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COMPARATIVE EVALUATION OF EFFECT OF 17% ETHYLENEDIAMINETETRAACETIC ACID AND CHITOSAN ON FRACTURE RESISTANCE OF ENDODONTICALLY TREATED TEETH: AN IN VITRO STUDY.

Dental Science

Dr. Anirudha Sharma* PG Student, Department of Conservative and Endodontics, Bharati Vidyapeeth Deemed University Dental College and Hospital, Pune, Maharashtra, India. *Corresponding Author

Dr. Varsha Pandit PG guide, MDS, Department of Conservative and Endodontics, Bharati Vidyapeeth Deemed University Dental College and Hospital, Pune, Maharashtra, India.

Dr. Anupam Sharma PG guide, MDS, Department of Conservative and Endodontics, Bharati Vidyapeeth Deemed University Dental College and Hospital, Pune, Maharashtra, India.

Dr. Rajlaxmi Patil Assistant Professor MDS, Department of Conservative and Endodontics, Bharati Vidyapeeth Deemed University Dental College and Hospital, Pune, Maharashtra, India.

Dr. Shivani Bhatia PG Student, Department of Conservative and Endodontics, Bharati Vidyapeeth Deemed University Dental College and Hospital, Pune, Maharashtra, India.

Dr. Esha Kedia PG Student, Department of Conservative and Endodontics, Bharati Vidyapeeth DeemedUniversity Dental College and Hospital, Pune, Maharashtra, India.

ABSTRACT

Effect of solutions of 0.2% Chitosan [Chitoirrigant] and 17% EDTA on microhardness of root dentin was evaluated comparatively in this study. 50 sound human single canaled teeth were selected and decoronated at the CEJ. Cleaning and shaping of root canals was done using ProTaper instruments till F2. 2 ml of 1% NaOCl was used as an intracanal irrigant between uses of each succeeding file. Canals were finally rinsed with saline to remove any dentin debris remained in canal after instrumentation. Teeth were randomly assigned into 5 groups, Group 1 the samples were irrigated with saline. Group 2,the samples were irrigated with 17% EDTA for 1min followed by 10 ml of 1% NaOCl for 1 min. In Group 3, the samples were irrigated with 17% EDTA for 3min followed by 10 ml of 1% NaOCl for 3 min.In Group 4, the samples were irrigated with 0.2% Chitosan for 1min followed by 10 ml of 1% NaOCl for 1 min. In Group 5, the samples were irrigated with 10 ml of 0.2 % Chitosan for 3min followed by 10 ml of 1% NaOCl for 3 min. After the use of irrigants roots were obturated with Gutta-percha points, and AH Plus (Dentsply) root canal sealer was used in the single-cone technique. All the teeth roots were set into rapid polymerization acrylic resin and the resin block was fitted to the universal testing machine to check the fracture resistance of each tooth. Analysis of variance (ANOVA) was used to find significance of study parameters between the groups. Post Hoc analysis was carried out along with ANOVA test. Fracture resistance test showed that the EDTA had detrimental effect on dentin. In conclusion it can be said that Chitosan has lesser detrimental effects on root canal dentin when compared to 17% EDTA.

KEYWORDS

Chitosan, fracture resistance, 17% EDTA

INTRODUCTION:

Successful root canal treatment depends on proper technique, quality of instrumentation, irrigation, disinfection, and three-dimensional obturation of the root canal system. [1]

Use of mechanical instrumentation and chemical irrigation has been recommended to obtain effective elimination of biofilms from the root canal system.[2,3] Chelating agents such as Ethylenediaminetetraacetic acid (EDTA) is the most commonly used during biomechanical preparation.[4,5,6]

Though it is commonly used as a chelating agent for removal of smear layer on dentin, it reduces the micro hardness of dentin, alters the structural characteristic of the dentin resulting in a compromised mechanical integrity and an increased potential for bacterial adherence on the collagen. Chitosan is a non-toxic cationic biopolymer usually obtained by alkaline deacetylation from chitin, which is the principal component of crustacean exoskeletons. The covalent immobilization of Chitosan on dentinal collagen has been proposed to induce the remineralization of the exposed and demineralized dentin structure because its functional phosphate groups might bind to calcium ions to form a favorable surface for crystal nucleation, resulting in the formation of a calcium phosphate layer. [7-10]

Chitosan has attracted a great deal of attention in dental research because of its biocompatibility, biodegradability, bioadhesion and lack of toxicity. [10,11,12].

So, in the present study we have evaluated the effect of routinely used chelating agent 17% EDTA with Chitosan on fracture resistance of endodontically treated teeth.

MATERIALS AND METHODOLOGY:

The present study was conducted in the Department of Conservative Dentistry and Endodontics , Bharati Vidyapeeth Deemed University Dental College and Hospital, Pune. The study has been reviewed and approved by the institutional ethics committee.

MATERIALS AND INSTRUMENTS:

Experimental Groups Materials : 17% EDTA [Prime dental company] and CHITOIRRIGANT RC SUPPORT [Everest Biotech] [O.2% Chitosan =chitosan + acetic acid]

EQUIPMENTS

Universal Testing Machine [JANITICS]

METHOD

Sample collection: 50 human single-canal teeth extracted for orthodontic or periodontal reasons were selected for this in vitro study, obtained from the Department of Oral and Maxillofacial Surgery, Bharati Vidyapeeth Deemed University Dental College and Hospital, Pune. Consent form along with subject information sheet was given to patients explaining the entire procedure. Identity of all patients whose teeth were used for the study was kept confidential, Strict anonymity was maintained. Sound non-carious human single-rooted teeth , teeth with mature apices and single rooted teeth with single canal were included in this study. Teeth with visible caries, cracks, defects, curved roots and multiple canals, root resorptions ,previous endodontic treatment and restorations were excluded.

Specimen Preparation: After extraction of single-canaled teeth, the soft tissues, dental calculus, and stains was immediately removed from teeth and stored in normal saline for further use.[1,13]

The crown of each tooth were sectioned perpendicular to the long axis of the root below the cementoenamel junction using a straight handpiece so that the length of root was adjusted to 13 mm. The

working length of each root were determined by inserting #10 K-file (Mani, Japan) until it just exited the apical foramen and then 1 mm was subtracted from the obtained length.[12,14] Later, cleaning and shaping of root canals was done using ProTaper instruments till F2. Along with instrumentation, 2 ml of 1% NaOCl was used as an intracanal irrigant between uses of each succeeding file. Further, the canals were finally rinsed with sterile saline to remove any dentin debris remained in canal after instrumentation.[12]

Teeth were randomly assigned into 5 groups, each containing 10 samples.

Experimental groups: Teeth will be randomly assigned into five groups

Table 1: ***Experimental groups***

Groups	*Chelating Agent*	*Time*	*Sample size*
(Control group) Group 1	10 ml of saline	-	10
Group 2	17% EDTA	1 minute	10
Group 3	17% EDTA	3 minutes	10
Group 4	Chitosan	1 minute	10
Group 5	Chitosan	3 minutes	10

Obturation: After the use of irrigants, roots were obturated with ProTaper F2 Gutta-percha points, and AH Plus (Dentsply) root canal sealer was used in the single-cone technique. Excess coronal Gutta-percha was removed with a ball burnisher 1 mm below canal opening and sealing was done with Coltosol F. Specimens were stored in an incubator at 37°C and 100% humidity for 7 days to allow the sealer to set completely.

Fracture resistance testing: Apical 5 mm of all specimens were embedded along the long axis in self-curing acrylic blocks with 8 mm of each root exposed. Fracture resistance of the specimen was tested using a Universal Testing Machine (JANATICS). A custom made stainless steel loading indenter with a round tip (r = 2 mm) was used to deliver force directed along the long axis of the tooth. The load increased at the rate of 1 mm/min until fracture took place. Fracture was evidenced by an audible crack or a sudden drop in load as seen on the graph. The force necessary to fracture each root was recorded in Newtons (N). The peak load to fracture the samples were recorded. All the data of forces at which the teeth fractured were recorded in Newtons and were subjected to statistical analysis.[1]

STATISTICALANALYSIS:

Results on continuous measurements were presented on Mean -+0.5 SD. Level of significance was fixed at p=0.05 and any value less than or equal to 0.05 was considered to be statistically significant.

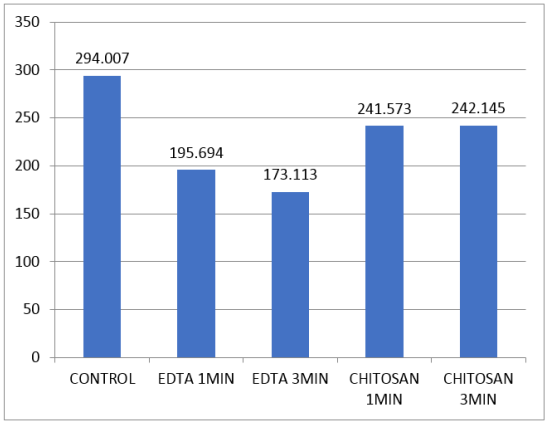
Analysis of variance (ANOVA) was used to find the significance of study parameters between the groups (Inter group analysis). Further Post Hoc analysis was carried out as the values of ANOVA test were significant.

The Statistical software IBM SPSS statistics 20.0 (IBM Corporation, Armonk, NY, USA) was used for the analyses of the data and Microsoft word and Excel were used to generate graphs,tables etc.

RESULTS

Table 2: ***Fracture Resistance values of samples, calculated under Universal Testing Machine of different groups.***

	CONTROL GROUP	17% EDTA GROUP [1minute]	17% EDTA GROUP [3minute]	CHITOSAN GROUP [1minute]	CHITOSAN GROUP [3minute]
1	290.981	197.823	170.329	246.123	246.876
2	289.460	198.123	169.461	249.456	245.291
3	295.393	189.479	171.339	239.319	249.591
4	300.463	196.812	173.461	234.210	240.441
5	297.396	195.396	171.129	251.292	240.876
6	299.763	199.421	176.981	237.456	239.876
7	295.333	194.881	174.871	230.789	239.591
8	289.291	193.211	172.811	244.312	237.467
9	290.871	194.321	175.293	242.456	241.321
10	291.119	197.476	175.461	240.321	240.123



Graph 1: *Comparison of effect of mean fracture load on different material*

DISCUSSION

Chelation is a physico-chemical process which involves the uptake of multivalent positive ions by specific chemical substances. Chelating agents were first introduced by Nygaard Ostby in 1957. Chelating agents when used as irrigants allow adequate removal of smear layer, and also provide antimicrobial action . Various chelating agents have been used in endodontics for irrigation e.g. EDTA, EDTAt ,Citric acid, Maleic acid etc. [1-5,13-15].

EDTA is a polyaminocarboxylic acid with the formula [CH2N (CH2CO2H)2]2. It is a complex molecule which binds to metal ions such as calcium and aluminium to form a stable ring structure. EDTA when applied to root canal surfaces removes the inorganic as well as organic components from the canal walls. Studies have shown that 17% EDTA is more efficient in smear layer removal than other decalcifying agents. [5,9,13]

Chitosan is a natural polysaccharide, prepared by the deacetylation of chitin, which is obtained from the shells of crabs and shrimps. It has attracted attention in dental research because of its biocompatibility, biodegradability, bioadhesion and lack of toxicity. It has a high chelating ability for various metal ions in acidic conditions. The antibacterial property of chitosan is due to the electrostatic interactions between NH3+ that binds to bacterial cell surface components and results in the leakage of intracellular components. Therefore, newly introduced Chitosan is now being recommended as a root canal irrigant due to its chelating property and anti-bacterial action. [11,12,13]

EDTA has an erosive effect on dentin. Thus, in addition to EDTA, Chitosan was used in this study.[12]

Various studies have been conducted have shown chelating efficacy of EDTA at different time intervals (1 minute, 3 minutes and 5 minutes). The application time of Chitosan were based on the studies of (Spanó et al.; Machado Silveiro et al; Silva PV, 2012). [12,15]

Silva PV concluded that 17% EDTA used for more than 5 min, did not increase the capacity for calcium removal. 0.2% and 0.37% solutions of Chitosan, used for 5 min, promoted degradation of dentin as the concentration increased. Thus, 17% EDTA and 0.2% Chitosan was used for 1 minute and 3 minutes in this study. [12]

Root canal treatment with different irrigants or chelators causes changes in the microstructure of the dentine which in turn change the microhardness, permeability, and solubility characteristics of dentin and then the fracture resistance of the teeth.[6]

Thus this study evaluated and compared the effect of routinely used chelating agent 17% EDTA and recently introduced chelating agent Chitosan at two different time intervals (1 minute and 3 minutes) on the fracture resistance of endodontically treated teeth. In present study, 50 Single-rooted single-canaled maxillary or mandibular teeth were used . Teeth were decoronated till CEJ to get a standardized root length of 13 mm.[1,16]

Biomechanical preparation was performed with ProTaper hand files in a crown down manner as this technique allows for adequate cleaning

and penetration of irrigant to the apical third of the root canal. 1% NaOCl was selected for its antimicrobial and tissue dissolving property, as, at this low concentration, it has minimal effect on the mechanical properties of dentin.[1]

Teeth were randomly divided into 5 groups of 10 depending on the irrigant used at different time intervals.

In Group 1 [Control group] ,saline was used for irrigation purpose. In group 2 and 3 ,17% EDTA was used as chelating agent for 1 and 3 minutes respectively. In group 4 and 5, Chitosan was used as chelating agent for 1 and 3 minutes respectively.After biomechanical preparation of the sample, single cone technique of obturation was used in the study as it excluded both the wedging forces of the spreaders during lateral compaction and the excessive dentin removal required to facilitate the plugger's insertion during vertical compaction.[11,10,12]

After obturation teeth were subjected to fracture resistance testing under universal testing machine which had a stainless steel fixture with tip of radius 2 mm and compressive forces were applied in a vertical direction at the rate of 1 mm/min. Studies have reported that applying the force vertically to the long axis of the tooth transmits the force uniformly. [1,16]

Analyses of results showed that samples from Group 1 showed the highest fracture resistance while the roots that were irrigated with 17% EDTA for 3minutes [Group 3] showed lowest fracture resistance. Table 3 shows Fracture Resistance values of all the samples, calculated under Universal Testing Machine of different groups using Anova test. Control group performed better than all the other experimental groups.Saline was used as an irrigant in control group. It showed better results than other groups because it has no chelating capacity when compared to 17% EDTA and 0.2% Chitosan. [12]

In this study, Group 2 [EDTA 1 minute] performed better than Group 3 [EDTA 3 minutes].Results obtained within the experimental conditions of the present study indicates that, canal irrigation with 17% EDTA solution is time dependent, increased irrigation time leads to structural changes, as evidenced by reduction of fracture resistance compared with the control group. This effect is related to the demineralizing effect of the irrigating solutions on the root canal dentin. Therefore, in Group3 there is decreased fracture resistance as compared to that of Group 2. [7,8,10]

It was reported that due to longer exposure of root canal dentin to 17% EDTA, it leads to more amount of calcium loss ultimately leading to decrease in fracture resistance of tooth. [7,8] All these can be attributed as reasons for reduction in fracture resistance in group 2 and 3 [EDTA 1 minute and 3 minutes] in our study. This observation is similar to the study done by, Ayudin et al, De-Deus et al, Eldeniz et al, Sayin et al. [7,9]

Shreetha et al and Cruz et al evaluated the effect of different chelating solutions on the microhardness of the most superficial dentin layer from the root canal lumen and reported that EDTA reacts with calcium ions in hydroxyapatite crystals and removes them from the dentin by forming stable water soluble complexes.[1,10]

The fracture load required to fracture the samples in Chitosan 1 and 3 minutes groups is more as compared to 17% EDTA 1 and 3 minutes. Fracture load required to fracture the teeth is more in Chitosan 1 minute than Chitosan 3 minute. But there is no statistically significant difference between these two groups. Chitosan performed better than 17% EDTA experimental group's. [where $p>0.001$]

Though the exact mechanism of action of Chitosan is still not known, it is believed that adsorption, ionic exchange and chelation are responsible for the formation of complexes between the substance and the metallic ions. [11,12]

Currently there are two theories that explain the possible chelating mechanism of chitosan. One theory, known as the bridge model,states that two or more amino groups of a chain of Chitosan bind to the same metal ion.[12]

The second theory supports that only one amino group of the structure of the substance is involved in the binding, which is the metal ion "anchored" to the amino group.[13,14]

All of these could be the reasons for 0.2% Chitosan Group [Groups 4 and 5] showing higher fracture resistance in this study.

There was a statistically significant difference between 0.2% Chitosan and 17% EDTA groups. Group 4 and 5 showed higher fracture resistance in comparison to Group 2 and 3. Erosion can be avoided by using chelators at low concentrations or shorter chelating times; therefore, chelators that are effective at low concentrations and in a lesser time should be preferred, and this is the reason why 0.2% Chitosan performed better. [15,16]

Hence from the findings of this study it can be said that Chitosan has lesser detrimental effects on the root canal dentin when compared to 17% EDTA, as 17 % EDTA has binding capacity to that of the calcium ions of the dentin which leads to lesser fracture resistance of endodontically treated teeth.

CONCLUSION

Based on the results of the present in vitro study, it may be concluded that

1] Among the experimental groups 0.2% Chitosan performed better than 17% EDTA as it had less effect on the fracture resistance of the teeth when compared to 17% EDTA .
2] 17% EDTA when used for more than 1 minute leads to significant decrease in fracture resistance of tooth.
3] 0.2% Chitosan whether used for 1 minute or 3 minutes had no difference on the fracture resistance of the teeth.

REFERENCES

1] Bhandary S, Kakamari S, Srinivasan R, Chandrappa MM, Nasreen F, Junjanna P. A comparative evaluation of the effect of 8% and 17% EDTA exposure for 1 min and 10 min on the fracture resistance of endodontically treated roots: An in vitro study. J Conserv Dent 2017;20: 21-4.
2] Ingle's Endodontics 6th edition. Pathways of pulp. Chapter irrigation and intracanal medicaments .Pg 1000-1002.
3] Hülsmann M, Heckendorff M, Lennon A. Chelating agents in root canal treatment: Mode of action and indications for their use. Int Endod J 2003;36:810-30.
4] Scelza MF, Pierro V, Scelza P, Pereira M. Effect of three different time periods of irrigation with EDTA-T, EDTA, and citric acid on smear layer removal. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;98:499-503.
5] Sen BH, Ertürk O, Piskin B. The effect of different concentrations of EDTA on instrumented root canal walls. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:622-7.
6] Zhang K, Kim YK, Cadenaro M, Bryan TE, Sidow SJ, Loushine RJ, et al. Effects of different exposure times and concentrations of sodium hypochlorite/ethylenediaminetetraacetic acid on the structural integrity of mineralized dentin. J Endod 2010;36:105-9.
7] De-Deus G, Paciornik S, Mauricio MH. Evaluation of the effect of EDTA, EDTAC and citric acid on the microhardness of root dentine. Int Endod J 2006;39:401-7.
8] Meryon SD, Tobias RS, Jakeman KJ. Smear removal agents: A quantitative study in vivo and in vitro. J Prosthet Dent 1987;57:174-9.
9] Sayin TC, Serper A, Cehreli ZC, Otlu HG. The effect of EDTA, EGTA, EDTAC, and tetracycline-HCl with and without subsequent NaOCl treatment on the microhardness of root canal dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:418-24.
10] Cruz-Filho AM, Sousa-Neto MD, Savioli RN, Silva RG, Vansan LP, Pécora JD. Effect of chelating solutions on the microhardness of root canal lumen dentin. J Endod 2011;37:358-62.
11] Sinha VR, Singla AK, Wadhawan S, Kaushik R, Kumria R, Bansal K, Dhawan S. Chitosan microspheres as a potential carrier for drugs. Int J Pharm 2004;274:1-33.
12] Silva PV, Guedes DFC, Nakadi FV, Pecora JD, Cruz-Filho AM (2013) Chitosan: a new solution for removal of smear layer after root canal instrumentation. Int Endod 46: 332-338.
13] Semra et al .Evaluate the effects of EDTA on smear layer removal and on the structure of dentin, after 1 and 10 min of application. January 2002 Volume 28, Issue 1, Pages 17–19.
14] Perez-Heredia M, Ferrer-Luque CM, Gonzalez-Rodrıguez MP, Martın-Peinado FJ, Gonzlez-Lopez [2007].Decalcifying effect of irrigating solutions.Med Oral Patol Oral Cir Bucal. 2013 Jan 1;18 (1):e158-61.
15] Mazen Doumani et al . A Review: The Applications of EDTA in Endodontics2279-0861.Volume 16, Issue 9 Ver. V (Sep. 2017), PP 83-85
16] Geethapriya, et al. Chitosan-EDTA – A potential endodontic irrigant473Journal of Conservative Dentistry | Sep-Oct 2016 | Vol 19 , Issue 19 Ver. V (Sep. 2019), PP 83-87