INTRODUCTION

INTRODUCTION

Successful root canal treatment depends on the thorough debridement of the root canal system, the elimination of pathogenic organisms and finally the complete sealing of the canal space to prevent ingress of bacteria from the oral environment and its spread to the periapical tissue. Inadequate obturation may result in the movement of oral fluids into voids in the obturated root canal and the induction of a periapical inflammatory reaction.[1]

The physical properties necessary for this function include adaptation and adhesion of the filling material to the root canal wall.[2] Gutta percha used for obturation does not bond to the sentinel wall due to poor adhesiveness and is therefore, used in conjunction with root canal sealers to accomplish this goal.[3]

Studies have shown that large areas of root canal walls remain untouched despite using hand and rotary instruments during canal preparation. This shows the importance of disinfecting the root canal system by chemical means. For this, the combined irrigating solutions used in a specific sequence can achieve this goal.[4]

During mechanical preparation of the canal, smear layer is formed on the instrumented canal walls and debris on the un-instrumented walls.[5] Studies have shown that these may impede sealer penetration into the dentinal tubules, thereby compromising the seal required during obturation.[6]

During instrumentation of the canal system, a superficial smear layer containing organic and inorganic particles; namely, pulpal remnants, dentinal debris, odontoblastic processes and bacteria are left behind on the dentinal walls.[7] A study concluded that significantly greater number of bacteria were found to adhere to those teeth in which a smear layer was present. Smear layer produced during root canal preparation promoted adhesion and colonization of P. nigrescens to the dentin matrix; it might also increase the likelihood of canal reinfection.[8]

The literature is inconclusive as to whether the smear layer should be removed prior to obturation. Some studies suggest that the removal of smear layer is advantageous because it eliminates trapped bacteria, allows for a higher quality seal, and decreases bacterial leakage. Other studies do not recommend smear layer removal because it increases dentin permeability, creates an additional avenue for bacterial leakage or disrupts the apical seal.[9,10,11] Studies advocating leaving the smear layer intact have theorized that its presence may prevent the initial penetration of bacteria into dentinal tubules.[12]

These conflicting studies may explain a 2001 survey that revealed that more than three-fourths of the dental students and nearly two-thirds of the endodontic residents are not being taught to routinely remove smear layer.[13]

The smear layer is tenaciously attached to the dentinal wall and cannot be removed by rinsing with saline alone.[14] Suchithra M S et al. showed that the use of saline as the only irrigant, left a typical amorphous smear layer on the root canal walls.[15]

Sodium hypochlorite (NaOCl) is the most widely used irrigant in endodontics. Among the irrigants, NaOCl solution is considered as the gold standard because of its exceptional qualities as an antiseptic and tissue dissolving effects.[16] However, it is toxic to the periapical tissue and has been suggested to degrade the micromechanical characteristics of dentine.[17] Furthermore, it has no effect on the inorganic part of the smear layer[18], as such a decalcifying agent should be used.[19] Sodium hypochlorite is used as an irrigant because it combines important properties such as tissue dissolving capability and microbicidal activity. The organic tissue-dissolving activity of NaOCl is well known aand its action increases with rising temperatures.[20] It is used in combination with chemical agents such as EDTA and Chitosan which aid in the elimination of smear layer and debris.

In addition to NaOCl, a chemo mechanical instrumentation regimen that incorporates the chelating agent Ethylene diamine tetra acetic acid (EDTA) has been shown to effectively remove the smear layer and expose dentinal tubules.[2,14] EDTA is a polyaminocarboxylic acid that is water soluble in a neutral or alkaline pH. It is used in endodontics because of

its chelating property whereby it interacts with calcium ions present in dentin to form soluble chelates of calcium. It is the most widely used chelating agent in endodontics.[21]

The literature supports using 1ml of 17% EDTA over a 1minute exposure followed by 3 ml of full strength sodium hypochlorite (NaOCl) as a final irrigation protocol prior to obturation.[22] This combination effectively removes the smear layer while minimizing erosion of the dentinal walls.[23] Other irrigants and techniques reported to remove the smear layer include hydrogen peroxide, citric and other weak acids, Bio Pure® MTAD®, Qmix® and activated irrigation using ultrasonics and lasers. However, these methods have been found to be less effective than the combination of EDTA and NaOCl.[2,14,24,25,26]

Etidronic acid (also known as 1-hydroxyethane 1,1-diphosphonic [HEDP]) is a biocompatible chelator that can be used in conjunction with NaOCl. It is a ‘‘soft’’ chelator that is less aggressive on dentin than EDTA.[27] Studies have shown that it has a weak chelating capacity when used alone.[28] This property can be used to the advantage of using NaOCl and HEDP as a single irrigant during and after root canal preparation. It was shown that a freshly mixed irrigant, containing HEDP and NaOCl, dissolves the smear layer. The combination is also shown to reduce the accumulation of hard tissue debris in the isthmus area.[29] Dual Rinse (9% HEDP) (Medcem GmbH, Weinfelden, Switzerland) is a medical device (product approved for use in the root canal) based on this chemistry. It comes in a capsule containing 0.9 g etidronate powder, which should be mixed immediately with 10 ml of the NaOCl solution of choice directly before treatment. This solution remains useful for 1 hour with all the desired properties of NaOCl remaining intact.[30] Additionally, this combined solution of HEDP and NaOCl inhibits smear layer formation during instrumentation as well as conditions the root canal wall for subsequent obturation.[21,31] A mixture of HEDP and NaOCl can be used not only during root canal instrumentation but also as a final irrigant.

Chitosan (2-amino-2-deoxy-β-D-glucan), a naturally acquired polysaccharide that is prepared by the deacetylation of chitin, is mainly obtained from crab and shrimp shell and has emerged as a potential material for bio dental applications.[32] Chitosan is a natural polysaccharide, which has attracted the attention of Dental research because of its

biocompatibility, biodegradability, bioadhesion and lack of toxicity.[33] It has a high chelating ability for various metal ions in acidic conditions and has been used widely for the removal of metal ions in different industrial areas.[34]

Bioceramic selaers like BioRoot RCS along with gutta-percha help to provide 3 dimensional seal of the root canal increasing the potential and obtaining more reliable results. BioRoot RCS (Septodont, Saint Maur des Fosses, France) is a tricalcium silicate sealer used as a new range of dental material that exhibits superior mechanical properties besides biocompatibility and bioactivity.[35]

This bioceramic material contains hydrophilic polymer which improves the adhesion property of the material. Even though the exact mechanism of bioceramic sealer to root dentin is unknown it is been suggested that the tubular diffusion[36] and the formation of hydroxyapatite (moisture in dentinal tubule)[37]along the mineral infiltration zone by the sealer[38] result in the close bond of the sealer to the dentine in the root canal.

Bond strength of endodontic sealers to dentin is an important property of filling materials because it minimizes the risk of detachment of the filling materials from dentin during restorative procedures or masticatory function ensuring that sealing is maintained and, consequently, leading to the clinical success of endodontic treatment.[39]

It has been suggested that the push-out test provides a better evaluation of bonding strength than the conventional shear test because in pushout test, the fracture occurs parallel to the dentine bonding interface, which makes this a true shear test for parallel-sided samples. Interfacial strength and dislocation resistance between the root filling material and the intra-radicular dentine can be evaluated using thin slice pushout tests.[40] The pushout bond strength test is a practical and reliable method that evaluates the adaptation of a restorative material to the root canal dentin. This study measures the difference in pushout bond strength of BioRoot RCS to the root dentin by using relatively new chelating agents such as Etidronate and Chitosan.

The null hypothesis is that there is no significant difference in pushout bond strength of BioRoot RCS after irrigating the root canal with different chelating agents such as 17% EDTA, 0.2% Chitosan and 18% HEDP.

AIM AND OBJECTIVES

AIM

The aim of this study was to evaluate and compare the pushout bond strength of BioRoot RCS in instrumented root canals, after irrigation using chelating agents-17% EDTA, 0.2% Chitosan, 18% HEDP.

OBJECTIVES

1. To evaluate the pushout bond strength of BioRoot RCS in root canals treated with different chelating agents.
2. To compare the effectiveness of different chelating agents in removing the smear layer in instrumented root canals.
3. To assess the patterns of fracture of BioRoot RCS with the root canal wall after pushout testing.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

- **Dorothy McComb et al. (1975)[7]** in their literature on preliminary Scanning Electron Microscopic study of root canals after endodontic procedure, concluded that the most effective cleaning procedure was the use of REDTA sealed in the canal for 24 hours. Canals treated in this way were free of a smear layer, superficial debris and the use of irrigating agents assumed greater importance.[7]

- **Russell S. Yamada et al. (1983)[14]** in their literature compared high volume final flush with several irrigating solutions using SEM and concluded that final flush with 10 ml of 17% EDTA buffered to pH 7.7 followed by 10 ml of 5.25% NaOCl solution was the most effective to clean the root canal after completion of instrumentation.[14]

- **Pashley DH et al. (1992)[41]** in his overview of smear layer: structure and function stated that smear plugs, together with the smear layer decrease dentin permeability, dentin sensitivity and surface wetness. Bonding to smear layers appears to limit the theoretical bond strength unless the smear layers are loosened or partially removed.[41]

- **Leonidas Vassiliadis et al. (1996)[42]** in his study on the effect of smear layer on coronal microleakage stated that the removal of smear layer significantly improved the sealing in the coronal area.[42]

- **Keisuke Kurita et al. (1998)[33]** in their study found that chitin and chitosan were considerably versatile and promising biomaterials. The de-acetulated chitin derivative, chitosan was a more useful and interesting bioactive polymer. Despite its bio-degradability, it had many reactive amino acid side groups which offered possibilities of chemical modifications, formation of a large variety of useful

derivatives that were commercially available. The reactivity of Bchitin was examined and confirmed to be much higher than that of ordinary or Achitin. The resulting chitin derivatives were evaluated in terms of affinity for solvents, lysozyme susceptibility and antimicrobial activity, and the effects of substituents were discussed.[33]

- **H. Dwight Moss et al. (2001)[13]** in his survey on philosophies and practices regarding the management of the endodontic smear layer proposed that there was no clear consensus in the endodontic community, either academically or clinically, as to whether the smear layer should be removed or be allowed to remain before obturation of the root canal space.[13]

- **Arash Shahravan et al. (2002)[43]** in his review on effect of smear layer on sealing ability of canal obturation: a systematic review and meta-analysis concluded that smear layer removal improved the fluid-tight seal of the root canal system whereas other factors such as the obturation technique or the sealer did not produce significant effects.[43]

- **Arias-Moliz et al. (2002)[44]** evaluated the antimicrobial activity on Enterococcus faecalis that grew in biofilms and a dentinal tubule infection model of 9% etidronic acid (HEBP) / 2.5% sodium hypochlorite (NaOCl) irrigant solution. They concluded that in biofilms and inside dentinal tubules, HEBP did not interfere with the ability of NaOCl to kill E. faecalis.[44]

- **Semra Calt et al. (2002)[23]** in their literature of time-dependent effects of EDTA on dentin structures proposed that the results showed that 1 min EDTA irrigation was effective in removing the smear layer. However a 10-min application of EDTA caused excessive peritubular and intertubular dentinal erosion. Therefore, they suggested that this procedure should not be prolonged >1 min during endodontic treatment.[23]

- **M. Hulsmanm et al. (2003)[45]** stated that the mechanical instrumentation of the root canal produced a smear layer that covered the dentinal tubules. There was a controversy over whether to remove or maintain the smear layer, but a recent systematic review and meta-analysis of leakage studies concluded that the removal of the smear layer improved the fluid tight seal of the root canal system.[45]

- **Scelza et al. (2004)[46]** evaluated the smear layer removal from root canal dentin by 17% EDTA, EDTA-T and 10% citric acid after final irrigation for 3, 10, and 15 mins. They concluded that these 3 irrigants were effective at the shortest time tested and with an increase in time, they did not demonstrate an improved effect.[46]

- **Teixeira et al. (2005)[47]** verified under the scanning electron microscope (SEM), the influence of irrigation time with sodium hypochlorite (NaOCl) and ethylene diaminetetraacetic acid (EDTA) on intracanal smear layer removal. They concluded that canal irrigation with EDTA and NaOCl was equally effective in removing the smear layer from the canal walls of straight roots for 1, 3 and 5 min.[47]

- **Brent J. Crumpton et al. (2005)[22]** quantified the volume of 17% ethylene diamine tetra-acetic acid (EDTA) needed after rotary instrumentation to efficiently remove the smear layer and to determine if additional irrigation had any effect on debris removal. They concluded that EDTA irrigation volume greater than 1 ml did not improve debris removal. Efficient removal of the smear layer was accomplished with a final rinse of 1 ml of 17% EDTA for 1 min, followed by 3 ml of 5.25% NaOCl.[22]

- An SEM analysis by **C. S. Teixeira et al. (2005)[48]** on the effect of application time of EDTA and NaOCl on intracanal smear layer removal concluded that canal irrigation with EDTA and NaOCl for 1, 3 and 5 min was equally effective in removing the smear layer from the canal walls of straight roots.[48]

- **Berber et al. (2006)[49]** evaluated the efficacy of 0.5%, 2.5% and 5.25% sodium hypochlorite (NaOCl) as intracanal irrigants against Enterococcus faecalis within root canals and dentinal tubule associated with hand and rotary instrumentation techniques. They found that 5.25% NaOCl was shown to be the most effective irrigant solution tested, when dentinal tubules were analysed at all depths and thirds of the root canals and for all techniques used, followed by 2.5% NaOCl. No differences among concentrations in cleaning the canals were found.[49]

- **Julio Cesar Emboava Spano et al. (2009)[24]** in their evaluation of Atomic Absorption Spectrometry and Scanning Electron Microscopy evaluation of concentration of calcium ions and smear layer removal with root canal chelators concluded that the use of 15% EDTA resulted in the greatest concentration of calcium ions followed by 10% Citric acid. 15% EDTA and 10% Citric acid were the most efficient solutions for the removal of smear layer.[24]

- **Zhang et al. (2010)[50]** studied the impact on the elastic modulus and flexure strength of standardized human root dentin bars of different irrigation sequences of EDTA (17%; 3 minutes) and NaOCl (2.5% w/v; total exposure time, 24 minutes). They found that the deleterious effects attributed to the use of NaOCl on dentin are time-dependent and concentration-dependent and they were not associated with the demineralization caused by the use of EDTA as the final active irrigant.[50]

- **Chen et al. (2011)[51]** evaluated the effect of paste and liquid type EDTA during rotary root-canal instrumentation using an incremental crown-down technique on root-canal debris removal. They concluded that the use of paste/gel-type chelators during rotary nickel titanium instrumentation in the coronal and middle parts of the root canal resulted in improved cleanliness. They recommended using liquid EDTA during root canal preparation as a final flushing solution because it provided a better smear layer-free condition before 3 dimensional root canal obturation.[51]

- **Deborah Clark-Holke et al. (2012)[10]** stated in his study that bacterial penetration through canals of endodontically treated teeth in the presence or absence of the smear layer proposed that the removal of the smear layer reduced the leakage of bacteria through the root canal.[10]

- **Joseph Dutner et al. (2012)[52]** in his web-based survey on irrigation trends among American Association of Endodontist members concluded that most of the respondents were using full strength Sodium hypochlorite and were routinely removing the smear layer during endodontic treatment. In addition, almost half of the respondents were using an adjunct, such as ultrasonic activation, to aid in their irrigation technique.[52]

- **Paque et al. (2012)[53]** investigated short-term compatibility of etidronate with sodium hypochlorite (NaOCl) which could reduce debris accumulation when applied in an all in one irrigant during root canal instrumentation. They concluded that a compatible chelator – Etidronate can reduce but not completely prevent hard-tissue debris accumulation during rotary root canal instrumentation.[53]

- **P. V. Silva et al. (2012)[54]** in his evaluation on Chitosan: a new solution for removal of smear layer after root canal instrumentation concluded that 15% EDTA, 0.2% Chitosan and 10% Citric acid effectively removed smear layer from the middle and apical thirds of the root canal. Chitosan is a natural polysaccharide, obtained by the deacetylation of chitin. Chitosan has attracted attention in dental research because of its biocompatibility, biodegradability, bioadhesion and lack of toxicity.[54]

- **Luiz Fernando Machado Silveiraa et al. (2013)[55]** in their analysis by scanning electron microscopy concluded that, the cleaning efficacy of a 2.5% sodium hypochlorite (NaOCl) and a 17% ethylene diamine tetra-acetic acid (EDTA) solution with the two solutions either applied alternately or mixed together for smear layer removal after the use of each endodontic file during the shaping stage

of root canal preparation has been shown to be the most effective form of irrigation in the removal of the smear layer.[55]

- **Tartari et al. (2013)[56]** investigated the effect of sodium hypochlorite (NaOCl), ethylene diamine tetra acetic (EDTA), etidronic (HEBP), and citric acid (CA) on root dentin microhardness. They concluded that except saline, all tested irrigation regimens reduced the microhardness of human root dentin. Despite being structurally different, the root thirds behaved similarly, when subjected to the same irrigation regimen.[56]

- **Poudyal S et al. (2014)[57]** evaluated the effectiveness of solution form of 17% ethylene diamine tetra acetic acid (EDTA) on removing smear layer of root canals at different exposure time periods. When the chelating agent was applied for 7 min, irrigation with 17% EDTA and 2.5% NaOCl could remove the smear layer with no significant alteration in dentinal structure. Partial removal of smear layer was observed at 3 and 5 min of application and negligible removal of smear layer at 1 min was achieved.[57]

- **Nawfal A. A. Zakarea et al. (2014)[58]** in his study on a newly prepared solution for the removal of the smear layer concluded that MCP solution had the ability to remove the smear layer partially at three levels of a root canal without dentin erosion, while EDTA had the ability to remove the smear layer completely at the three levels of the canal with obvious dentinal erosion. Still the apical area had mechanical and anatomical limitations in root canal irrigation.[58]

- **Aggarwal Vineet S et al. (2014)[59]** Hydroxyethylidene bisphosphonate (HEDP) also known as Etidronic acid or Etidronate can be used as a possible alternative to EDTA. De-Deus et al. reported that soft chelating irrigation protocol (18% HEDP) optimized the bonding quality of root canal sealers.[59]

- **Nikhil Vineeta, Sachin Gupta et al. (2014)[60]** This study compared the amount of aqueous based and oil based calcium hydroxide remaining in the canal, after removal with 2 different chelators 17% EDTA and 0.2 % Chitosan in combination with ultrasonic agitation. Both the chelators failed to remove aqueous based as well as oil based calcium hydroxide completely from the root canal. Aqueous based was easier to remove. The authors concluded that a combination of 0.2% Chitosan and ultrasonic agitation resulted in lower amount of calcium hydroxide remnants.[60]

- **Shigenori Suzuki1 et al. (2014)[61]** Chitosan has been reported to have broad spectrum of antibacterial properties, high chelating ability for various metal ions in acidic conditions, biocompatibility and biodegradability. Therefore, this study focused on citric acid as a solution to dissolve chitosan. The purpose of this study was to determine whether chitosan-citrate solution shows antibacterial properties against Enterococcus faecalis and removes the smear layer when used as a root canal irrigant. In conclusion, chitosan-citrate solution showed antibacterial activity and enabled the removal of smear layer. As this ability depended on chitosan, it is considered that the action was enhanced by chitosan.[61]

- **Guiotti FA et al. (2014)[62]** An in-vitro study was designed to study the interactions of newly developed tricalcium silicate cement (BioRoot RCS; Septodont, Saint Maur Des Fosses, France) with apical tissue compared with a standard zinc oxide eugenol sealer (Pulp Canal Sealer [PCS]; SybronEndo, Orange, CA). BioRoot RCS had less toxic effects on PDL cell than PCS and induced a higher secretion of angiogenic and osteogenic growth factors than PCS. The authors concluded that the calcium silicate cement (BioRoot RCS) has a higher bioactivity than the zinc oxide eugenol sealer (PCS) on human PDL cells.[62]

- **Camps J et al. (2014)[63]** An in vitro study was performed to investigate the ability of BioRoot RCS, a tricalcium silicate based root canal sealer and AH Plus to effectively fill the root canals of contralateral teeth using three evaluation methods, and to investigate the correlation between the methods. BioRoot RCS exhibited

significantly more percentage of voids than AH Plus. BioRoot RCS exhibited a different pattern of sealer penetration and interaction with the dentine walls compared to AH Plus. Micro CT analysis revealed a higher void volume for BioRoot RCS.[63]

- A study on the effect of ultrasonic activation of irrigants on smear layer removal by **Schmidt TF et al. (2015)[26]** concluded that passive ultrasonic irrigation by using 1% NaOCl and ultrasonic tip placed within 1 mm of the apical foramen did not show higher efficacy in smear layer removal compared with conventional irrigation.[26]

- **Karan Yash Bhargava et al. (2015)[64]** in his comparative evaluation of the efficacy of three antioxidants vs. NaOCl and EDTA: used for root canal irrigation in smear layer removal sem study concluded that Neem, Triphala and Amla showed the potential to remove smear layer. EDTA showed the maximum efficacy in removing the smear layer.[64]

- **Aby Kuruvilla et al. (2015)[65]** in his comparative evaluation of smear layer removal by using EDTA, Etidronic acid and Maleic acid as root canal irrigants: an in vitro Scanning Electron Microscopic study concluded that final irrigation with 7% Maleic acid is more efficient than 17% EDTA and 18% Etidronic acid in the removal of smear layer from the apical third of root canal.[65]

- **Dr. Koppolu Madhusudhana et al. (2015)[66]** in his study on comparison of the effect of Chitosan and Morinda citrifolia on smear layer removal: An in-vitro study concluded that 0.2% Chitosan and 17% EDTA effectively removed the smear layer when compared to Morinda citrifolia juice.[66]

- **Leal F et al. (2015)[67]** An in-vitro study was performed to compare the solubility, radiopacity and setting times of a tricalcium silicate containing (BioRoot RCS; Septodont, St Maurdes- Foses, France) and a mineral trioxide aggregate containing

sealer (MTA Fillapex; Angelus, Londrina, Brazil) with an epoxy resin based sealer (AH Plus; DENTSPLY De- Trey, Konstanz, Germany). After immersion for 1 minute in distilled water, BioRoot RCS was significantly less soluble than AH Plus and MTA Fillapex . At all other exposure times, AH Plus was significantly less soluble than BioRoot RCS, whereas BioRoot RCS was significantly more soluble than the other 2 sealers ($P < .05$).[67]

- **Kiran S et al. (2016)[68]** in his comparative evaluation of smear layer and debris on the canal walls prepared with a combination of hand and rotary ProTaper technique using scanning electron microscope concluded that none of the instrumentation techniques in the present study could completely eliminate the smear layer and debris from the canal walls. Instrumentation of the canals with hand files after automated rotary preparation could result in cleaner canal walls.[68]

- **Arias-Moliz et al. (2016)[69]** studied the influence of dentin powder on the concentration, pH and antimicrobial activity of sodium hypochlorite (NaOCl) alone and combined with etidronic acid (HEBP). They concluded that the presence of dentin powder significantly decreased the available chlorine and antimicrobial activity of 1% NaOCl / HEBP irrigating solutions, 1% NaOCl and 2.5% NaOCl. The antimicrobial activity of 2.5% NaOCl / HEBP after a 3 minute contact time against E. faecalis biofilms was not affected by the dentin powder.[69]

- **Morago et al. (2016)**[70] evaluated the influence of the antimicrobial activity of a 2.5% sodium hypochlorite (NaOCl) / 9% etidronic acid (HEBP) irrigating solution against bacteria growing inside dentin tubules of the smear layer. They concluded that the presence of the smear layer reduced the antimicrobial activity of 2.5% NaOCl, wheras the smear layer di nott reduce the antimicrobial activity of the combination of 2.5% NaOCl / 9% HEBP.[70]

- **Shabnam Hosseini et al. (2016)[71]** in her research work on a new Nano Chitosan irrigant with superior smear layer removal and penetration concluded that Nano chitosan (Nano-CS) appeared to be relatively more effective in penetrating the root canal as an irrigant than EDTA, NaOCl and regular Chlorhexidine.[71]

- **Dr. Suchithra M S et al. (2017)[15]** evaluated the effectiveness of four different irrigation regimes on the removal of smear layer and smear plugs at the tubular apertures in the middle and apical thirds of the root canals by using Scanning Electron Microscope. They concluded that the use of EDTA effectively removed smear layer from the root canals without inducing erosion of the tubules, the most effective irrigation regime was the use of EDTA in combination with NaOCl and H_2O_2, as it completely removed the smear layer from both the middle and the apical thirds.[15]

- **Ramya Raghu, Geethu Pradeep et al. (2017)[72]** This in vitro study compared the amount of aqueous based and oil based calcium hydroxide remaining in the canal, after removal with two different chelators 17% EDTA, 20% Citric acid and 0.2% Chitosan in combination with ultrasonic agitation. $Ca(OH)_2$. was removed using either 17% EDTA, 20% Citric acid or 0.2% Chitosan in combination with ultrasonic agitation. It was concluded that combination of 0.2% Chitosan and ultrasonic agitation resulted in lower amount of $Ca(OH)_2$ remnants than 17% EDTA, 20% Citric acid irrespective of the type of vehicle present in the mix.[72]

- **Uzunoglu-Özyürek E et al. (2018)[73]** The study evaluated the effect of calcium hydroxide dressing on the dentinal tubule penetration of epoxy resin–based sealer, AH 26 and tricalcium silicate–based sealer, Bioroot RCS. Bioroot RCS presented a higher dentinal tubule penetration than AH 26 even in the presence of $Ca(OH)_2$ residues. $Ca(OH)_2$ remnants decreased both dentinal tubule penetration depth and

the percentage of the tested sealers; however, a more drastic effect was observed for AH 26.[73]

RELEVANCE

RELEVANCE

The success of endodontic therapy mainly depends upon the proper cleaning of the root canal space. Instrumentation cannot solely remove all the debris and contaminants. This highlights the importance of chemical cleaning and disinfection of the root canal system. Moreover, the smear layer, dentin mud and debris produced during mechanical preparation can hinder the sealer penetration into the dentinal tubules. Different chelating agents are used in conjunction with NaOCl to remove smear layer and improve penetration of sealant. Conventional chelating agents like EDTA have various drawbacks including reduced efficacy in the removal of smear layer in the apical third. Newer agents like Chitosan & Etidronate are recently proposed as alternatives to EDTA.

Bioceramic selaers like BioRoot RCS provide 3 dimensional seal of the root canal thus increasing the potential and obtaining more reliable results. BioRoot RCS (Septodont, Saint Maur des Fosses, France) is a tricalcium silicate sealer used as a new range of dental material that exhibits superior mechanical properties besides biocompatibility and bioactivity. They have excellent biological properties and sealing abilities. In order to achieve these properties to the full extent, the dentin wall should be devoid of smear layer.

The pushout bond strength test is a practical and reliable method that evaluates the adaptation of a restorative material to the root canal dentin. This study measures the difference in pushout bond strength of BioRoot RCS to the root dentin after using relatively new chelating agents such as Etidronate and Chitosan.

MATERIALS AND METHODS

MATERIALS AND METHODS

Research Approach

Qualitative and Quantitative analysis

Study design

In vitro study

Study Setting

Study was conducted at

- St. Gregorios Dental College, Chelad, Kothamangalam.
- Department of Polymer Science & Rubber Technology, CUSAT, Kalamassery.
- School of Marine Sciences, Pallimukku, Kochi.

SAMPLE AND SAMPLE SIZE

- The sample size was calculated using statistical package G*Power (3.1.5).
- The sample size required for this study was n=40, 10 samples per group.

The materials and methodology used for this study are described under the following headings:

1. Selection of specimens

2. Armamentarium

3. Root canal preparation

4. Irrigation Protocol

   - Irrigation Regimen
   - Sectioning

5. Pushout bond strength measurement

6. Stereomicroscopic analysis

7. Statistical analysis

1) SELECTION OF SPECIMENS

Human second premolars extracted for orthodontic purposes were collected from the Department of Oral and Maxillofacial Surgery, St. Gregorios Dental College, Kerala.

- **SAMPLE PREPARATION**

- Forty mandibular second premolars were selected.
- The teeth were radiographed at two angulations.
- Soft tissue fragments and calcified debris on the specimens was removed using ultrasonic scalers.
- The specimens were stored in a solution of 0.2% sodium azide at $4^{o}C$ until use.
- The tooth was decoronated diamond disc before cleaning an shaping.

INCLUSION CRITERIA

Single rooted premolars

Non carious teeth

Teeth with complete root formation

EXCLUSION CRITERIA

Immature teeth with open apex or other structural anomalies.

Canals with moderate or accentuate curvature.

Calcifications in the pulp chamber.

Internal resorption.

Previous endodontic treatment and metallic dental restorations in the crown or root.

Root perforations or resorption.

STUDY GROUPS:

- Group 1 (G1): 17% EDTA group with BioRoot RCS (n=10)
- Group 2 (G2): 0.2% Chitosan group with BioRoot RCS (n=10)
- Group 3 (G3): 18% HEDP group with BioRoot RCS (n=10)
- Group 4 (G4): 5.25% NaOCl with BioRoot RCS (Control group) (n=10)

2) ARMAMENTARIUM

Materials

40 single rooted human mandibular second premolars.

0.30-mm-thick diamond disc (KG Sorensen, São Paulo, SP, Brazil)

Airotor hand piece X-Smart endomotor (Dentsply Maillefer)

Size 10-k file, 15-k file (Dentsply, Mani)

Protaper gold (Dentsply Tulsa Dental, Tulsa, OK, USA)

5.25% NaOCl (VIP, Vensons, India)

17% EDTA (Avue prep, India)

0.2% Chitosan (Everest biotech, India)

18% HEDP (TCI Chemicals, Japan)

Normal Saline

Side vented irrigation needle,

Size F2 GP cones,

BioRoot RCS (Septodont).

Equipments

Universal Testing Machine
Stereomicroscope (Labomed CZM 4, Novel Technologies)

- 40 samples were equally divided into four groups.

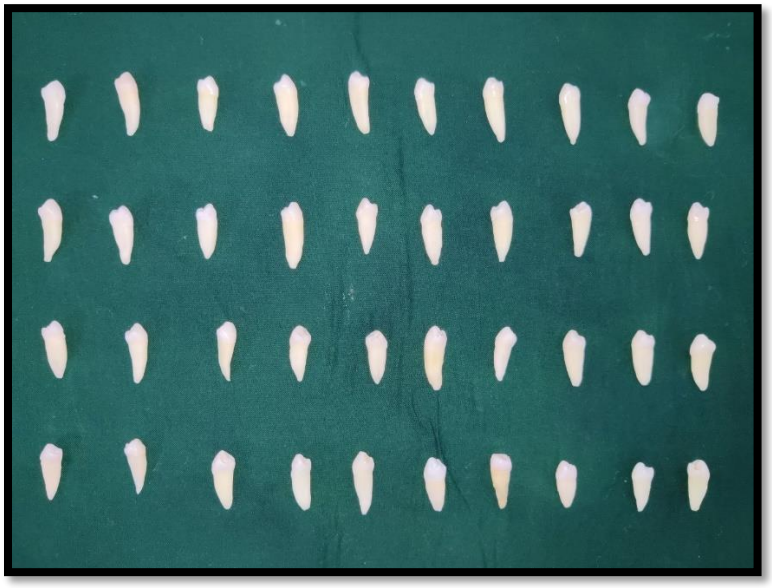


FIGURE 1: EXTRACTED TEETH SAMPLE: 40 Nos.

FIGURE 2: ARMAMENTARIUM FOR DECORONATION OF TOOTH SAMPLES

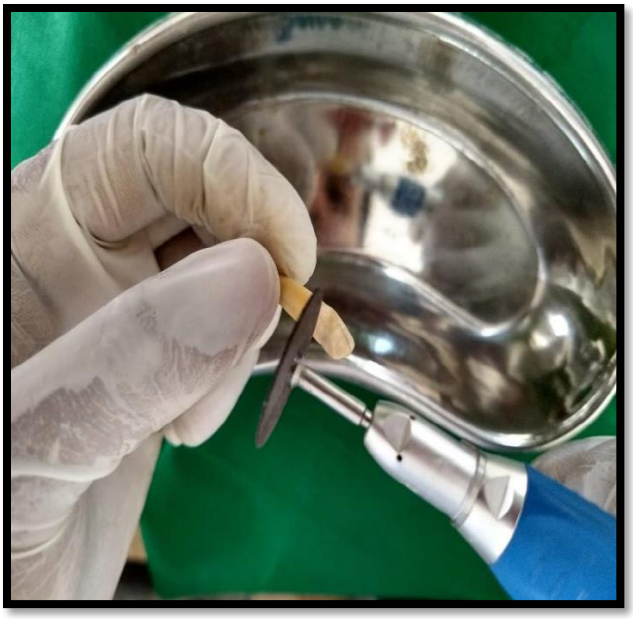


FIGURE 3: TEETH BEING DECORONATED

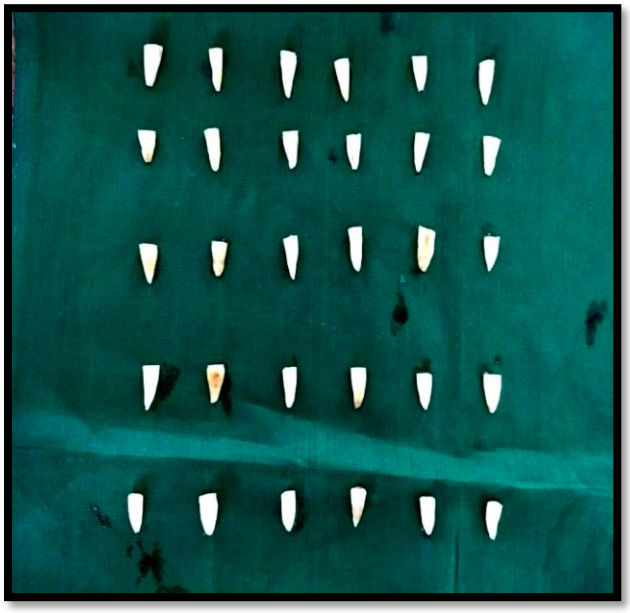


FIGURE 4: DECORONATED SAMPLES

FIGURE 5: 17% ETHYLENE DI-AMINE TETRA ACETIC ACID

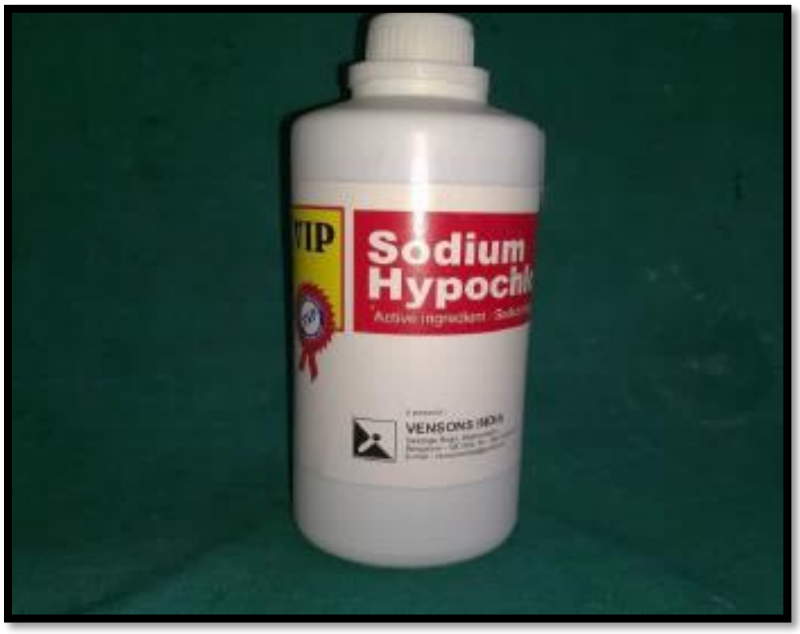


FIGURE 6: 5.25% SODIUM HYPOCHLORITE

FIGURE 7: 0.2% CHITOSAN

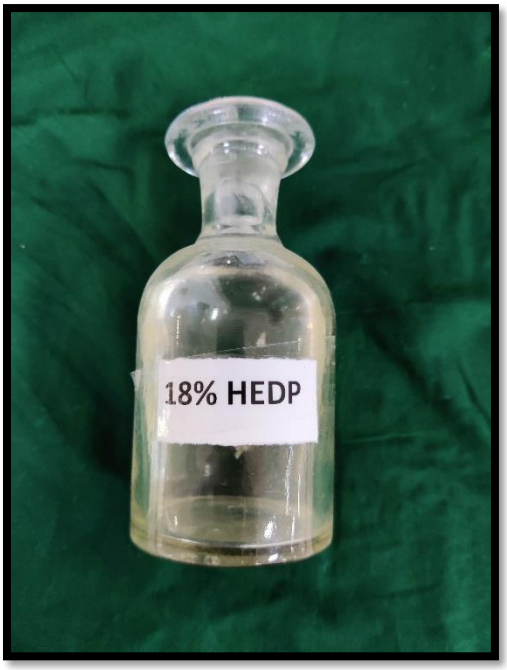


FIGURE 8: 18% HEDP

FIGURE 9: K FLEX FILE 15-40 SIZE 2% TAPER

FIGURE 10: BIOROOT RCS

3) ROOT CANAL PREPARATION

After decoronating the samples, the working length was established with the introduction of a #10 K flex file (MANI Inc.Japan) in the root canal, which was visualized in the foramen; this measurement was reduced by 1 mm to obtain the working length of each sample. The apices of all the teeth will be sealed with sticky wax to prevent the flow of irrigants through the foramen and to allow an effective reverse flow of the irrigant to simulate a closed end system.

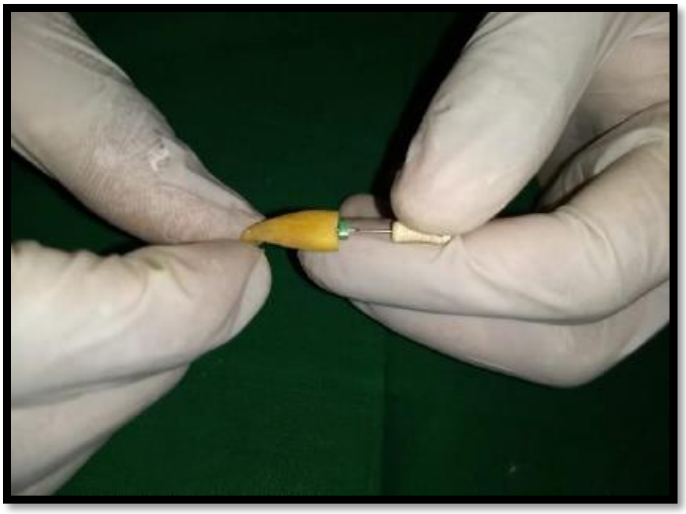


FIGURE 11: WORKING LENGTH DETERMINATION

4) IRRIGATION PROTOCOL

• Group 1: 17% EDTA used during BMP

• Group 2: 18% HEDP used during BMP

• Group 3: 0.2% Chitosan used during BMP

• Group 4: Control group in which 5.25% NaOCl was used as the irrigant.

• All samples were irrigated with 5.25% NaOCl and distilled water after each instrument change.

- **IRRIGATION REGIMEN**

The auxiliary chemicals used in this study were 5.25% Sodium Hypochlorite (VIP, Vensons India), 17% Ethylenediaminetetra-acetic acid (Avue prep, India) , 0.2% Chitosan (Everest biotech, India) and 18% Etidronic acid (TCI Chemicals, Japan). The irrigation was performed with a plastic syringe and needles of #30 gauge Pro-rinse (Dentsply Sirona, USA), inserted 1 mm short of the working length. The solutions were combined for the following proposed irrigation schemes.

G 1: 5 ml 5.25% NaOCl for 1 minute after each instrument change / 5 ml 17% EDTA for 1 minute/final rinse of 5 ml distilled water for 1 minute.

G 2: 5 ml 5.25% NaOCl for 1 minute after each instrument change / 5 ml 18% HEDP for 1 minute/final rinse of 5 ml distilled water for 1 minute.

G3: 5 ml 5.25% NaOCl for 1 minute after each instrument change / 5 ml 0.2% Chitosan for 1 minute/final rinse of 5 ml distilled water for 1 minute.

G4: 5 ml 5.25% NaOCl for 1 minute after each instrument change / 5 ml 5.25% NaOCl for 1 minute/final rinse of 5 ml distilled water for 1 minute.

FIGURE 12: IRRIGATION PERFORMED WITH PRO-RINSE IRRIGATION TIP

The root canals were cleaned and shaped using the ProTaper system (Dentsply Sirona Endodontics, Tulsa, OK) to size F3. After the final irrigation, the canals were dried with paper points (Dentsply Sirona Endodontics).

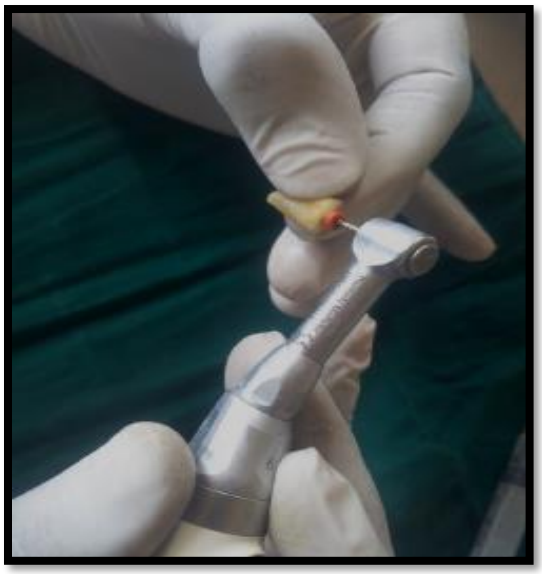


FIGURE 13: BIOMECHANICAL PREPARATION

In groups G1, G2, G3, G4, the root canals were obturated with gutta-percha and BioRoot RCS. BioRoot RCS was then mixed according to the manufacturer's instructions and placed in the canal using an amalgam carrier and condensed with hand pluggers (Hu-Friedy, Chicago, IL). Obturated roots were then to ensure that the canals were densely obturated without any voids. All the teeth were stored at room temperature for 1 week to allow complete setting of BRCS. The tooth is to be sectioned horizontally in the middle third to obtain a slice of approximately 2 mm thickness.

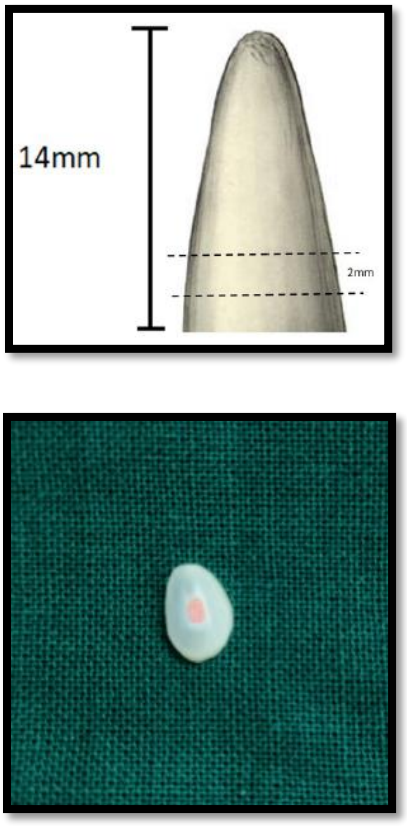


FIGURE 14: TRANSVERSE SECTIONING OF SPECIMEN

5) PUSHOUT BOND STRENGTH MEASUREMENT

Each root was embedded in cold cure acrylic (Dentsply India, Gurgaon, India). The middle third was sectioned horizontally using a hard tissue microtome (Leica Biosystems, Nussloch, Germany) with continuous water cooling to obtain a slice of 2 mm thickness. The root canal diameter as well as the height of each slice was recorded using a digital caliper. The adhesion surface area was calculated by the following equation:

Adhesion surface area (mm^2) = D1 + D2/ 2 x π x H

where "D1" and " D2" are the largest and smallest canal diameter, respectively

"π" is the constant 3.14, "H" is the thickness of the root slice.

The push out test was performed using a universal testing machine (Instron, Norwood, MA). The force applied was in the apico-coronal direction at a crosshead speed of 1 mm/min using a stainless steel plunger of 0.6 mm, positioned such that it contacted only the filling material. The maximum force (F) applied for bond failure was recorded in Newton (N).

The POBS was calculated in Megapascal (MPa) using the following formula: POBS (MPa) = Force (N) /Adhesion surface area (mm^2)

The data of the POBS of BioRoot RCS to root canal walls is presented as means +/- standard deviation (SD).

FIGURE 15: STAINLESS STEEL PLUNGER 0.6mm

FIGURE 16: SECTIONED SPECIMENS

FIGURE 17: DIGITAL CALIPER

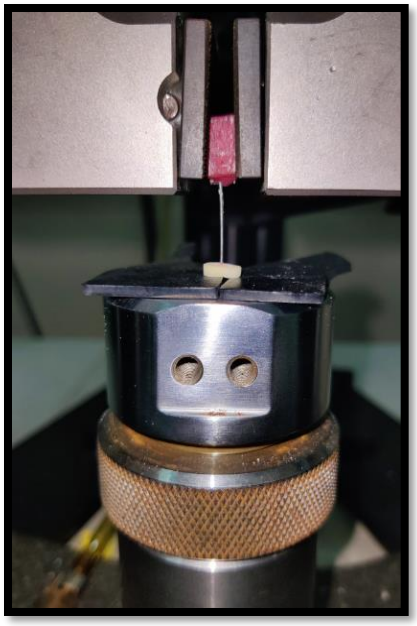


FIGURE 18: UNIVERSAL TESTING MACHINE

6) STEREOMICROSCOPIC ANALYSIS

Following the pushout test, the samples were viewed at 40x magnification in a Stereomicroscope, so that the failure types could be determined. The failure type was classified into three categories:

i. Adhesive failure between cement and dentin

ii. Cohesive failure within cement

iii. Mixed failure which include cement and dentin together

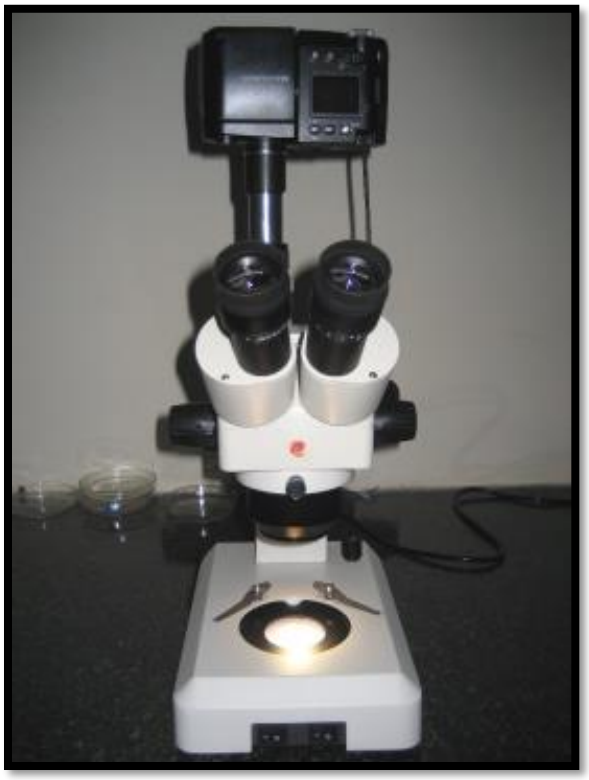


FIGURE 19: STEREOMICROSCOPE FOR VIEWING THE CROSS SECTION

7) STATISTICAL ANALYSIS

Data was analyzed using the statistical package **SPSS 22.0** (SPSS Inc., Chicago, IL) and the level of significance was set at **p<0.05**. **Descriptive statistics** was performed to assess the mean and standard deviation of the respective groups. Normality of the data was assessed using **Shapiro Wilkinson test**. **Inferential statistics** to find out the difference between the groups was done using **ONE WAY ANOVA and TUKEY'S POST HOC TEST**. **CHI SQUARE test** was used to find out the association between the groups.

TABLE 1- DESCRIPTIVE DATA (MICROTENSILE BOND STRENGTH)

GROUPS	N	MEAN	SD
17%EDTA+ BioRoot RCS (G1)	10	29.37	2.25
18%HEDP+ BioRoot RCS (G2)	10	33.29	2.89
0.2% CHITOSAN+ BioRoot RCS (G3)	10	45.99	2.77
5.25%NaOCl+ BioRoot RCS (G4)	10	19.31	1.76

TABLE 2-THE RESULTS OF THE ONE-WAY ANOVA ARE GIVEN BELOW.

	Sum of squares	df	Mean Squares	F	sig
Between Groups	3653.3680	3	1217.7893	201.4115	**0.0001***
Within Groups	217.6659	36	6.0463		
Total	3871.0339	39			

***P<0.05 is statistically significant (ANOVA TEST)**

TABLE 3-TUKEY'S HSD POST HOC TEST (PAIRWISE COMPARISON)

GROUP(I)	GROUP(J)	MEAN DIFFERENCE(I-J)	95% OF CONFIDENCE INTERVAL		P VALUE
			LOWER	UPPER	
Group 1	Group 2	3.9200	0.9584	6.8816	**0.0001***
	Group 3	16.620	13.6584	19.5816	**0.0001***
	Group 4	-10.060	-13.0216	-7.0984	**0.0001***
Group 2	Group 3	12.70	9.7384	15.6616	**0.0001***
	Group 4	-13.98	-16.9416	-11.0184	**0.0001***
Group 3	Group 4	-26.68	-29.6416	-23.7184	**0.0001***

***P<0.05 is statistically significant (TUKEY'S POST HOC TEST)**

TABLE 4- PERCENTAGE OF MODE OF FAILURE

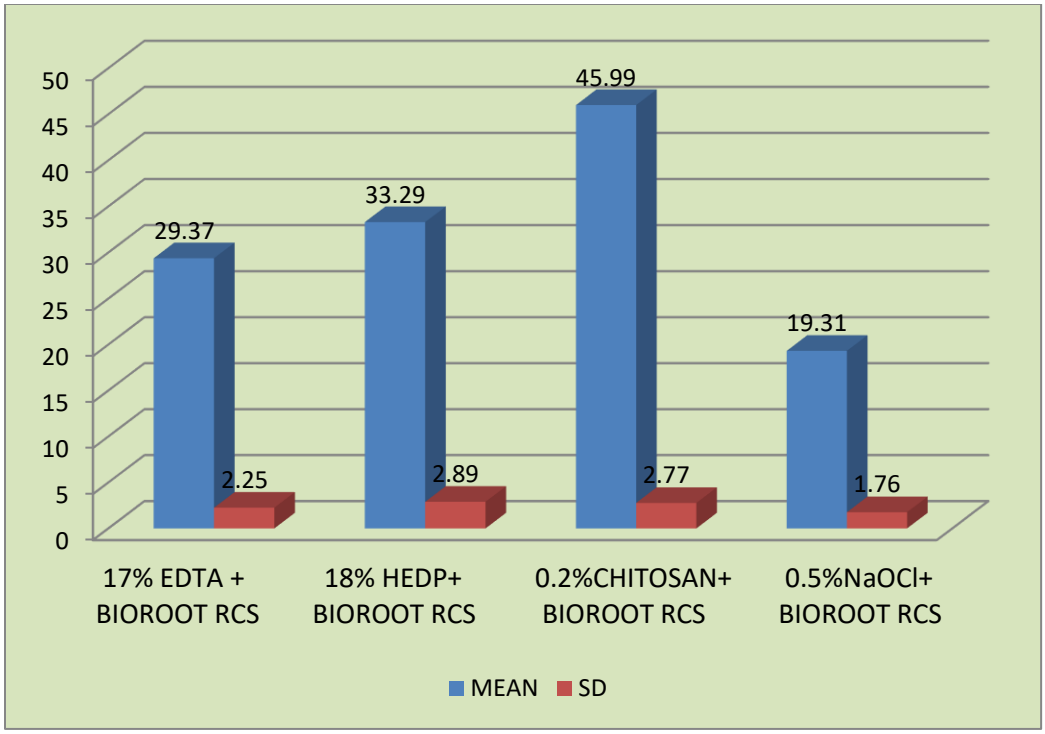
GROUPS	ADHESIVE	COHESIVE	COMBINED	X2 value	P value
17%EDTA+ BioRoot RCS	3(30%)	3(30%)	4(40%)	103.33	0.0001*
18%HEDP+ BioRoot RCS	2(20%)	5(50%)	3(30%)		
0.2%CHITOSAN+ BioRoot RCS	1(10%)	7(70%)	2(20%)		
5.25% NaOCl+ BioR9oot RCS	6(60%)	1(10%)	3(30%)		

***P<0.05 is statistically significant (CHI SQUARE TEST)**

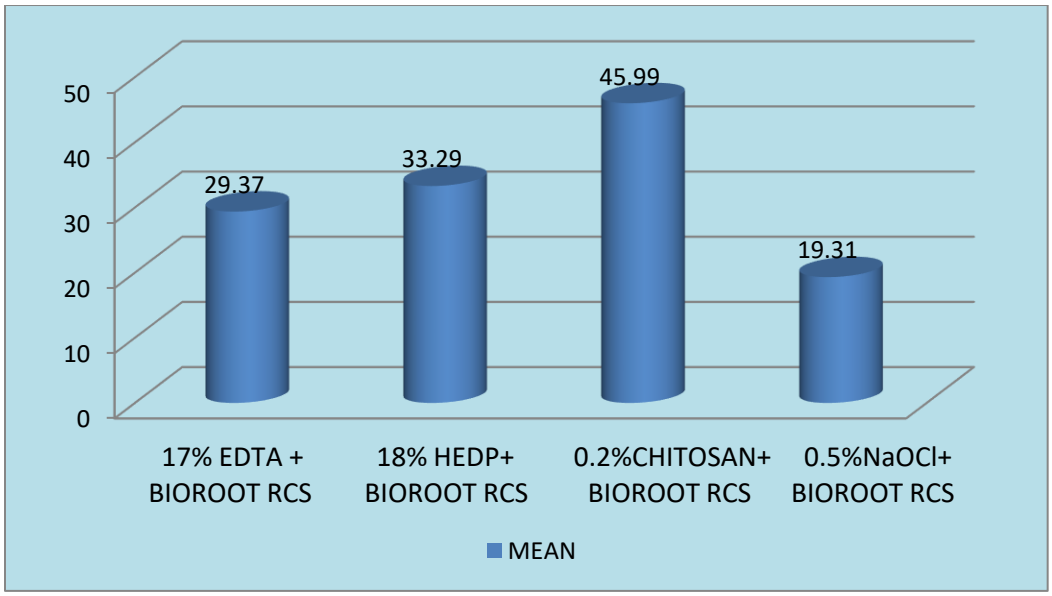
TABLE 5- HOMOGENEOUS SUBSETS

BONDSTRENGTH					
Tukey HSD[a]					
		Subset for alpha = 0.05			
Treatments	N	1	2	3	4
G4	10	19.3160			
G1	10		29.3740		
G2	10			33.2930	
G3	10				45.9930
Sig.		1.000	1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed.					
a. Uses Harmonic Mean Sample Size = 10.000.					

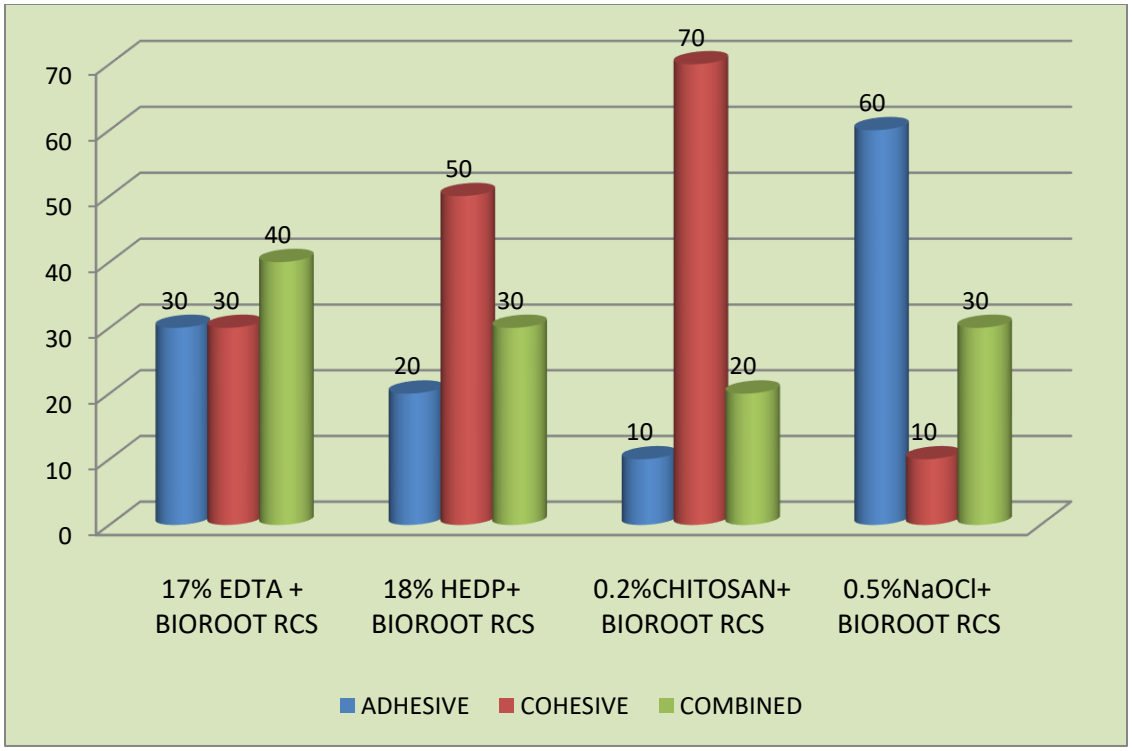
GRAPH 1- MEAN AND STANDARD DEVIATION



GRAPH 2- MEAN PUSHOUT BOND STRNGTH



GRAPH 3- FRACTOGRAPHIC ANALYSIS:



RESULTS

RESULTS

In the current study, the mean scores of pushout bond strength (POBS) of different groups is shown in Table.1. Here, G3 (0.2% Chitosan + BioRoot RCS) showed superior POBS (45.99 MPa) when compared with other groups. Also, it showed a significant difference when compared with G1 (17% EDTA+ BioRoot RCS) and G2 (18% HEDP+ BioRoot RCS). The POBS for G4 (5.25% NaOCl + BioRoot RCS was significantly the lowest among all other groups.

However, POBS was significantly higher in relatively new chelating agents used in this study as seen in G3 (0.2% chitosan + BioRoot RCS) (45.99 MPa) and G2 (18% HEDP+ Bioroot RCS) (33.29 MPa), when compared with conventional irrigants as seen in G1 (29.37 MPa) and G4 (19. 31 MPa).

Mean POBS values were ranked as follows; G3>G2> >G4.

In the present study, Table 2 shows comparisons among the groups using One-way ANOVA (Analysis of Variance) Welch test. Analysis of variance showed significant statistical differences among the tested groups ($p<0.05$). From ANOVA we can see that the significance value is 0.000($p=0.0001$) which is below 0.05 (i.e $\alpha=0.05$.) therefore there is a statistically significant difference between groups determined by $F(3,36) = 201.4115$ and $p=.0001$.

Multiple intergroup comparisons using Post Hoc Tukey Test is shown in Table 3. POBS was significantly higher in G3 (0.2% Chitosan + BioRoot RCS) (45.99 MPa) than G2 (18% HEDP+ BioRoot RCS) (33.29 MPa) and G1 (17% EDTA + BioRoot RCS) (29.37 MPa).

The multiple comparison table 3 which contains the result of Tukey post hoc test,
Compares the p value given in the table with ($\alpha = 0.05$)
if $p < \alpha$,there is statistical difference between the groups.

- There is statistically significant difference between Group 1 and Group 2 since p= .0001 which is less than 0.05 ie α.
- There is statistically significant difference between Group 1 and Group 3 since p = 0.0001 < α.
- There is statistically significant difference between Group 1 and Group 4 since p= 0.0001 which is lessthan 0.05 ie α.
- There is statistically significant difference between Group 2 and Group 3 since p= 0.0001 which is lessthan 0.05 ie α.
- There is statistically significant difference between Group 2 and Group 4 since p= 0.0001 which is lessthan 0.05 ie α.
- There is statistically significant difference between Group 3 and Group 4 since p= 0.0001 which is lessthan 0.05 ie α.
- Group 4 (Control group) is statistically different from other groups.

In this present study, Table 4 shows the percentage of mode of failure. Results of mode of failure indicates that most of the samples showed cohesive and mixed failures. The failure patterns for G3 (0.2% Chitosan + BioRoot RCS) (70%), followed by and G2 (18% HEDP + BioRoot RCS) (50%) were predominantly cohesive, for G1 (17% EDTA + BioRoot RCS) (40%) were mixed pattern and for G4 (5.25% NaOCl + BioRoot RCS) (60%) were predominantly adhesive failure.

STEREOMICROSCOPE OBSERVATIONS:

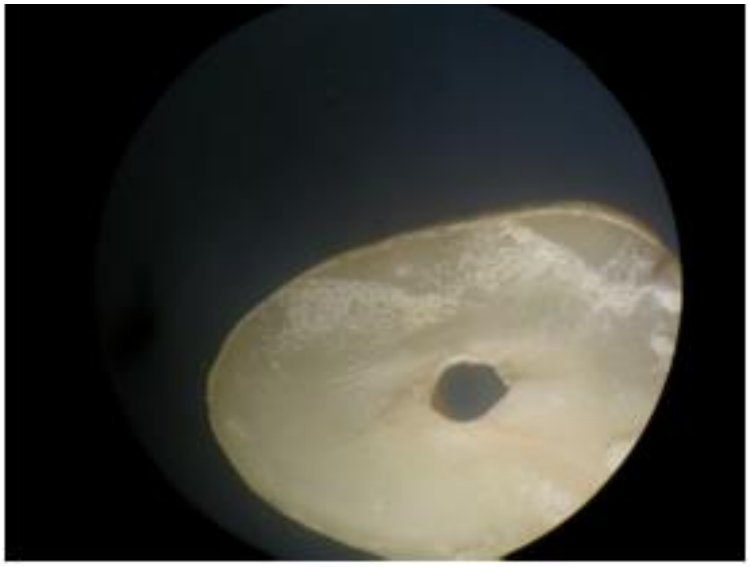


FIG 20: ADHESIVE FAILURE AT 25X

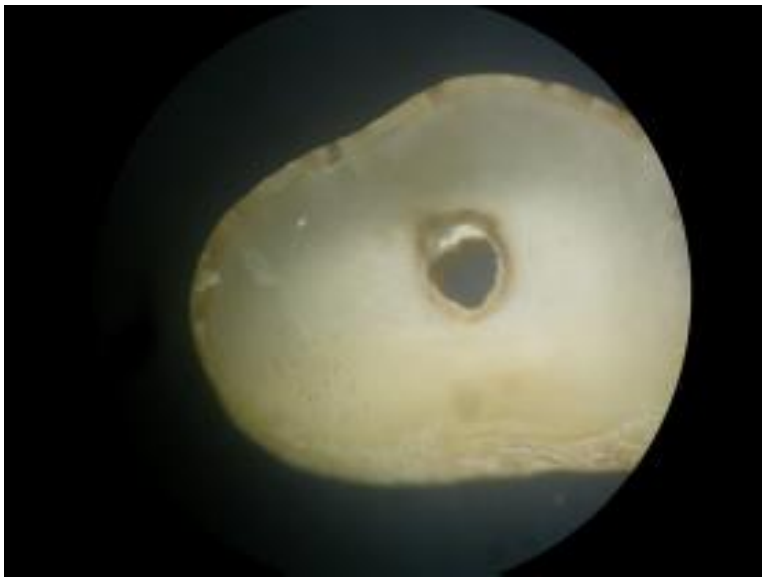


FIG 21: MIXED FAILURE AT 25X

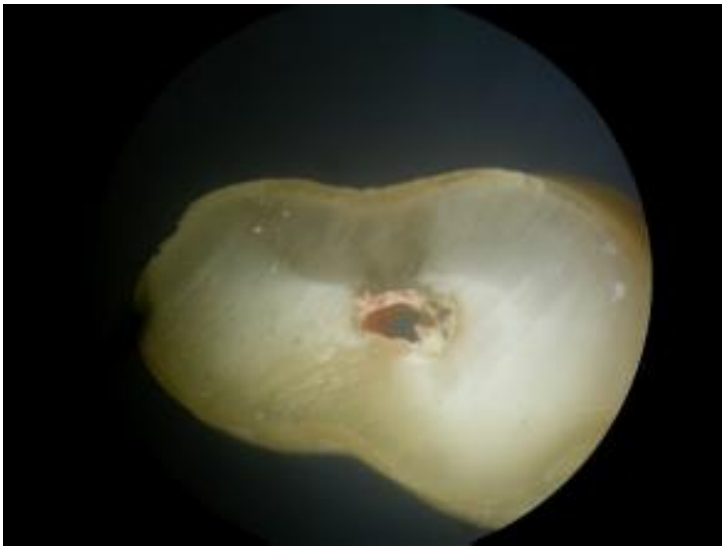


FIG 22: COHESIVE FAILURE AT 25X

DISCUSSION

DISCUSSION

One of the greatest challenges in endodontic therapy is rendering a complex root canal system and its ramifications completely clean of organic and inorganic debris, thereby creating a healthy environment for the tooth to achieve maximal healing. The elimination of microorganisms from the root canal is an important step in the success of endodontic therapy.[74] The colonization of dentinal walls with biofilm, along with the anatomical complexity of the root canal and the possibility of invasion of dentinal tubules, can compromise the success of endodontic therapy.[42]

Chemo mechanical preparation plays an important role in the success of endodontic treatment.[75] However instrumentation of root canal results in accumulation of organic and inorganic material known as smear layer.[76] Pashley found that the smear layer contains organic and inorganic substances that include fragments of odontoblastic process, microorganism and necrotic materials.[77] McComb & Smith (1975) were the first researchers to describe smear layer on the instrumented root canal surface.

The root canal wall, when submitted to the action of each instrument (manual or rotary), becomes coated with a layer predominantly composed of grinding debris and reported as the smear layer. Because it is of dentinal origin, it is composed of organic and inorganic matter.[78] The morphology of the smear layer is composed of two layers. The superficial layer is firmly adhered to the dentine surface and the deep layer is formed by smaller particles that are compacted into the dentinal tubules, making the deep layer difficult to remove.[79] This compaction causes the reduction of dentine permeability by 25–49%, which would protect the bacteria previously installed inside the dentinal tubules.[80]

There was a high controversy regarding the removal of smear layer. Many studies favoured the retention of smear layer which may block the dentinal tubules and limit bacterial or toxin penetration by altering the dentinal permeability.[81] But many studies reported that removal of smear layer prevents apical/coronal micro leakage by a better adherence and

penetration of sealer into the dentinal tubules and provides better disinfection by allowing intracanal medicaments to penetrate into the dentinal tubules.[82] It improves the bonding of resins to the tooth structure.

Regarding the chemical composition of smear layer, it can be effectively and totally removed by only agents combining both organic and inorganic solvents.[83] There are various methods to remove smear layer like chemical, ultrasonic and laser techniques. None of the methods remove smear layer throughout the length of the canal completely.[84] Kalyoncuoğlu E and Demiryürek EÖ evaluated the efficacy of smear layer removal from teeth following root canals using lasers (Er:YAG and Nd:YAG), NaOCl, 17% EDTA, and MTAD by scanning electron microscopy (SEM). They concluded that although improvement was observed in removal of the smear layer using alternative materials and techniques, application of a combination of EDTA and NaOCl remains an effective technique.[85] Thus in our study we used NaOCl and EDTA as irrigants.

The present in vitro study evaluated the pushout bond strength of BioRoot RCS to root canal dentin when irrigated using different irrigation protocols. Penetration of the root canal sealer into the dentinal tubule can provide a mechanical interlocking between the sealer and root dentin.[86] The results showed that the use of various irrigation protocols had a differential effect on the pushout bond strength of BioRoot RCS. This is in congruence with earlier studies.[87,88] Hence, the null hypothesis has been rejected.

Since 1920, NaOCl is one of the most commonly used endodontic irrigants. It is known for its antibacterial activity and for its capacity of dissolving organic tissue in root canal.[89] It results in the formation of hypochlorous acid (HOCl) which shows antibacterial properties, when it reacts with organic debris. HOCl disrupts the microbial metabolism by oxidation of sulphydryl groups within bacterial enzyme systems.[90] Strong basic pH and high percentage of free chlorine in solution are its two peculiar actions related to the antibacterial and solvent actions of NaOCl.[91] It has limited activity on the inorganic components of the smear layer and this required the use of chelating agents.[92]

Nygaard Ostby was the first to introduce chelating agents in endodontics. Chelating agents decalcify the dentine by combining with calcium ions of the tooth.[93] Chelating agents and acids have been reported to remove the smear layer from the root canal, because the components of this loosely bound structure are very small particles with a large surface-mass ratio that makes them very soluble in acids.[94] Chelating solutions have been used as a part of the final irrigation regimen in various studies.

EDTA is a commonly used irrigant because it can chelate and remove the mineralized portion of smear layers. EDTA is a colourless, water soluble semi solid and it is the widely used acronym for the chemical compound ethylene diamine tetra acetic acid. EDTA is a polyamino carboxylic acid with the formula [CH2N (CH2CO2H) 2]2. Chelating action occurs by its ability to extract di- and tri-cationic metal ions such as Ca^{2+} and Fe^{3+}.[95] It is a synthetic, non-biodegradable material which is considered as a pollutant in root canal system and reported to be cytotoxic to macrophages. It lacks antimicrobial properties.[96]

Even though the combination of EDTA and NaOCl appears to be the most effective agent for smear layer removal, the combination however cannot be simultaneously used because EDTA solution is able to chemically interact with NaOCl and reduce the amount of free chlorine.[97] This combination allows a synergistic interaction allowing easy penetration of EDTA into the intertubular and peritubular dentine expediting its disintegration and is responsible for a pronounced canal wall erosion.[63]

Different chelating agents are used in conjunction with NaOCl to remove smear layer and improve penetration of sealant. Conventional chelating agents like EDTA have various drawbacks including reduced efficacy in the removal of smear layer in the apical third[97], a reduction in dentin microhardness[98] and cytotoxicity.[99] Furthermore, it also reduces the bond strength of resin cements[100], brings about a reduction in active chlorine when combined with NaOCl[101] and forms a precipitate in combination with chlorhexidine.[102]

Hence, newer agents like Chitosan & Etidronate have been used in this study and are recently proposed as alternatives to EDTA.

Chitosan has been employed in concentrations of 0.1%, 0.2% and 0.3% for the removal of smear layer in endodontics. At a concentration of 0.1% it is not effective in completely removing the smear layer. On the other hand, 0.3% concentration of Chitosan causes greater erosion of dentinal tubules. A study by Silva et al. showed that 0.2% concentration of Chitosan has the same chelating effect as 15% EDTA. A study by Vilaca P et al.[103,104,105] on the time dependent effects of Chitosan on dentin concluded that 0.2% Chitosan removed smear layer adequately while causing less erosion of the dentinal tubules. It is also more economical. Hence this concentration was employed in the present study.

In the present study, 0.2% Chitosan in combination with 5.25% NaOCl was more effective than 17% EDTA + 5.25% NaOCl and 18% HEDP + 5.25% NaOCl) in removing smear layer and higher pushout bond strength (45.99 MPa) of BioRoot RCS. This result is consistent with studies done by other researchers.

The chelating mechanism of Chitosan is due to its own properties rather than because of 1% acetic acid in which it is prepared.[104] The presence of acetic acid in Chitosan mmainlyis to prevent the reprecipitation of bound calcium by maintaining an acidic pH of the solution in the canal. Adsorption, ionic exchange and chelation are probably the mechanisms responsible for the formation of complexes between Chitosan and metal ions.

Two models have been used to explain the chelating mechanisms of Chitosan:

1. Bridge model: This is based on the theory that two or more amino groups of a Chitosan chain link to the same metal ion.
2. Pendant model: This suggests that only one amino group of the structure of the substance is involved in the bridging.[106,107]

Recently, Etidronic acid also known as Etidronate (HEBP), a substance that prevents bone resorption has been used in medicine for patients suffering from osteoporosis or Paget´s disease. This was suggested as a substitute for traditional chelators due to fewer effects observed on dentin structure.[108] The advantage of etidronate is that it can be mixed with NaOCl without interfering in its antimicrobial activities.[109] HEDP is a weak chelator, therefore it can be less aggressive on dentin than EDTA.[110] However these solutions may need longer time for removal of smear layer. It is biocompatible with periapical tissues.

In this study, the pushout bond strength of BioRoot RCS was significantly higher when the root canal was irrigated with a combination of 5.25% NaOCl and 18% HEDP than with a combination of 5.25% NaOCl and 17% EDTA (33.29 MPa). This is in conjunction with the findings of Neelakantan et al.[111]

The improved performance of HEDP could be attributed to the fact that it has no adverse effect on the hydration properties of calcium silicate cements.[111] Also, smear layer removal by NaOCl and HEDP also known as continuous chelation, has been shown to be comparable with that of EDTA. Hence, the current study showed that the removal of the smear layer has a direct correlation with the adhesion of BioRoot RCS to the root canal dentin. Furthermore, an irrigating protocol using the NaOCl + HEDP combination has been shown to be able to optimize the bonding quality of Resilon/Epiphany (Sybron Endo, Glendora, CA) root fillings.[35]

The lower pushout strength obtained in the specimens treated with only 5.25% NaOCl (19.31MPa) could be attributed to the inferior smear layer removal property of NaOCl, thereby reducing the bonding of BioRoot RCS to the root canal walls. Also, studies have shown that NaOCl interacts with calcium silicate cement, which, in turn, can affect its adhesion.[28]

EDTA has been shown to impart a negative influence on the hydration properties of calcium silicate cements because of its acidic nature.[112] This causes dissolution of the binding phase of the cement, which, in turn, inhibits its adhesion to materials. This can occur if the final rinse after the use of EDTA is insufficient resulting in some residual EDTA on the root canal dentin, which may chelate the calcium ions released from the tricalcium silicate cement during hydration, thereby disturbing the formation of hydrated products.[113] EDTA also decreases the hardness and flexural strength of tricalcium silicate cements.[114] Furthermore, the sealing ability of tricalcium silicate cement was reported to reduce when the final irrigation was performed using EDTA, which could have a direct correlation to its adhesion to the root canal dentin. These could be the reasons why the groups treated with NaOCl + EDTA had poor bond strength when compared with NaOCl + 18% HEDP in the present study.

Tricalcium silicate cements can be placed in the root canals using hand files, pluggers, Lentulo spirals, and ultrasonic activation. In the current study, BioRoot RCS was condensed into the root canal using hand pluggers because studies have shown that manual compaction of calcium silicate cement resulted in more densely obturated root fillings [115] with superior marginal adaptation compared with ultrasonic activation.

Bioceramic root canal sealers have extremely small particle size (less than two microns) which may improve the flow of the sealer into the dentinal tubules and anatomic irregularities. Moreover, they exhibit minimal or no shrinkage during the setting phase because of the calcium silicate ingredient, which utilizes the moisture in the dentinal tubules to initiate and complete the setting reaction. In addition, they exhibit 0.2% expansion during the setting period. These characteristics support the spread of the sealer over the dentinal walls of the root canal and in filling the lateral canals.[116]

BioRoot RCS is a new calcium silicate based sealer with good biocompatibility.[117] Manufacturers claims that it can provide 3 Dimensional and durable sealing of the entire root canal system. BioRoot RCS (Septodont, France) is a powder / liquid hydraulic

tricalcium silicate based cement recommended for single cone technique or cold lateral condensation root filling. The powder contains tricalcium silicate, povidone and zirconium oxide; the liquid is an aqueous solution of calcium chloride and polycarboxylate. The release of calcium hydroxide after hydration and the contact with phosphate from tissue fluids leads to precipitation of calcium phosphate or calcium carbonate on the surface. The formation of hydroxyapatite on BioRoot RCS after contact with phosphate buffered saline solution was reported. Calcium silicates form an interfacial layer at the dentin called the "mineral infiltration zone" with increased mineralization.[118]

A study done by Altemah et al. concluded that EDTA influenced the push out bond strength of calcium silicate based cements negatively. The reduction of calcium at the sealer dentin interface or a degradation of the calcium silicate fraction in the sealer might hinder the formation of the "mineral infiltration zone" which may result in weaker interaction between root canal sealer and dentin wall.[118]

However, limited data is available on the bonding performance of the newer endodontic sealers. Hence, the present study was designed to assess the bond strength of the newer endodontic sealer to root dentin using push out bond strength testing. A laterally compacted gutta percha filling technique was selected for the current study. Lateral condensation and warm vertical compaction may exert a certain impact on the POBS and are less reproducible than the single-cone technique.[119]

Warm gutta percha filling techniques have been developed to improve the adaptation of the filling material to the root canal geometry, as thermoplasticized gutta-percha can fill canal irregularities better than solid gutta percha points.[120] This technique was not employed as the manufacturer of BioRoot RCS recommends the use of cold lateral condensation. Furthermore, sealer properties are affected by the application of heat during warm vertical compaction for BioRoot RCS [121], which exhibited reduced flow and setting time.

Different tests have been reported for bond strength evaluation. Shear strength, microtensile and even pull out or pushout tests have been described as reproducible and effective for direct comparison of the results. Pushout test allows an accurate standardization of the specimens. However, it is clear that test models cannot reproduce the exact clinical conditions, mainly because root dentin is not uniform and the surface of the canal walls prepared during the endodontic treatment differ considerably.[122,123]

In the present study, 2 mm thick slices were used in order to prevent premature debonding which is in accordance to the study by Kreimer et al.[124]

The bond strength of root canal sealers to the radicular dentin helps to maintain the integrity of the sealer dentin interface without being disrupted in the long term. Bond strength testing has become a popular method for determining the effectiveness of adhesion between endodontic materials and tooth structure. There are many methods for measuring the adhesion of endodontic root canal sealers, but none have yet been widely accepted.

The pushout test is an efficient and reliable technique to assess bond strength of root canal filling materials to root dentin. Probable reasons are that with this design, it is easy to align samples for testing. It is less sensitive to small variations among specimens and to the variation of stress distribution during load application. Another advantage of this method is that it allows root canal sealers to be evaluated even when bond strengths are low. In the present study too, the bond strength between the root canal sealers to the radicular dentin was evaluated by POBS using universal testing machine.[125]

Analysis of the mode of bond failure, which was analyzed in the current study, showed 70% cohesive type of bond failure in 0.2% Chitosan + 5.25% NaOCl group. This is in accordance with earlier studies.[126,127] This may be attributed to the good adhesion of BioRoot RCS to the root canal walls because of its finer particle size, which, in turn, enhances the infiltration of the cement into the dentinal tubules.[128] It can also be attributed to its biomineralization property through the formation of tags.[129]

In this study there is a correlation between the failure modes and POBS of the tested materials. The failure modes were predominantly cohesive for G3 (0.2% Chitosan + BioRoot RCS) showing 70% cohesive failure, which is because of greater adhesion of sealer to dentin which makes the sealer resistant to displacement. This explains the highest adhesion of BioRoot RCS to the dentin when root canals were irrigated with 0.2% Chitosan when compared with other root canal irrigants used in this study. On the other hand, G1 and G2 showed adhesiveness of 30% and 50% and a combined failure of 40% and 30% respectively. G4 (5.25% NaOCl + BioRoot RCS) showed a mixed failure of 30% and an adhesive failure of 60%, indicating that the sealer did not penetrate sufficiently into the tubules, which showed the least POBS.

Within the limitations of this study it can be concluded that, the pushout bond strength of root canal sealer is influenced by their properties and various dentine surface treatments. Removal of smear layer increases the POBS to root dentine as smear layer removal is critical for the better adaptation of the endodontic sealer.

CONCLUSION

CONCLUSION

Summarizing, according to the results of this in vitro study performed, the null hypothesis can be rejected as statistically significant difference was found in the POBS value between the groups.

Based on the results of this study, following conclusions can be drawn within the limitations of the experimental design:

- Highest POBS was seen in Group 3, followed by Group 2 followed by Group 1.
- Highest efficacy in removal of smear layer was seen in Chitosan, followed by Etidronate, followed by EDTA
- Group 3 has shown predominantly (70%) cohesive failure followed by Group 2 (50%), whereas Group 1 showed predominantly mixed failures (40%).

SUMMARY

SUMMARY

Mechanical instrumentation of the root canal produces a smear layer that covers the dentinal tubules. There is a controversy as to whether smear layer must be removed or maintained. A recent systematic review and meta-analysis of leakage studies concluded that the removal of the smear layer improves the fluid tight seal of the root canal system. Conditioning of the root surface with various acids and chelating agents has been advocated as an effective procedure for smear layer removal and detoxification. Till now a variety of chemicals like EDTA, citric acid, tetracycline have been used but none have come up to the gold standard[2].

EDTA is a polyaminocarboxylic acid that is water soluble in a neutral or alkaline pH. It is used in endodontics because of its chelating property whereby it interacts with calcium ions present in dentin and forms soluble calcium chelates. Hydroxyethylidene bisphosphonate (HEDP) also known as Etidronic acid or Etidronate can be used as a possible alternative to EDTA. De Deus et al. reported that soft chelating irrigation protocol (18% HEDP) optimized the bonding quality of root canal sealers. Chitosan is a natural polysaccharide, obtained by the deacetylation of chitin. Chitosan has attracted attention in dental research because of its biocompatibility, biodegradability, bioadhesion and lack of toxicity. The properties of chitosan that provide its chelating capacity on canal walls have not been assessed, and the possibility for its use as an irrigant in root canal treatment is yet to be investigated.

The aim of this study was to evaluate and compare the pushout bond strength of BioRoot RCS in instrumented canals, after irrigation using chelating agents-17% EDTA, 0.2% Chitosan, 18% HEDP.

Forty mandibular second premolars were selected. The teeth were radiographed at two angulations. Soft tissue fragments and calcified debris on the specimens were removed using ultrasonic scalers. The specimens were stored in a solution of 0.2% sodium azide at

4°C until use. The teeth were decoronated using a diamond disc. The working length was established by inserting a size 10-K file. The apices of all the teeth will be sealed with sticky wax to prevent the flow of irrigants through them and to allow an effective reverse flow of the irrigant to simulate a closed end system.

STUDY GROUPS:

- Group 1: 17% EDTA group with BioRoot RCS (n=10)
- Group 2: 0.2% Chitosan group with BioRoot RCS (n=10)
- Group 3: 18% HEDP group with BioRoot RCS (n=10)
- Group 4: 5.25% NaOCl with BioRoot RCS (control group) (n=10)

IRRIGATION PROTOCOL

• Group 1: 17% EDTA throughout BMP

• Group 2: 18% HEDP throughout BMP

• Group 3: 0.2% Chitosan throughout BMP

• Group 4: Control group in which 5.25% NaOCl is used as the irrigant.

• All samples were irrigated with 5.25% NaOCl and distilled water after each instrument change.

In Groups 1, 2, 3 & 4, the root canals were obturated with gutta-percha and BioRoot RCS and obturated using hand pluggers. The tooth was sectioned horizontally in the middle third to obtain a slice of approximately 2 mm thickness. The pushout bond strength was performed using a universal testing machine (Autograph AG-1). The force was applied using a stainless steel plunger of 0.6 mm diameter at a speed of 1mm/min. The plunger was positioned in such a way that it contacted only the filling material. The maximum force applied at bond failure for each tooth was recorded.

CONCLUSION

Based on the results of this study, the following conclusions can be drawn within the limitations of the experimental design:

- Highest POBS was seen in Group 3, followed by Group 2 followed by Group 1.
- Highest efficacy in removal of smear layer was seen in Chitosan, followed by Etidronate, followed by EDTA
- Group 3 has shown predominantly (70%) cohesive failure followed by Group 2 (50%), whereas Group 1 showed predominantly mixed failures (40%).

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ANNEXURE

ST. GREGORIOS DENTAL COLLEGE

UNDER THE MANAGEMENT OF MJSCE TRUST, PUTHENCRUZ

CHELAD , KOTHAMANGALAM, ERNAKULAM DIST, KERALA - 686681

ETHICAL CLEARANCE CERTIFICATE

SGDC//152/2018/3379/2 Date:- 20-12-2018

To,

Dr. Jesline Maria Jose

St. Gregorios Dental College

Chelad, Kothamangalam

Dear Dr. Jesline Maria Jose

Subject:- Ethics Committee Clearance Reg.

Protocol- Effect of root dentin conditioning using different chelating agents on pushout bond strength of Bioroot RCS: An *in-vitro* study.

After the Institutional Ethics Committee (TEC) held on 19th of December 2018, this study was examined and discussed. After the consideration, the committee had decided to approve and grant clearance for the aforementioned study.

The members who attended the meeting at which the protocol was discussed were:

1. Dr. CKK Nair – Former BARC science
2. Dr. OmmenAju Jacob – Dean, St. Gregorios Dental College, Chelad
3. Dr. Cinu Thomas A – Scientist, Senior Lecturer, Department of Pharmaceutical Sciences Center for Professional and Advanced Studies
4. Rv. Fr. Shanu K. Paulose
5. Lissy Jose – Former Member Women's Welfare Association
6. Adv. Jose Aranjani – Advocate
7. Dr. Sauganth Paul – Senior Lecturer, Department of Biochemistry, St. Gregorios Dental College
8. Dr. Eapen Cherian – Secretary
9. Dr. Jain Mathew – Principal and Head of the Department, Department of Conservative Dentistry and Endodontics
10. Dr. George Francis – Head of the Department, Department of Prosthodontics Crown & Bridge
11. Dr. Binaoy Kurian – Head of the Depaertment, Department of Orthodontics & Dentofacial Orthopeadics

Dr. CKK Nair
Chairman Institutional Ethics Committee
St. Gregorios Dental College, Chelad

Dr. Eapen Cherian
Secretary

Phone : 0485-2572529, 530, 531, 2571429, Fax : 0485-2572530,
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PUSHOUT BOND STRENGTH MEASUREMENTS (MPa)

17% EDTA+ BioRoot RCS G1	18% HEDP+ BioRoot RCS G2	0.2% CHITOSAN+ BioRoot RCS G3	5.25% NaOCl+ BioRoot RCS G4 Control group
30.56	28.13	48.81	22.73
27.16	32.18	48.64	18.27
29.43	29.71	46.67	19.28
26.13	36.26	47.51	21.61
28.62	34.32	43.22	18.42
29.75	35.35	42.35	20.54
28.21	34.41	47.31	18.91
34.46	32.47	46.24	18.08
30.32	37.57	48.11	17.11
29.10	32.53	41.07	18.21

FACTOGRAPHIC ANALYSIS (%)

GROUPS	ADHESIVE FAILURE	COHESIVE FAILURE	MIXED FAILURE
17% EDTA (G1)	3	3	4
18% HEDP (G2)	2	5	3
0.2% CHITOSAN (G3)	1	7	2
5.25% NaOCl (G4)	6	1	3

LIST OF ABBREVIATIONS USED

(in Alphabetical order)

NO.	ABBREVIATIONS	DESCRIPTIONS
1.	ANOVA	Analysis of Variance
2.	BMP	Biomechanical Preparation
3.	EDTA	Ethylene diamine tetra acetic acid
4.	NaOCl	Sodium Hypochlorite
5.	POBS	Pushout bond strength
6.	P- VALUE	Probability Value
7.	RCS	Root canal sealer
8.	SD	Standard Deviation
9.	HEDP	Hydroxyethylidene Disphosphonic Acid