Original Article

The Effect of Different Irrigation Protocols on Elastic Modulus of Dentine and Biomechanics of Single-Rooted Premolar Tooth: A Nano-Indentation and Finite Element Analysis Study

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Abstract

Aim: This study evaluated the effect of different irrigation protocols on elastic modulus and biomechanics of single-rooted premolar tooth using with nano-indentation and finite element analysis (FEA). **Materials and Methods:** Root canals of single-rooted human teeth were prepared, divided into eight groups, and irrigated with (1) 2.5% NaOCl + 17% EDTA; (2) 2.5% NaOCl + 17% EDTA + 2.5% NaOCl; (3) 2.5% NaOCl + SmearClear; (4) 2.5% NaOCl + 2% chlorhexidine; (5) 1.3% NaOCl + MTAD; (6) 5.25% NaOCl; (7) 17% EDTA; and (8) saline. The roots were vertically sectioned, and elastic modulus of the root dentine was measured using nano-indenter device at coronal, middle, and apical third. Data were recorded as megapascal and statistically analyzed (one-way analysis of variance, Tukey tests). Three-dimensional FEA model of a premolar tooth was created, and the inner root dentine was modified to simulate the effect of irrigation protocols on root dentine. The elastic properties of inner root dentine layer in the FEA models were modified for each group according to the data obtained with nano-indentation. A 300-N load was applied at the buccal cusp and central fossa of the models with a 45° angle. The stresses were calculated using von Mises stress criteria. **Results:** All irrigation protocols affected the elastic modulus of root dentine. Groups 2 and 3 showed similar elastic modulus values ($P > 0.05$), whereas the lowest values were obtained in group 7 ($P < 0.05$). No statistically significant differences were found between groups 4, 5, and 8 ($P > 0.05$). **Conclusion:** Despite the effect of different clinically used irrigation protocols on elastic modulus of the inner dentine, this does not affect the biomechanics of the roots.

Keywords: *Dentine, elastic modulus, finite element analysis, irrigants, nano-indentation*

Introduction

Cleaning with root canal irrigants is the most accepted method for the removal of tissue remnants and dentine debris during instrumentation.[1] The most commonly used irrigating solutions are sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA), and chlorhexidine (CHX);[2] SmearClear (SybronEndo, Orange, CA, USA) (contains 17% EDTA, cetrimide, and a specific surfactant)[3] and MTAD (Dentsply, Tulsa, OK, USA) (an irrigant consisting of tetracycline, citric acid, and detergent) are other widely used solutions.[4]

Studies have indicated that irrigation solutions affect the mechanical properties of dentine.[5,6] There are several techniques and tools for analyzing the mechanical

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properties of materials. Nano-indentation is one of the widely used one among these. The advantage of nano-indentation test over the others is that it is capable of probing the mechanical properties and behavior of small volumes of dental structures.[7]

The biomechanics of the tooth is an important parameter for its survival.[8] Structure loss because of several reasons is a major problem in endodontically treated teeth because changes in the elastic properties of dentine may affect the biomechanics of the tooth, since there is limited dentine when compared with sound teeth.[8] Finite element analysis (FEA) is a well-known technique that is widely used in all fields of science and dentistry. Some dental and medical research that cannot be conducted on live tissues or simulated in a laboratory can be carried out using FEA.[9]

The effect of irrigation protocols on the elastic modulus of dentine was studied by several researchers in the past.[5,6] To our knowledge, none of those studies conducted a detailed measurement from coronal to apical and from canal lumen to the inner root dentine. Furthermore, clinically accepted and widely used irrigation protocols were not compared in detail. The effect of irrigation solutions on the elastic modulus of dentine was simulated in a previous FEA study;[8] however, the elastic properties of the structures were provided from the manufacturers or from previously published articles. The effect of changes in the elastic properties of dentine because of widely used clinical irrigation protocols was also not simulated in the past. Thus, this study was planned to evaluate the effect of different clinically used irrigation protocols on the elastic modulus of root dentine from coronal to apical and from canal lumen to inner root dentine, and to evaluate the effect of these changes on stresses within the tooth under loading using FEA. The first hypothesis to be tested in the study was that different irrigation protocols do not change the elastic modulus of root dentine from coronal to apical and from canal lumen toward the inner root dentine. The second hypothesis of the study was that changes in the elastic modulus of dentine because of different clinically used irrigation protocols do not affect the stress distributions within the endodontically treated tooth.

Materials and Methods

This study protocol was approved by the Ethical Committee of Medical School, Selçuk University, Konya, Turkey (protocol number 2012/10). Forty single-rooted extracted premolar teeth were selected with similar dimensions, and without caries, coronal or root filling, or cracks. The soft tissue remnants covering the root surfaces were removed using 0.01% NaOCl with gauze and a fine brush. The teeth were then stored until use in distilled water at 4°C for a maximum of 1 month. The crowns were removed with a high-speed bur under water cooling, and root lengths were standardized to 15 ± 1 mm. The roots were randomly divided into five groups according to the irrigation protocol (n = 5) and three control groups (n = 5). Root canals were then prepared using ProTaper rotary files (Dentsply, Maillefer, Ballaigues, Switzerland) to size F4 of the ProTaper instrument. The sequence followed was SX, S1, S2, F1, F2, F3, and F4.

The irrigation protocols were done with a 30-gauge slot-tipped needle, and the study groups were as follows:

Group 1: 2 mL 2.5% NaOCl (Çağlayan Kimya, Konya, Turkey) after each file change and final 5 mL 17% EDTA (Werax, Spot Diş Deposu, İzmir, Turkey) for 5 min.

Group 2: 2 mL 2.5% NaOCl after each file change, final 5 mL 17% EDTA for 5 min, and then 5 mL 2.5% NaOCl.

Group 3: 2 mL 2.5% NaOCl after each file change and final 5 mL SmearClear for 5 min.

Group 4: 2 mL 2.5% NaOCl after each file change and final 5 mL 2% CHX (Klorhex, Drogsan, Ankara, Turkey) for 5 min.

Group 5: 2 mL 1.3% NaOCl (Çağlayan Kimya) after each file change and final 5 mL Biopure MTAD for 5 min.[6]

Group 6: 2 mL 5.25% NaOCl (Wizard, Rehber Kimya, İstanbul, Turkey) after each file change and final 5 mL 5.25% NaOCl for 5 min.

Group 7: 2 mL 17% EDTA after each file change and final 5 mL 17% EDTA for 5 min.

Group 8: 2 mL saline after each file change and final 5 mL saline for 5 min.

Before CHX and Biopure MTAD irrigations, root canals were irrigated with 2 mL of distilled water to avoid colored precipitate. All roots were irrigated with 10 mL of distilled water finally to remove the remaining irrigants.

Nano-indentation testing

The roots were split in the buccolingual direction with a low-speed diamond saw (Accutom-50; Struers, Copenhagen, Denmark) under constant water irrigation. Samples were embedded in epoxy resin (Epon 815; Nissin, Tokyo, Japan). After setting, exposed surfaces were polished using carbide paper with a grit size of 600–2000. This was followed by polishing with 3 and 1 μm polishing diamond paste in sequence. Samples were stored in distilled water at 4°C until nano-indentation testing (CSM Instruments, SA, Switzerland). The indenter used was a pyramidal-triangular-shaped Berkovich indenter, and the maximum force applied was 20 mN. Each indent was accomplished at a 40-mN/min loading ratio and a 20-s delay of the maximum load at the intertubular dentine. The maximum penetration depth was 1400 nm. The nano-indentation test was performed in each sample from apical to the cervical region at 2.5, 5.5, and 8.5 mm for the measurement of the elastic modulus. Three measurements were done in each test region starting from the root canal lumen toward the cement [at 10, 50, and 90 μm; Figure 1]. Each group had a total of 45 indentations (3 regions × 3 deepness × 5 samples).

FEA

A three-dimensional FEA model of a mandibular premolar tooth was allocated based on the anatomical data described by Nelson.[10] The FEA model included enamel, dentine, cementum, pulp, and periodontal ligament and consisted of 105,443 nodes and 68,036 elements. Assuming that the root canal was prepared using a rotary file, it was enlarged from 1 mm less than the apex to the coronal with a 6% taper. The FEA study models were modified based on this initial model. In these models, a 90-μm-thick additional layer associated with the root canal lumen was created within the root dentine. All the materials and structures used in this study were assumed to be homogeneous, isotropic, and linear elastic except for this 90-μm-thick dentine layer associated with the root canal lumen. Data, which were obtained from the nano-indentation test for each irrigation protocol for each region and deepness (total of 45 indentations [3 regions × 3 deepness {10, 50, and 90 μm} × 5 samples]), were averaged and used as the elastic modulus of this dentine layer to simulate the affected dentine. The elastic modulus of the remaining root dentine structure was achieved from the measurements, which was obtained from the saline group. The elastic modulus and Poisson’s ratios of all the other materials and structures used were obtained from the literature[8,9] or from the manufacturers [Table 1]. Initially, the root canal models were assumed to be prepared with irrigation protocols and unfilled, and then the same models were assumed to be root-filled and coronally restored [Figure 2].

A 300-N load was applied to the functional buccal cusp and central fossa at a 45° angle to the long axis of the tooth to calculate the stress patterns.[9] Nodes at the outer surface of the roots were assumed as fixed in all directions to calculate the stress distributions. To simulate adhesion between the structures, all interfaces were considered as completely bonded. The finite elements on the *x*, *y*, and *z*-axes for each model were assumed as fixed for the boundary conditions. FE modeling was accomplished using the SolidWorks software program (SolidWorks 2009; SolidWorks Corp., Concord, MA, USA), and analyses were run using the COSMOSWorks structural-analysis program (SolidWorks Corp., Waltham, MA, USA).

The stresses were recorded using von Mises criteria. A standard view of a mid-sagittal section from each model was provided. The stress distribution scale range was limited to 0–10 MPa. To provide better visualization, calculated numeric data were transformed into color images.

Statistical analysis

Elastic modulus values were statistically analyzed using SPSS 15.0 (SPSS, Chicago, IL, USA) for Windows, using one-way analysis of variance with a subsequent Tukey’s multiple comparison test ($P < 0.05$).

Results

Nano-indentation test

Table 2 shows the mean elastic modulus values after treatment with different irrigation protocols. The results of the statistical analysis according to the regions were as follows:

Coronal region: No difference was found among the elastic modulus of dentine in groups 4, 5, and 8 ($P > 0.05$). These three groups were found to be significantly different from the others ($P < 0.05$). No difference was found among groups 1, 2, 3, and 6 ($P > 0.05$). Elastic modulus of the dentine was found to be lowest in Group 7 (Cosmos <0.05).

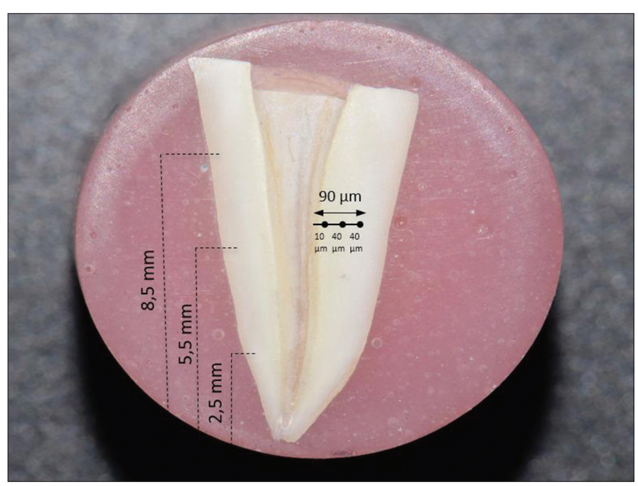

Figure 1: Nano-indentaion measurement points at three regions from root canal lument to cementum

Midcoronal region: No difference was found among groups 1, 4, 5, 6, and 8 ($P > 0.05$). The elastic modulus values of these groups were significantly higher than the other test groups ($P < 0.05$). Groups 2 and 3 showed similar elastic modulus values ($P > 0.05$), while the lowest values were obtained in group 7 with 17% EDTA ($P < 0.05$).

Table 1: Mechanical properties of the isotropic and unisotropic materials used

	Elastic modulus (GPa)	Poisson's ratio (v)
Dentine		
Group 1[a]	17.25	0.31
Group 2[a]	15.56	0.31
Group 3[a]	16.48	0.31
Group 4[a]	18.52	0,31
Group 5[a]	18.31	0.31
Group 6[a]	17.11	0.31
Group 7[a]	14.01	0.31
Group 8[a]	19.64	0.31
Enamel[9]	41	0.31
Pulp[9]	0.003	0.45
Periodontal ligament[9]	0.0000689	0.45
Composite resin[8]	12	0.30
Gutta-percha[9]	0.14	0.45

[a]Acquired from the manufacturer

Table 2: Mean elastic modulus values of root dentine obtained at totally 90 μm (mean±SE)

Groups	Elastic Modulus (GPa)			
	Region			Mean
	Apical	Middle	Cervical	
1	17.5±0.81[b]	17.13±0.57[a]	17.12±0.73[b]	17.25±0.4
2	14.88±0.68[b]	16.65±0.63[b]	15.16±0.48[b]	15.56±0.35
3	17.71±0.82[b]	16.52±0.7[b]	15.2±0.5[b]	16.48±0.41
4	19.17±0.66[a]	17.92±0.86[a]	18.46±0.67[a]	18.52±0.42
5	18.6±0.53[a]	18.5±0.64[a]	17.82±0.59[a]	18.31±0.33
6	16.76±0.78[b]	17.21±0.69[a]	17.34±0.76[b]	17.11±0.42
7	14.42±0.75[c]	13.89±0.63[c]	13.71±0.66[c]	14.01±0,38
8	19.8±0.87[a]	20.12±0.9[a]	18.99±0.99[a]	19.64±0.52

The common letters on the columns are not statistically significant (a > b > c > d; $P<0.05$). SE=Standard error

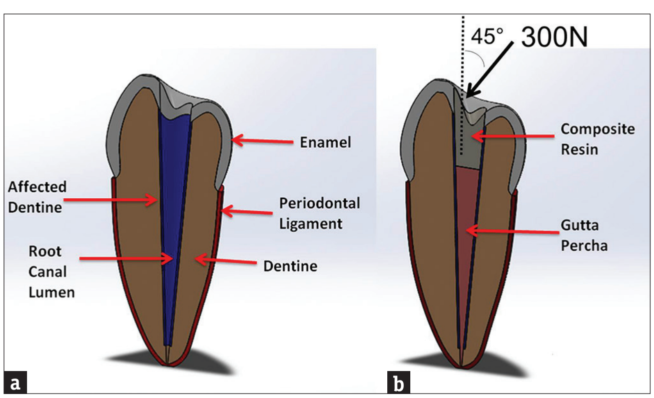

Figure 2: The structures, materials, and the loading conditions of the FEA models. (a) The unfilled model and (b) the model assumed to be filled with gutta-percha and restored using composite resin

Apical region: Similar to the coronal region, the elastic modulus values were higher in groups 4, 5, and 8 ($P < 0.05$). Groups 1, 2, 3, and 6 were also found to be similar to each other ($P > 0.05$) and significantly different from the other groups ($P < 0.05$). The lowest values were found with 17% EDTA ($P < 0.05$).

FEA

Table 3 shows the maximum and total von Mises stress values for each group. The maximum stress values in root dentine were found to be similar in groups 4 (2.5% NaOCl + 2% CHX), 5 (1.3% NaOCl + MTAD), and 8 (Saline), whereas affected dentine showed higher maximum stress values in the models that were assumed to be prepared but kept unfilled. The models that were assumed to be root-filled showed almost identical maximum stress values in dentine. The affected dentine showed higher stress values in groups 4, 5, and 8. The affected dentine showed lower values in groups 2 (2.5% NaOCl + 17% EDTA + 2.5% NaOCl) and 7 (17% EDTA) in the unfilled models and higher values in groups 4, 5, and 8. Nevertheless, total stress values were similar in all groups.

Figures 3 and 4 show the stress distributions and accumulations within the models, which represent teeth treated with different irrigation protocols. von Mises stress values and domains in the unfilled models did not show any differences among the groups. It was observed

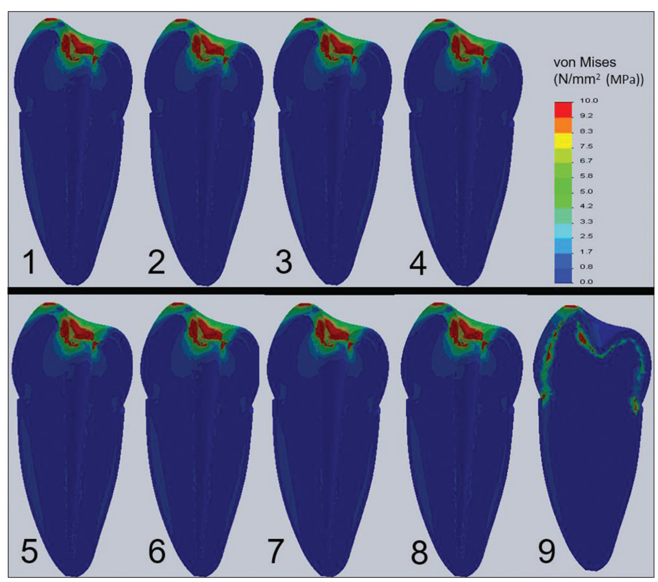

Figure 3: Von Mises stress patterns calculated in the corresponding FEA models within the prepared models. Stress values were specified in the color scale. (1) 2.5% NaOCl + 17% EDTA, (2) 2.5% NaOCl + 17% EDTA + 2.5% NaOCl, (3) 2.5% NaOCl + SmearClear, (4) 2.5% NaOCl + 2% chlorhexidine, (5) 1.3% NaOCl + MTAD, (6) 5.25% NaOCl, (7) 17% EDTA, (8) saline, (9) natural tooth

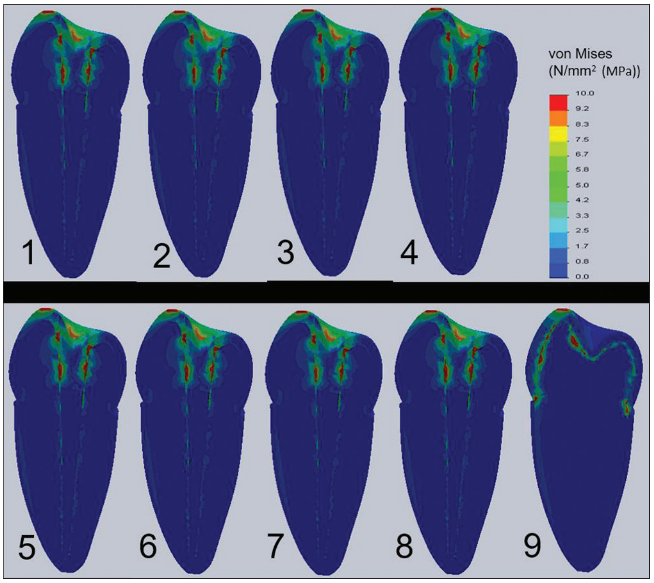

Figure 4: Von Mises stress patterns calculated in the corresponding FEA models within the filled/coronally restored. Stress values were specified in the color scale. (1) 2.5% NaOCl + 17% EDTA, (2) 2.5% NaOCl + 17% EDTA + 2.5% NaOCl, (3) 2.5% NaOCl + SmearClear, (4) 2.5% NaOCl + 2% chlorhexidine, (5) 1.3% NaOCl + MTAD, (6) 5.25% NaOCl, (7) 17% EDTA, (8) saline, (9) natural tooth

Table 3: Maximum and total von Mises stress values obtained within the FEA models representing prepared but unfilled and root-filled teeth

Groups	Region	Prepared model Maximum/total	Restored model Maximum/total
1	Dentine	47.22/25339	28.27/22747
	Affected dentine	38.07/6215	213.5/9944
	Enamel	44.46/65188	70.19/53992
2	Dentine	47.35/25446	28.47/22797
	Affected dentine	35.76/5841	202.14/9286
	Enamel	42.04/65331	68.01/53997
3	Dentine	47.34/25379	28.35/22766
	Affected dentine	37.19/6077	209.4/9701
	Enamel	43.57/65239	69.4/53993
4	Dentine	46.82/25232	28.09/22692
	Affected dentine	40.53/6595	224.51/10606
	Enamel	46.90/65058	72.25/53987
5	Dentine	46.87/25247	28.11/22700
	Affected dentine	40.18/6542	222.99/10514
	Enamel	46.55/65075	71.97/53987
6	Dentine	47.19/25333	28.26/22744
	Affected dentine	38.22/6238	214.2/9984
	Enamel	44.61/65180	70.31/53991
7	Dentine	47.33/25562	28.68/22852
	Affected dentine	33.48/5446	189.61/8584
	Enamel	39.44/65502	65.53/54003
8	Dentine	46.60/25170	27.99/22658
	Affected dentine	42.03/6820	230.87/10995
	Enamel	48.35/64986	73.41/53984
9	Dentine	42.49/36429	
	Enamel	338.96/37638	

A natural tooth was added as ninth group for comparison; values are given in MPa

that the stress intensity of the input cavity enamel dentine junction consisted of, but was dispersed in, the natural tooth models that stress coronal dentine and enamel [Figure 3].

von Mises stress values and domains in the models that were considered filled were almost identical [Figure 4]. High stress accumulations were observed along the root canal dentine walls and access cavity.

Discussion

The penetration of different irrigation solutions into dentine has shown different results in previous studies because distinct testing protocols were used. Kuga *et al.*[11] found the depth to be 107 µm when dentine bars were placed in the irrigation solution for 20 min, while Zou *et al.*[12] recorded a depth of 77–123 µm when using different concentrations for 2 min. In this study, the measurements were performed at 10, 50, and 90 µm, starting from the root canal lumen and considering that the maximum penetration depth of irrigation protocols is around 100 µm.[11] Three measurements were carried out for each region (coronal, median, and apical) and a distance of 40 µm was preserved between the measurement points to avoid residual stresses, as mentioned by He *et al.*[13] The results indicated that different irrigation protocols affected the elastic modulus of dentine; thus, the first hypothesis of the study that different irrigation protocols do not change the elastic modulus of root dentine from coronal to apical and from canal lumen toward the inner root dentine should be rejected.

In this study, irrigation protocols were applied at room temperature; thus, the effect of temperature was eliminated. In contrast to previous studies, which used high concentrations of irrigation solutions for longer periods,[5,6] this study's irrigation protocols were conducted in accordance with clinical protocols to get more clinically relevant results. For this reason, lower concentrations of NaOCl, Biopure MTAD, SmearClear, and CHX were used as final irrigating solutions and then compared with control groups of EDTA, 5.25% NaOCl, and saline.

The effect of NaOCl on the elastic modulus of root dentine has previously been published.[14] This effect may change depending on the concentration of the solution.[5] In this study, dentine bars[6] or extended application times for the irrigation solutions[12] were not used; instead, clinical protocols were followed. The results indicated that the elastic modulus of dentine decreased with increased concentration of NaOCl [5.25% vs. 1.3%; Table 2]. Conversely, Grigoratos *et al.*[5] found that NaOCl concentrations did not significantly decrease dentine's elastic modulus. These different results might be because of the conditions mentioned above.

The common concentrations used for EDTA are 15%–17%.[15] When the elastic modulus of dentine was evaluated in this study according to the regions, the effect of 17% EDTA on root dentine from coronal to the apical was found to be similar [Table 2]. The lowest values were achieved with 17% EDTA [Group 7; Table 2], while its effect on elastic modulus of root dentine was changed when used in combination with NaOCl [groups 1 and 2; Table 2]. NaOCl irrigation followed by 17% EDTA opens the dentinal tubular orifices, erodes the intertubular dentine, and reduces dentine microhardness.[16-18] Several *in vitro* studies have shown that this combination removed the inorganic and organic phases of the dentine.[19-21]

The toughness of dehydrated dentine is significantly lower than when in the hydrated state.[22,23] When the dentinal tubules lose water, the "water-induced effects" such as inherent plasticity and distinct strain response in the directions parallel and perpendicular to the dentinal tubules are lost.[19,24] Kinney *et al.*[25] reported different values when the elastic modulus of dentine was measured under dry or wet conditions (23.9 vs. 20 GPa, respectively). This water loss was reported to be fully restorable by rehydration. In this study, the dentine samples were kept in wet conditions between the measurements; however, the testing procedure was applied under dry conditions with dynamic loading. Considering the rapidity of the testing procedures, the possible effect of dehydration on the elastic modulus of dentine was ignored.

FEA is a widely used technique; however, many details are idealized, simplified, or ignored.[9] Three-dimensional modeling is therefore necessary. However, it is not possible to simulate all of the structures because they are not homogeneous; this is the case with dentine. Therefore, in this study, all the structures except dentine were assumed to be homogeneous.[8] A perfect simulation still could not be carried out for dentine because an affected dentine layer was modeled and the elastic modulus of the affected dentine layer (average of coronal, middle, and apical values) was achieved from the first part of the study. While doing this, the effects of dentinal tubules, intrapulpal hydrostatic pressure, and the elastic modulus gradient on the mechanical properties of dentine were ignored, as in the previous FEA studies.[8] This is one other important limitation of this study.

The stress distributions or accumulations within the FEA models were found to be similar in this study, showing that the outcome of the elastic modulus change does not affect the biomechanics of the roots. Therefore, the second hypothesis that changes in the elastic modulus of dentine because of different irrigation protocols do not affect the stresses within an endodontically treated tooth must be accepted.

Belli *et al.*[8] reported that both MTAD and 17% EDTA changed the stress dynamics within the root dentine when the clinical protocol was not simulated. As a result, these authors concluded that MTAD should be used according to the clinical protocol as advised by Machnick *et al.*[6] Therefore, in this study, a clinical protocol of 1.3% NaOCl with MTAD was applied. MTAD showed similar results with physiological saline, confirming the results of this previously published article. In addition, this result should be related to the concentration of 1.3% NaOCl.

The maximum stress was noticeably reduced toward the apical region of the root [Figure 4]. This decreased stress distribution in the middle/apical third of the root was attributed to the shape of the tooth and its interaction with the supporting bone.[19] Also, a number of studies have suggested that there are no major differences in the mechanical properties of dentine from teeth with vital pulp and root-filled teeth.[26,27]

The maximum and total stress values were similar in CHX, MTAD, and saline [Table 3]. In accordance with previous studies, the results of this study showed that using the clinical protocol for MTAD and CHX causes no adverse effects on the mechanical properties of root canal dentine.[6,14] Considering the other advantages of CHX such as remineralizing the demineralized dentine[28] or other positive properties of MTAD,[4,6] the results of this study suggest that CHX and MTAD have beneficial qualities as root canal irrigants.

Conclusion

Within the limitations of this study it was concluded that

(1) Despite the effect of different clinically used irrigation protocols on elastic modulus of the inner dentine, this does not affect the biomechanics of the roots

(2) CHX and MTAD showed a similar effect with saline on the elastic properties of root dentine when used according to the clinical protocol.

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Conflicts of interest

There are no conflicts of interest.

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