EUROPEAN ENDODONTIC JOURNAL

ORIGINAL ARTICLE

Effect of Final Irrigation Solutions on Mechanical Properties of Root Canal Dentine

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ABSTRACT

Objective: The final irrigation solution should have the capability of a chelation agent to remove the smear layer. The purpose of this study was to determine the effect of Ethylenediaminetetraacetic acid (EDTA), novel silver citrate, and chitosan nanoparticles as a final irrigation solution on the mechanical properties of root canal dentine, namely the flexural strength and elastic modulus.

Methods: Thirty-two intact, single, and straight roots, with no caries, no cracks, and no restoration of premolars, were assigned into four groups of 8 teeth each. Group 1: irrigated with EDTA; Group 2: irrigated with novel silver citrate; Group 3: irrigated with chitosan nanoparticles; Group 4: irrigated with saline as control. Using a Universal Testing Machine, each sample was tested for flexural strength and elastic modulus with a three-point bend test. The data were analysed using one-way ANOVA and followed by the Tukey's test with a significance level of 0.05 ($p=0.05$).

Results: Chitosan nanoparticles generated the highest flexural strength and elastic modulus of root canal dentine (212.03±1.64 and 16.40±1.05, respectively) than EDTA (127.10±0.90 and 7.37±0.94) and novel silver citrate (177.87±2.03, and 12.27±1.01) ($p<0.05$). However, chitosan nanoparticles have similar flexural strength and elastic modulus of root canal dentine when compared to saline (213.76±1.48, 17.02±1.14, respectively) ($p>0.05$).

Conclusion: Among final irrigations used, chitosan nanoparticles produced the highest flexural strength and elastic modulus of root canal dentine compared to EDTA and novel silver citrate.

Keywords: Chelating solution, chitosan nanoparticles, elastic modulus, final irrigation, flexural strength

HIGHLIGHTS

- Chitosan nanoparticles have the potential to be proposed as an alternative to EDTA and novel silver citrate.
- Chitosan nanoparticles produced the highest flexural strength and elastic modulus than EDTA and novel silver citrate.
- Chitosan is a vulnerable chelating agent, influencing an insignificant quantity of the superficial root canal dentine compared to EDTA and novel silver citrate, and can generate remineralization of demineralized dentine.

Please cite this article as:
Ratih DN, Widyastuti A, Monika A. Effect of Final Irrigation Solutions on Mechanical Properties of Root Canal Dentine. Eur Endod J 2024; 9: 405-10

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Received : February 24, 2024,
Revised : May 31, 2024,
Accepted : June 03, 2024

Published online: October 31, 2024
DOI 10.14744/eej.2024.63308

INTRODUCTION

The success of root canal treatment (RCT) is the accomplishment of the endodontic triad, namely biomechanical preparation (cleaning and shaping), including chemical root canal cleaning using irrigation solutions, root canal sterilization, and root canal filling (1). Irrigation of the root canal system is one of the crucial stages in RCT. The purpose of root canal irrigation is to clean the root canal system from necrotic tissue remains and abolish bacteria and their products (2).

Endodontic instruments utilized either in a manual or mechanical manner in RCT produces a smear layer on the root canal walls and a smear plug in the dentinal tubules. The smear layer is formed during root canal instrumentation with a file, and its thickness ranges from 0.5–2 µm. Even though the thickness is only a small number of microns, the existence of a smear layer can hinder the infiltration of the obturation sealer into the dentine tubules and the adhesion of the sealer to the dentine walls of the root canal system (3). Removing the smear layer from root canals system is highly recommended to achieve success in RCT in clinical condition (4).

The usage of the final irrigation solution is crucial during the root canal irrigation phase. One of the requirements of the final irrigation solution is that it must have chelating properties that can remove the smear layer, particularly the inorganic components smear layer, from the root canal system (5). However, removing the smear layer from the root canal system is supposed not to influence the mechanical properties, particularly flexural strength and elastic modulus, which could lead to fracture of the tooth following RCT.

Ethylenediaminetetraacetic acid (EDTA) is a final irrigation agent that is the gold standard used for final irrigation materials in clinics (6), due to its capacity to act with calcium ions in dentine and form calcium chelation; thus, it can dissolve the smear layer (7). As a chelating agent, EDTA is likely to change the structure of dentine; hence, it can affect the mechanical properties of root canal dentine. Previous research stated that EDTA as a final root canal irrigation material has a positive effect; it can dissolve the smear layer but has a negative effect, causing dentine erosion (8). Another main drawback of EDTA is that it does not have antibacterial efficacy (5). Dentine erosion may reduce flexural strength and elastic modulus, making teeth prone to fracture after root canal treatment (3). Therefore, a new irrigation solution is required to eliminate the smear layer without influencing mechanical properties of root canal system, particularly the flexural strength and elastic modulus.

Novel silver citrate (NSC) is one of the new irrigation materials on the market today. An NSC solution has been tested as an innovative biomaterial that can be utilized as a root canal irrigation material. The advantage of this irrigation material is due to the presence of a silver compound that has antibacterial properties, while the citric acid contained in this solution is a weak organic acid with chelation properties (9). However, until now, there has not been much research on NCS in relation to its chelation property.

At present, chitosan has been broadly utilized in the health sector due to its many benefits, such as antimicrobial activity, biocompatibility, biodegradability, fungistatic, haemostatic potential, non-carcinogenicity, remarkable affinity to proteins, and promotion of cell adhesion (10, 11). In addition, chitosan can be used for endodontic regenerative due to its biological properties, such as proliferation and differentiation, and it is not toxic (11, 12). Chitosan is a natural, non-toxic polysaccharide produced from the deacetylation of chitin, which is obtained from the shells of marine animals, crustaceans, and shrimp. Chitosan contains b-(1–4)-glucosamine and N-acetyl-D-glucosamine monomeric units (13). It is also widely found in nature, cheap, and has chelating properties against metal ions (11). Preceding studies have shown that chitosan nanoparticles are antibacterial against E faecalis (14). Other research explains that chitosan has high antibacterial against E faecalis and C albican (15). Silva et al. (16) stated that chitosan possesses chelation capabilities, which can dismiss the smear layer.

However, until now, there has been deliberation about the chelation properties of the final root canal irrigation that should not affect the mechanical properties of root canal dentine. Thus, the purpose of this study was to evaluate the effect of three final irrigation solutions, namely EDTA, novel silver citrate, and chitosan nanoparticle, as a final canal irrigation material on the flexural strength and elastic modulus of root canal dentine. The null hypothesis of this study was that no difference occurred in flexural strength and elastic modulus of root canal dentine among EDTA, NSC, and chitosan nanoparticles.

MATERIALS AND METHODS

The research procedure was agreed by the Universitas Gadjah Mada, Faculty of Dentistry, ethics committee under the number 101/UN1/KEP/FKG-RSGM/EC/2023 and accomplished in accordance with The Declaration of Helsinki.

Preparation of 0.2% Chitosan Nanoparticles

The 0.2% chitosan nanoparticle solution was created by dispersing 0.2 gram of chitosan powder (size 397.5 ± 98.5 nm) (NHI, Tangerang, Indonesia) in 1% acetic acid with the volume of 100 millilitres. Chitosan was synthesis from shrimp shells (degree of deacetylation >75%) using ionic glass method and Polyanion Tripolyphosphate (TPP) as crosslinker. The mixture was blended with a magnetic stirrer for two hours to achieve a uniform blend (17).

Sample Preparation

Thirty-two single root premolar teeth that were intact had no caries or cracks and had not undergone root canal preparation and restoration, which had previously been stored in distilled water, were used in this study. Dentine plates were made by cutting each tooth on its labial side with a cutting a cutting machine (Microtome, Leica, Wetzlar, Germany). The plate was rectangular with dimensions of length =8 mm width =2 mm, and height =3 mm. Silicon carbide grit no. 1500 (Multi-Tech Products, Anaheim, CA, USA) was used to level and smooth the surface of dentine plates. The dentine plate was examined for cracks under a light microscope at 24X magnification. Each plate was measured with a digital sliding calliper (Mitutoyo UK Ltd, Andover, Hampshire, UK) with a precision of 0.01 mm at three sites alongside the plate.

The dentine plates were then randomly assigned into six groups, each group consisting of 8 teeth: Group 1: dentine plates soaked in 17% EDTA (Pulpdent, Watertown, MA, USA) irrigation solution; Group 2: dentine plates soaked in NSC (BioAKT endo, New Tech Solution, Brescia, Italy); Group 3: dentine plates soaked in 0.2% chitosan nanoparticles (NHI, Tangerang, Indonesia); Group 4: dentine plates soaked in saline (PT Otsuka Indonesia, Malang, Indonesia) as control. All samples were soaked in irrigation solution according to the group for 30 minutes. The irrigation solution is always replaced with a new solution (each with a volume of 5 mL) every three minutes to stimulate conditions in the clinic for applying the irrigation solution to the root canal (18).

The dentine plates were then taken from the irrigation solution bath and cleaned with distilled water to remove any remaining irrigation solution that was still attached to the dentine plate so that the effect of the irrigation solution would not continue. Before testing the flexural strength and elastic modulus, the dentine plates were soaked in artificial saliva (Analytical Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Yogyakarta, Indonesia) for 24 hours and stored in an incubator at 37°C.

Mechanical Properties Test

Each sample was tested for flexural strength and elastic modulus with a three-point bend test using a universal testing machine (Type AMU-5-DE, Tokyo Testing Machine, MFG, Co., LT, Tokyo, Japan) with a load speed of 1 mm/min. Each sample is then placed at two points; therefore, a distance of 6 mm was obtained with the load located precisely in the middle (Fig. 1). Each sample was then pressed with a load until it fractured, and the load required to fracture the sample was recorded. The fracture that occurs could be a complete fracture or a partial fracture. Figure 2 shows the flowchart of the experimental procedures of the study.

Flexural strength was calculated in MPa units (3) = 3 X Load X Length/2 X Width X Thickness^2. The elastic modulus was calculated in GPa units as follows: Deformation = Load X Length^3/4 X Elastic Modulus X Width X thickness.

Statistical Analysis

Data obtained showed normal (Shapiro-Wilk) and homogeneous (Lavene's test) distributions and were statistically analysed using one-way ANOVA and Tukey's test. The IBM SPSS Statistics Software, version 23 (IBM Corp., Armonk, NY, United States) was employed for data analysis. The significance level was 95%.

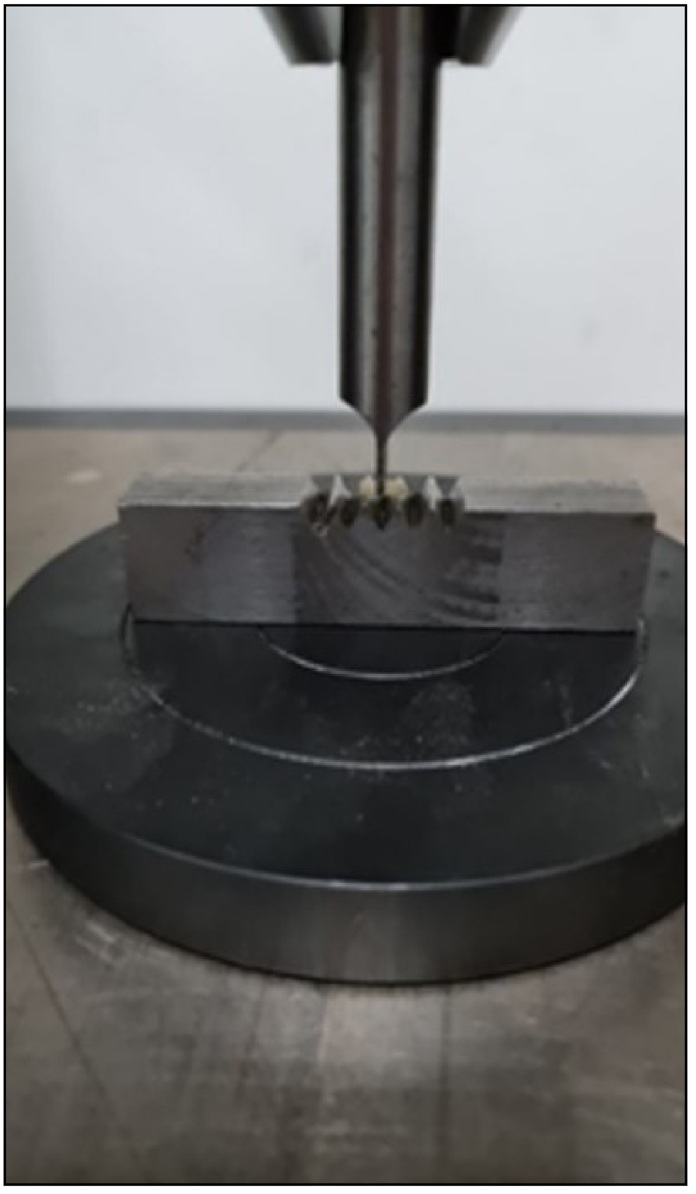

Figure 1. The load located precisely in the middle of sample

RESULTS

The statistical analysis results using one-way ANOVA demonstrated a significant difference in the effects of the final irrigation solution using EDTA, NSC, and saline on flexural strength or elastic modulus of root canal dentine ($p<0.05$).

Table 1 shows that chitosan nanoparticles produced the highest flexural strength and elastic modulus (212.03±1.64 and 16.40 ±1.05, respectively) compared to other final irrigation solutions, namely NSC (177.87±2.03 and 12.27±1.01) and EDTA (127.10±0.90 and 7.37±0.94) ($p<0.05$). However, compared to saline as a control, chitosan nanoparticles generated similar flexural strength and elastic modulus (213.76±1.48 and 17.02±1.14) ($p>0.05$).

DISCUSSION

The root canal system comprises inorganic elements of hard dental tissues, in which calcium and phosphorus are dispersed in the structure of hydroxyapatite crystals (17). Final irrigation solution, particularly with chelating properties, might induce changes in the inorganic and mechanical constitution of dentine due to the deproteinizing and decalcifying effects of chelating agents that are concentration and time-dependent, inducing alteration of surface and subsurface ultrastructural, chemical, and mechanical, as well as reducing Ca/P ratio of root canal system (19). As a result, it influences the flexural strength and elastic modulus of root canal system (20). This statement is in accordance with the results of the present study.

Dentine plates treated using EDTA demonstrated a reduction of flexural strength and elastic modulus that was statistically significant from the other groups. It can be elucidated that EDTA has chelation capability in dismissing inorganic components of the smear layer.; hence, the loss of calcium ion of inorganic component generated by the chelating action of EDTA induces the ratio change of Ca/P in the composition of root canal dentine. This phenomenon caused root canal dentine demineralization, which, in turn, lowered both the flexural strength and elastic modulus (21). The present study also revealed that final

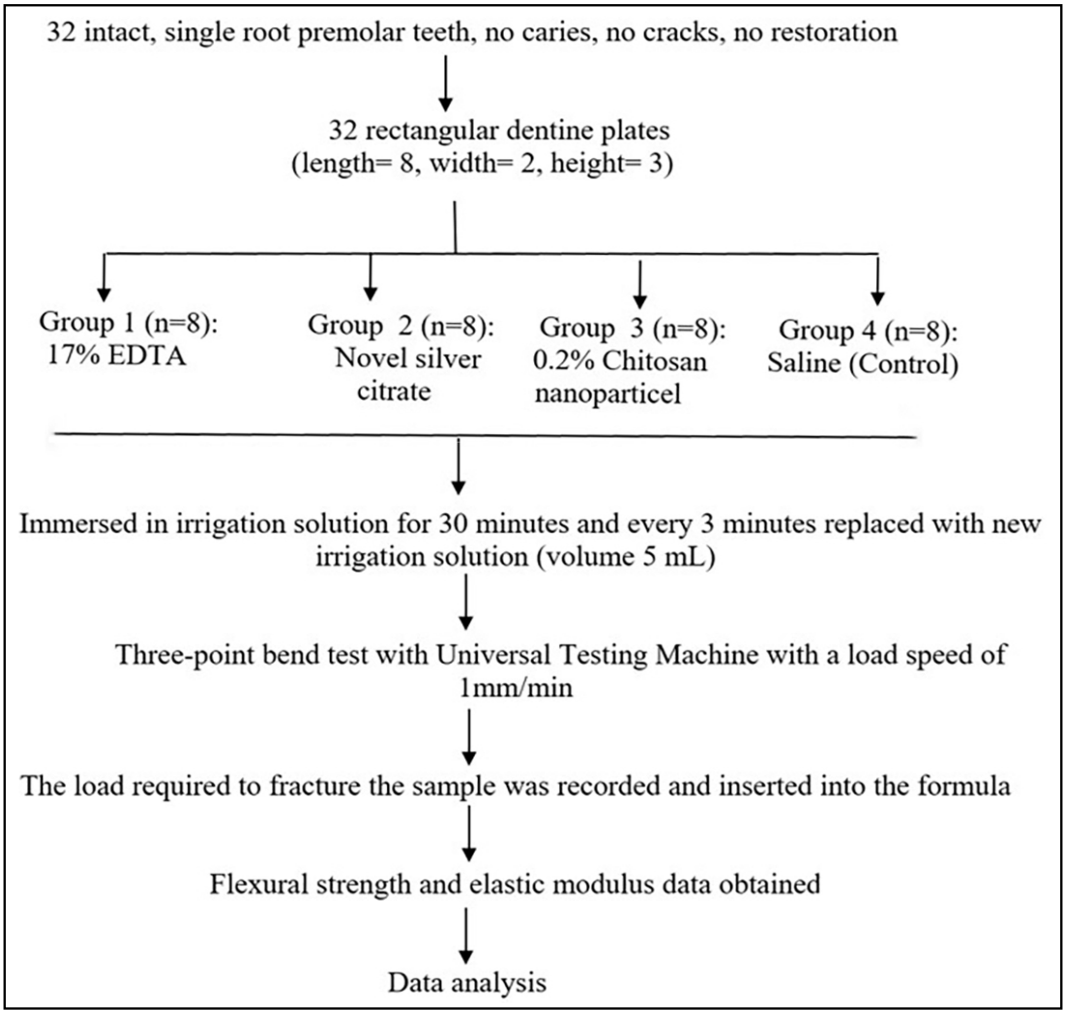

Figure 2. The experimental procedure flowchart

EDTA: Ethylenediaminetetraacetic acid

irrigation using EDTA produced the significantly lowest flexural strength and elastic modulus; it may be due to the strong chelating agent of EDTA compared to other irrigation solutions used in this study. If a potent chelating agent is applied for extended time to root canal dentine, it could eradicate the inorganic part of the smear layer and significantly remove the peritubular and inter-tubular dentine (22).

Based on a previous study, 30 minutes of exposure time was chosen for the maximum time, corresponding to the maximum time that the tooth would be exposed to irrigation solution during multi-visit endodontic treatment. In addition, to simulate conditions in the clinic for applying the irrigation solution to the root canal, the irrigation solution is always replaced with a new solution (each with a volume of 5 mL) every three minutes to stimulate conditions in the clinic (18).

The chelating ability of the NSC solution to eradicate the smear layer is due to the citric acid content in this solution, which causes the acidic pH of the NSC solution (23). Although the citric acid in the novel silver citrate is a weak organic acid, it can dissolve minerals in root canal dentine (6, 9).

TABLE 1. Mean and standard deviation (SD) of flexural strength and elastic modulus of root canal dentine after final irrigation solution using EDTA, novel silver citrate, chitosan nanoparticles, and saline (control)

Final irrigation	N	Flexural strength Mean±SD (MPa)	Elastic modulus Mean±SD (GPa)
EDTA	8	127.10±0.90[a]	7.37±0.94[a]
Novel silver citrate	8	177.87±2.03[b]	12.27±1.01[b]
Chitosan nanoparticle	8	212.03±1.64[c]	16.40±1.05[c]
Saline (control)	8	213.76±1.48[c]	17.02±1.14[c]

Different letters in each mean±SD of flexural strength and elastic modulus results indicate statistically significant differences ($p<0.05$). EDTA: Ethylenediaminetetraacetic acid

This study also showed that chitosan nanoparticles have chelating properties and can be depicted in three stages: adsorption, ion interchange, and chelation. Hydrophilic properties of chitosan generate this solution to be adsorbed to the superficial root canal dentine; consequently, ionic interaction between calcium in the dentine superficial and chelating substance might arise (24). The results indicate that chitosan nanoparticles produced the highest flexural strength and elastic modulus of the root canal dentine than to other groups excluding for the control group using saline. This is due to chitosan being a vulnerable chelating agent, accordingly, influencing an insignificant quantity of the superficial root canal dentine compared to EDTA (10). The chitosan used in this study was nanoparticle since it is better absorbed than chitosan in regular size. Modifying the physical form of chitosan into nanoparticles was done to optimize its effectiveness for final irrigation as it has improved absorption and diffusion (25).

Furthermore, chitosan could restrain the covalent link to the dentine collagen, creating remineralization of demineralized dentine. This phenomenon is described as the phosphate groups that could attract calcium ions to make crystal nucleation to procedure a calcium-phosphate layer (26). On the other hand, EDTA and NSC are incapable of remineralizing the demineralized dentine (27, 28). Therefore, this condition might elucidate why the chitosan solution generated the highest flexural strength and elastic modulus compared to EDTA and NSC. Conversely, saline as control has no chelating action (29); thus, it has no ability to eliminate the inorganic part of root canal dentine; as a result, flexural strength and elastic modulus were the highest compared to the treated groups.

This study has limitations. Firstly, dentine possesses an anisotropic characteristic. Thus, it can vary if the test for flexural strength and elastic modulus is altered in the location. Secondly, the immersion time and the volume of the irrigation solution in a root canal used in the clinic is less than when employed for immersing the sample. However, the utilization of consistent conditions permitted comparable results among the three final irrigation solutions. Thirdly, research on flexural strength and elastic modulus of root canal systems needs a thin rectangular block to be appropriate for a three-point bend test. Therefore, it is difficult to cut the root canal of each tooth without damaging the internal root canal system after root canal treatment procedures in the clinic, such instrumentation and irrigation procedures. Thus, further *in vitro* and *in vivo* studies are needed to evaluate the efficacy of chitosan nanoparticles based on their ability to remove smear layers from the root canal walls and their antibacterial efficacy.

CONCLUSION

The final irrigation solution affected the flexural strength and elastic modulus of root canal dentine. The final irrigation solution with chitosan nanoparticles produced the highest flexural strength and elastic modulus of root canal dentine compared to EDTA and novel silver citrate but was similar to saline.

Disclosures

Ethics Committee Approval: The study was approved by the Universitas Gadjah Mada Faculty of Dentistry Ethics Committee (no: 101/UN1/KEP/FKG-RSGM/EC/2023, date: 19/06/2023).

Authorship Contributions: Concept – D.N.R., A.W.; Design – D.N.R., A.W.; Supervision – D.N.R., A.W.; Funding – D.N.R.; Materials – D.N.R., A.M.; Data collection and/or processing – D.N.R., A.W., A.M.; Data analysis and/or interpretation – D.N.R., A.M.; Literature search – D.N.R., A.M.; Writing – D.N.R.; Critical review – A.W., A.M.

Conflict of Interest: All authors declared no conflict of interest.

Use of AI for Writing Assistance: All authors did not use artificial intelligence (AI)– assisted technologies (such as Large Language Models [LLMs], chatbots, or image creators) in the production of submitted work and assert that there is no plagiarism in their paper, including in text and images produced by the AI.

Financial Disclosure: This study was funded by Universitas Gadjah Mada, Faculty of Dentistry Grant number 3372/UN1/FKG/Set.KG1/ LT/2023.

Peer-review: Externally peer-reviewed.

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