndoFill sealer group had the lowest mean bond strength in the apical, middle, and coronal thirds. The fact that sealers with ZOE base stick to dentin by electrostatic bonding instead of entering the dentinal tubules could be one explanation. The zinc ion in zinc oxide may react with the mineral makeup of dentin as well as the zinc oxide component of gutta-percha. Furthermore, gutta-percha may become softer as a result of eugenol. As a result, a crisscross design is produced, which improves the materials' ability to stick together (*39*).

The root canal filler's resistance to displacement in the coronal- apical direction increased. The mean was lowest at the coronal and middle levels and highest at the apical level. The architecture of the root canal, the thickness of the sealer's film, and the root canal filling technique selected should all be taken into account. The circular cross-section of the root canal at the apical third may have offered greater resistance to dislodgment forces during the tests, although the tubule density drops from the coronal to the apical area. Conversely, there are round or even flattened root canal sections in the coronal and intermediate portions. These differences in the structure of the root canal may cause the primary gutta-percha to mismatch and weaken the connection. The middle and coronal thirds of the root canal, where the master cone is positioned correctly, must have a thicker coating of sealer than the apical third when employing a single-cone technique (without intra-canal gutta-percha compaction). In any case, the greater POBS values in the apical third may be explained by these reasons (40).

The results of the present study reject the hypothesis. Chitosan nanoparticle solution clinically has been proposed as a substitute for well-known root canal irrigants, such as sodium hypochlorite, which has some drawbacks, including increased toxicity and bad odor, CHX inability to remove the smear layer, and EDTA that causes dentine erosion and degradation. A chitosan nanoparticle solution has some properties, such as less toxicity and biocompatibility, so chitosan nanoparticles increase the push-out bond strength of bioceramic sealer.

This study has some limitations. First, only teeth with straight canals were involved in the study. Second, no assessment of the effect of chitosan nanoparticles on dentine degradation and microhardness. Further investigation is also necessary to evaluate the exposure time needed.

## CONCLUSIONS

Within the limitations of this study:

1. CNPs irrigation improves the bonding strength of AH Plus, MTA Fillapex and Endofill at the apical third compared to EDTA and NaOCl.

2. The chelating agent CNPs has a superior effect than EDTA on the bond strength of all sealers.
3. CHX has a comparable effect to CNPs at coronal and middle levels of AH Plus and MTA Fillapex, respectively.
4. The NaOCl at 3% showed the lowest efficiency on push-out bonding strength.
5. AH Plus has the greatest bond strength value, followed by MTA Fillapex, whereas EndoFill showed the least value of push-out bond strength.

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**Authors' Contribution**

The authors, Al-Hashimy and Al-Jadwaa, designed the study; Al-Jadwaa supervised the work. Al-Hashimy conducted the experiments, collected data, performed the data analysis, and wrote the manuscript. Al-Jadwaa contributed by providing scientific information and assisting in the study.

**Ethical statement**:

The study was reviewed and approved by the University Ethics and Research Committee in accordance with the principles of respect for the rights of human research participants (Approval Code**: UoM.Dent.23/73**).

**Conflict of interest**

The author declares that there are no conflicts of interest regarding the publication of this manuscript.

**Availability of data and materials**: All data generated or analyzed during this study are included in this published article and its supplementary information files.

**Declaration of Generative AI and AI-assisted technologies**

The authors declare that no generative AI or AI-assisted technologies were used in the preparation of this manuscript. The authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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## تأثير نانو كيتوزان كعامل ري نهائي على قوة الترابط الدفعي للخارج لمواد مانعة التسرب المختلفة المستخدمة في علاج لب الأسنان

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**الملخص**
**الأهداف**: هدفت هذه الدراسة إلى تقييم تأثير ري الجسيمات النانوية مادة كيتوزان بتركيزين مختلفين 0.2٪ و 0.5٪ ومقارنتها بعمليات ري أخرى على قوة الترابط الدفعي للخارج لسدادات اللب الحشوية. **المواد وطرائق العمل**: تم استخدام 54 ضرسًا بشريًا سفليًا بجذور مستقيمة واحدة. تم ازالة التيجان للأسنان بطول 12 ملم. تم إجراء التحضير البيوميكانيكي للقناة باستخدام نظام ProTaper Universal. تم تقسيم الأسنان إلى ست مجموعات بناءً على مادة الري النهائية: المجموعة الأولى: 0.2% من جزيئات الكيتوزان النانوية (CNP) والمجموعة الثانية: 0.5% من جزيئات الكيتوزان النانوية (CNP) والمجموعة الثالثة: 17% من مادة EDTA والمجموعة الرابعة: 3% من محلول هيبوكلوريت الصوديوم (NaOCl) والمجموعة الخامسة: 2% من الكلورهيكسيدين (CHX). والمجموعة السادسة: الماء المقطر كمجموعة سيطرة. تم تقسيم المجموعات لاحقًا إلى ثلاث مجموعات فرعية بناءً على المادة المانعة للتسرب المستخدمة: كالتالي: مادة AH Plus و مادة MTA Fillapex ومادة EndoFill . تم استخدام مخروط F3 gutta-percha وتقنية المخروط الواحد لملء جميع العينات بالسادة ومن كل عينة تم قطع ثلاث شرائح بسمك 2 ملم على مسافات 3 و7 و11 ملم من قمة الجذر وهي تمثل الجزء التاجي و الأوسط والقمي على التوالي. تم استخدام آلة اختبار عالمية لتحليل قوة الالتصاق بالضغط (POBS) ، وتم فحص نمط الفشل باستخدام مجهر ضوئي بتكبير X 40. ثم تم جمع البيانات وتحليلها إحصائيًا. **النتائج**: أظهرت جزيئات الكيتوزان النانوية (CNP) أعلى متوسط لقوة الالتصاق في الجزء القمي من الجذر، تليها مادة EDTA ثم محلول هيبوكلوريت الصوديوم ولوحظ فرق معنوي بين تركيز 0.5% و0.2% من الكيتوزان. أظهر الكلورهيكسيدين زيادة في قوة الالتصاق عند استخدامه مع السادة AH Plus في الجزء التاجي ومع MTA Fillapex في الجزء الأوسط. أقل متوسط لقوة الالتصاق تم تسجيله مع محلول هيبوكلوريت الصوديوم والماء المقطر. **الاستنتاجات**: تعتبر جزيئات الكيتوزان النانوية مادة ري جديدة تعمل على تحسين قوة الالتصاق بين الحشوات الجذرية والمواد السادة القائمة على الراتنج وMTA بشكل أفضل من مادة EDTA بتركيز 17%. كما أن الكلورهيكسيدين 2% فعال بنفس القدر مثل الكيتوزان في تحسين قوة الالتصاق مع المواد السادة AH Plus وMTA Fillapex وEndoFill.

**الكلمات المفتاحية**: غسول الفم، مغناطيسيًا، التشوه المرن، تقويم الأسنان المطاطية.