OriginalResearch Article

AComparativeStudyonDentinalTubulePenetrationofEndodonticIrrigants: Confocal Laser Microscopy Insights

Abstract

Aim: Thisinvitrostudyisaimedtoanalyseandcomparethedentinalpenetrationdepthofthree endodontic irrigants using Confocal laser maicroscope.
*StudyDesign:*Itisadescriptivestudy.
*Methodology:*17%EDTA, 17%REDTAand 17% Citric Acid at acidicpH (pH5.5) and distilled water as a control was taken as final root canal irrigants. 60 human permanent single rooted teeth were taken from a pool of extracted teeth, decoronated and mechanically shaped using waveone gold file systemandapexwaspreparedbyWaveOneGoldLarge(size0.45,taper0.07)file and5.25%sodium hypochlorite solution. The final rinsing was done with rhodamine B dye conjugated EDTA, REDTA, Citric acid and distilled water. After this, teeth were embedded in epoxy resin and sectioning was done at coronal, middle and apical third level using microtome and thin sections of approx.200µm thickness were prepared. Sections were examined under confocal laser microscope and statistical analysis was done using ANOVA and Post-Hoc Tests.
*Results:*Studyrevealedthatthecitricacidisthebestendodonticirriganthavingmaximumdentinal penetration depth at all three levels.

Keywords

EDTA,REDTA,Citricacid,Dentinalpenetrationdepth,pH, Confocallasermicroscope

Introduction

"The prognosis of a root canal treatment depends on many factors and one of the important factors is an endodontic irrigant chosen. An ideal solution is a non-viscous, non-irritating, germicidal solution with detergent qualities and abilities to dissolve necrotic tissue"[1]"The antibacterialeffect,achievedbyendodontictreatment,ismorelikelyaffectedbydegreeofthe penetration of irrigants to scavenge bacteria residing deeply inside infected dentinal tubules than by instrumentation of the root canal system because the shaping protocol revealed deficient debridement and area untouched by both manual K-files and rotary or reciprocating instruments".[2]" Thecalciumpresentinhydroxyapatitecrystalsisoneofthemaininorganic elements of dentin. Any change in the calcium ratio can significantly alter the original proportion of organic and inorganic components, which can alter dentin permeability, micro-hardness, and solubility".[3]"EDTA has self-limiting action, forms a stable with calcium and dissolve dentin. Citric acid is used in concentrations ranging from 1- 40% in endodontic practice to remove smear layer after root canal preparation. 10% citric acid have been proven to be more effective in removing smear layer and dentine dissolution when compared with EDTA and also has antimicrobial effects"[4-6] In order to increase the cleaning and bactericidal potentialofthesolution,detergentcanbeaddedtoEDTA.Anotheradvantageofthisaddition is reducing the surface tension of the irrigant, facilitating the wetting of the entire root canal wall and thereby increasing the ability of the chelators to penetrate the root canal dentin.[7-9] Kennedyetal[10]reportedthatoncethesmearlayerwasremovedbyusingREDTA(EDTA 17.00 g, cetyl trimethylammonium bromide 0.84 g, 5N sodium hydroxide solution 9.25 mL, and distilled water 100.00 mL), dentinal openings could be created more easily in younger teeth thanin physiologicallyolderteeth, especiallyin moresclerotic middleand apical thirds. Aliteraturereviewhasrevealedthatdifferentmixtures,concentrations,pH,workingtimeand methods have been used to evaluate the irrigating solutions.[11] Thus considering these factors, this studywill be conducted to evaluate in vitro the penetration depth into dentinal tubules of 17%EDTA, 17%REDTA, 17% Citric Acid and distilled water (control) at acidic pH.

Materialand Methodology

This in vitro studyis done by using final irrigants namely17% EDTA (WaldentAlchem), lab formulated 17% citric acid (Rankem), lab formulate 17% REDTA (EDTA 17.00 g, cetyl trimethylammonium bromide 0.84 g ((Loba chemicals), 5N sodium hydroxide solution 9.25 mL(Rankem), and distilled water 100.00 mL) and distilledwaterand pH5.5 was adjusted on pH meter by addition of Hydrochloric acid on digital pH meter (decibel) at department of Pharmacology, World College of Medical Sciences & Research and Hospital (WCMSRH), Jhajjar (Haryana).

60humanpermanentteethwithsingle,straight,oval-shaperootcanalwithfullyformedapices werechosenfromapoolofextractedteethandwerestoredin5%sodiumhypochloritesolution (Septodont) for 2 hrs for soft tissue dissolution and then the teeth were stored in .9% saline solution(HiLine-NS)tilltheiruse.Thenthecrownswereremovedbydiamonddisc.Thenthe adequacy of the working length (WL) and the presence of an open apical foramen were confirmedbyinsertingasize10K-file(Mani). A mechanical glidepathwasestablishedusing

WaveOneGoldGliderreciprocatingsinglefiles(Densply). Subsequently,eachrootcanalwas shaped using WaveOne Gold Medium (size 0.35, taper 0.06) followed by WaveOne Gold Large (size 0.45, taper 0.07) files. The instruments were employed with a slow in-and-out peckingmotionusinganEighteethenodomotor.TheWLwasreassessedwithasize10K-File. "Followingeachinstrumentchange,eachcanalwasirrigatedwith1mLof5.25%NaOCl (Safe Endo)usingasyringeequippedwitha30-gaugeside-ventedneedle.Theneedlewaspositioned atleast2mmawayfromtheworkinglength(WL).Atotalof10mLof5.25%NaOClwasused for each specimen. Following irrigation, the outer surface of the teeth was dried using paper towels. Then, a thin layer of dual composite was applied to cover the apical third of the teeth. After that the samples were randomly segregated into 4 distinct experimental groups. After that, the samples were finally rinsed with 5ml of each 17% EDTA, 17%REDTA, 17% citric acidanddistilledwaterusing30-gaugesideventneedle.Eachsolutionwaslabelledwith.1%wt rhodamine B dye and was left for 3minutes and then the canals were dried with Wave One Gold paper points of large size. After final irrigation, in laboratory (Lab Crystals, Aliganj, Lucknow, Uttar Pradesh), the teeth were embedded in methyl-methacrylate resin. After hardeningtheresin, 200µm transversesections ofteeth werecut at coronal, middleand apical third bymicrotome and slides were prepared. After fixing the slides, the slides were analysed underConfocalLaserMicroscopeLeica(TCSSP8C)at10XusingFIJIsoftwareandthendata wasanalysedintheMicrosoftExcel2007andusingtheSPSSstatisticalsoftware23.0Version (Department of Cell Biology, IIT Sonipat (a branch of Central Research Facility IIT Delhi), Haryana)" [2].

Result

The mean dentinal penetration depth (in µm) was shown maximum by citric acid group and least was shown by distilled group (Table 1). The maximum penetration depth at coronal, middleandapicalwasalsotoppedbycitricacidgroupandatbottomwasdistilledwatergroup. Confocal images of various groups at various levels are shown in table 2.

Statistical Analysis

The data was entered in the Microsoft Excel 2007 and analyzed using the SPSS statistical software 23.0 Version. Thedescriptivestatistics includedmean, standard deviation frequency and percentage. The level of the significance for the present study was fixed at 5%. The intergroup comparison was done using the One Way ANOVA test followed by post Hoc Analysisdependinguponthenormalityofthedata.TheShapiro–Wilktestwasusedto investigatethe distribution of thedataandLevene'stesttoexplore thehomogeneityofthe variables.

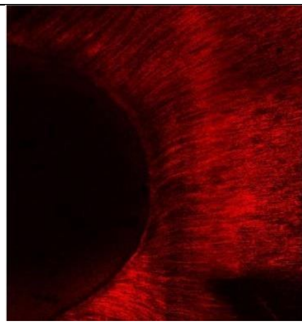
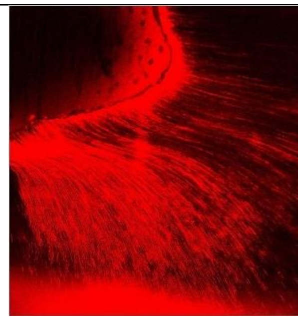
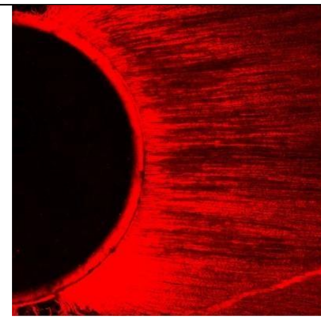
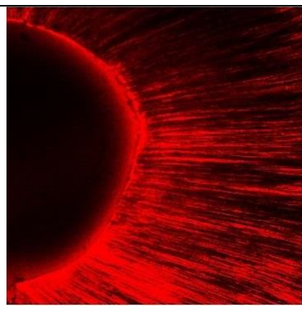
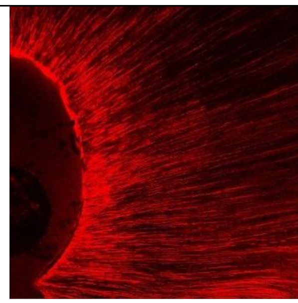
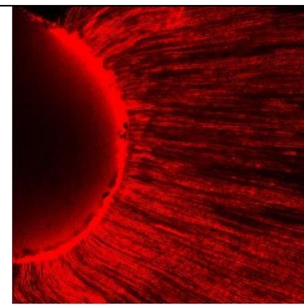
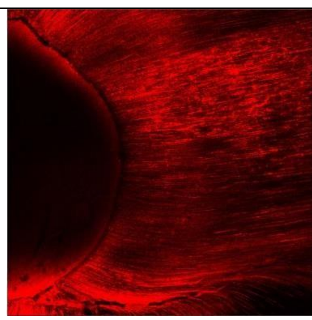
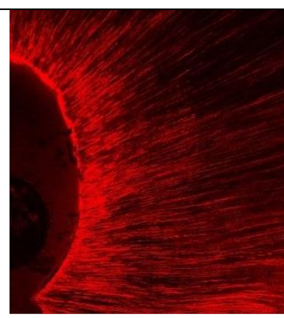
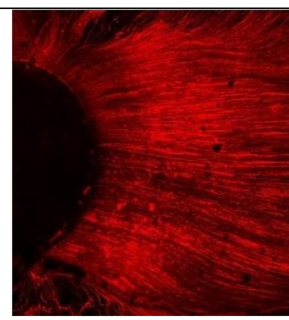
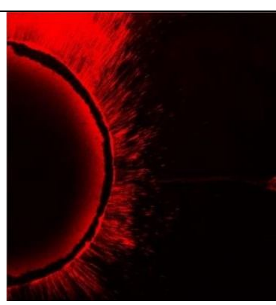
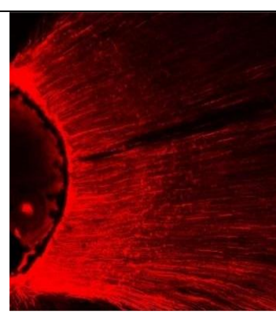
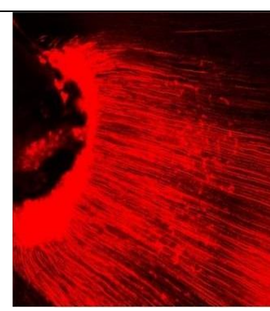
Table:1

Comparisonofmean(inµm)amongfour groups

	Apical	Middle	Coronal
Group Distilled Water	200.01±40.684	325.02±52.371	534.50±54.527
Group REDTA	293.31±43.018	544.78±56.139	824.90±54.605
Group EDTA	366.71±44.978	785.33±56.413	1216.40±51.247
Group Citric Acid	593.31±41.018	976.96±58.384	1648.00±60.593

Table:2

Confocallasermicroscopicimagesof variousgroupsatvariouslevel

Group Name	CoronalSection	Middle Section	ApicalSection
EDTA			
REDTA			
Citric Acid			
Distilled Water			

Discussion

In the present in vitro study, the penetration depth of four irrigating solutions at acidic pH (EDTA, REDTA, Citric Acid and distilled water as control) into dentine tubules is evaluated. The three irrigants used in the study except distilled water are chelating agents. This study indicateshigherpenetrationdepthsintodentinaltubulesinthecoronalandmiddlethirdsofthe teeth from all the groups and a lower penetration depth is in the apical third of all the root canals studied, confirming findings of previous studies.[12]

Thepresentfindingsmightbeattributedto theinfluenceoftherootcanal system's anatomical structure on the depth to which irrigants can penetrate into dentinal tubules. Indeed, dentinal tubules in the apical third of the root canals are less permeable than those in the coronal and middle thirds due to tubular sclerosis, the smaller diameter, and the reduced number.[13] The diameter of dentine tubules decreases from 1.2 μm at the pulp-dentine junction to 0.4μm at cemento-dentinaljumction.[14]Thenumberoftubules persquaremillimetre is also greaternear to pulp (58000mm^{-2}) than further away from the pulp (10000mm^{-2}).[15] As the tubules density reduces towards the apex, so does the dentine permeability.[16] Moreover, root dentine is not mineralised uniformly. Apical dentine is more sclerosed and mineralised.[17] Dentine permeabilityis directlyrelated to area of tubule lumina and indirectlyto wall thickness of the rootcanal.[18]AfterBMP,wallthicknessisreducedandsurfaceareaoftherootcanalisincreased but the smear layer acts as a diffusion barrier, reducing dentine permeability by 25-49%.[19-22]

Luciano Giardino[23-31], Generali, L.[32] and Peters OA[33] reported that lower surface tension enablestheirriganttopenetrateintodentinaltubules,microscopicirregularities,andaccessory canals.Taşmanetal[34]reportedthatonobservingtheaveragesurfacetensionvaluesofthetest solutions, it was noted that Ringer's solution, saline solution, and distilled water—which are knowntolackanychemicaleffect—hadthehighestvalues,questioningonceagaintheefficacy ofthesesolutionsinendodontictherapy.Ontheotherhand,therelativelylowersurfacetension values of NaOCl and EDTA may contribute to the high success rates achieved with the combined use of these irrigants as reported in previous reports. Abbaszadegan et al[35] and La Rosa GRM*, Scolaro C, Leanza G et al[36] also supported the high surface tension of distilled water,limitingthepenetrationdepthintodentinaltubules.DushanB.Naumovich[37]showedthe highest surface tension of distilled water amongst 22 endodontic irrigants used in the experimentandsupportedMarch,T.[38],whostatedthatAlongwithosmoticactivityand diffusibility (free diffusion), surface tension is considered one of the most important factors thatdeterminethepenetrabilityandspreadingofdrug.Theseabovestatementssupporttheleast penetration of distilled water into dentinal tubules.

In thisstudy, 2ndlastdentinalpenetrationdepthwasobtained fromREDTAgroup.REDTAis a chelating agent, formulated by addition of 0.84g Cetyl-trimethylammonium bromide (Cetrimde) to 17%EDTA in aqueous medium and buffered by NaOH at pH 8 and then made acidic (pH 5.5) by addition of HCl. Cetrimide {cetyltriethyl ammonium bromide (CTAB)} is both a disinfecting agent and a cationic surfactant, reduces the surface tension of the irrigant[26,39-40],improvesantibacterialeffectiveness[40-42],facilitatespenetrationoftheirrigantto thedentinsurface[43],andincreasesthewettabilityofthedentinsurface[26].Inaddition,cetrimide mayalsoalterthestructureofhydroxyapatitenano rodsinaconcentrationdependentmanner. It also elongatesthehydroxyapatitenano rods,therebythelength diameterratio ofthesenano rodsdecreaseswhenthecontentofCTABincreasespotentiallyalteringthephysicalproperties of dentin[44]. In 1991, Sterrett JD, Delaney B, Rizkalla A, Hawkins CH stated that the cationic surfactants are potent antimicrobial agents that have also been shown to act on biofilm components, but they have no decalcifying effects on root canal dentin.[44] Then again in 2011 by Tianyuan M, Zhigou X, Libing L.[45] reported that this low penetration might be due to the demineralizingactionofchelatingagentsresultsinincreasedexposureoforganiccontent.This organic matrix of dentine acts as a limiting factor for further dissolution of inorganic content, thus reducing the decalcifying action of chelating solutions over time. Again in 2014, same findingsweresupportedbyPoggioC,DagnaA,ColomboM,ScribanteA,ChiesaM.bystating that the presence of cetrimide in the irrigating solutions does not improve the extraction of Ca2+ from root dentine and it could be considered useful to complete antibacterial activityof irrigating solutions.[46] La Rosa GRM*, Scolaro C, Leanza G et al.(2021)[36] reported that there were no significant changes reported in surface tension and wetting ability regarding EDTA groupandEDTAPLUSgroup(EDTAcontainingsurfaceactivecompounds).Asweconsider thatlowsurfacetensionresultsinhighdentinalpenetration,sofromabovestatementitcanbe derivedthatEDTAplusmightberesultedinlow penetrationdepthintodentinaltubulesaswe analysed in our study. Many previous studies also reported the similar findings.[26,47] Giardino L,AmbuE,BecceC,RimondiniL,MorraM[23]hadstatedthattheactorsaffectingthedepthof penetration of root canal irrigants could be surface tension, viscosity and molecular size, so another reason of low dentinal penetration depth might be increased viscositydue to addition of surfactants in to EDTA.

The chelation effect typically involves the formation of multiple coordinate bonds between a multidentate ligand (electron pair donor) and a metal ion (electron pair acceptor). EDTA, a hexaprotic weak acid (H6Y2+) featuring four carboxylic acids and two ammonium groups, was initiallysuggested by Nygaard-Ostbyfor use in root canal preparation through chelation. When mixed with water, EDTA exhibits an acidic pH but cannot be utilized directly for irrigationduetoitspoorsolubility.Moreover,themechanismofEDTA'sactioninvolvesboth protonation and chelation, which vary depending on the pH level.,[48,10,49] At a low pH, the protonationofEDTAreducesthenumberofavailableelectronpairs,whichinturnslowsdown the dissociation of hydroxyapatite (HAp) and the process of demineralization. Conversely, at high or neutral pH levels, EDTA's ability to bind calcium ions promotes the dissociation of HAp, making it more available for chelation. To improve EDTA's water solubility while reducing the protonation effect, its pH must be adjusted to at least 7. EDTA chelates calcium ions, forming soluble calcium chelates. When disodium EDTA is added to the system, it removes calcium ions from the solution by forming these soluble chelates.[50] Ballal NV[51], TorabinejadMetal[52]andManciniM[53]revealedEDTAtobelesssuccessfulintheapicalthird comparedwiththecoronalandmiddlethirdsfortheremovalofSL. Ithasbeenshownthatthe dentinaltubulesprogressivelyscleroseintheapicalthird.[13,54]Thus,theactivityofEDTAmay not beas effectivein the apical third.[53] Calve,Medina,and Shnchez[29] has reported the almost similar findings. Cury JA[55] reported that the efficiency of EDTA solutions can be achieved between a pH of 5 to 6. However, EDTA preparations usually have an average pH of 7.3, possibly exerting a greater solubility effect on hydroxyapatite. At high pH values, the excess number of hydroxyl groups will slow down the dissociation of hydroxyapatite, thus limiting thenumberofavailable Ca^{2+}.Atalowomeutral pH,thebindingofCa^{2+}willtendtoincrease the dissociation of hydroxyapatite and its availabilityfor chelation.[56,57] Yılmaz et al[26] showed that plain EDTA solution mayonlybe suitable for wetting of dentin at a pH of 5.5 in 37□. It mightbesuggestedthatusingEDTAsolutionduringrootcanaltreatmentmightresultinbetter surface tension level because the body temperature is around 37□. “Furthermore, the ineffectiveness of NaOCl and Ethylenediaminetetraacetic acid (EDTA) in dissolving smear layerintheapicalthirdofrootcanalscanattributetotheirhighdynamicviscosity.[41]Moreover, Because the wettability of pure EDTA itself is poor, some reports have suggested that a surfactant should be added to pure EDTA solution”.[58-65] Further, Ravnik C[66] and Cury JA[55] stated that as pH decreases, the solutions might not be so penetrating.

"An alternative to EDTA, which is less widely recognized but still possesses strong chelating properties, is citric acid".[25,67-68] "Its ability to bind to metal ions makes it valuable in processes wheretheremovalorsequestrationofmetalsisnecessary.Therefore,EDTA" [48]andcitricacid[69] havebeenrecommendedasadjuvantsinrootcanaltherapy.The17%citricacidisusedinthis studybecauseinadditiontoitsbiocompatibility,ithastheabilitytoeliminateinorganicsmear layertissue.This is consistent with Schafer's[70] assertion that 1–40%citricacid can beused in root canal treatment because it is effective in removing smear layers, in dissolving the dentin powderandindemineralizingtheintertubulardentintoopenthedentinaltubules.Additionally, this is in accordance with research by Malheiros[71] showing that 17% EDTA has higher cytotoxicity than 10%, 15%, and 25% citric acid, so 10–25% citric acid had better biocompatibility than 17% EDTA. Sousa S.M., S. T (2005)[3] has conducted a study to biochemically compare the decalcifying effects of 1% EDTA (pH 7.4), 1% EGTA (pH 7.4), 1% CDTA (pH7.4), 1% citricacid solutions (pH 1.0 and 7.4) and saline solution (control) on root dentin and reported that 1% citricacid at pH 1.0 was the best solution to remove calcium fromtherootdentin.Takingtheinspirationfromtheirstudy,thisstudyisalsoaimedtocompare the dentinal penetration depth making the similar concentrations and pH of the root canal irrigants.Thisstudyrevealsthatthemaximumpenetrationdepthintodentinaltubulesisshown by 17% citric acid at pH 5.5.long-standing studies reported its best action at lower pH.[71-72] Both studies have demonstrated that the pH level of a citric acid solution is a more crucial factor for demineralization than its concentration. Sterrett et al.[44] suggested that "this phenomenon might result from a balance between the decreasing pH and the increasing viscosity of the solution as the constituent concentration rises. At high concentrations, citrate occupies a significant portion of the solvent, greatlyreducing the amount of solvent available for Ca2+ diffusion". Previous literature has been suggested that 10% citric acid has been as a good alternative to EDTA for better elimination of the smear layer from the root canal walls.[67,65,28]. The reason may be its acidic pH, which increases the elimination of calcium.[46]

Conclusion

This is the first study aimed to compare and analyse dentinal penetration depth using the 17%citricacid,17%EDTAand17%REDTAasanendodonticirrigantsatpH5.5anddistilled waterasacontrol.Inspiteoflimitationsofthisstudy,thisresearchreportsthe17%citricacid as a better root canal irrigant than EDTA, REDTA and distilled water and it also shows that the addition of a surfactant to EDTA has little effect on dentinal penetration.

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DataAvailabilityStatement:Thedataisavailablewiththemainauthoronrequest.

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ConflictsofInterest:Allauthorsdeclarenoconflictofinterest.

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