Evaluation of Effect of 17% EDTA and 5.25% Sodium Hypochloride Irrigating Solutions on Surface Hardness of Brasseler Endosequence Root Repair Material

Himanshu Sharma
Marquette University

Recommended Citation
http://epublications.marquette.edu/theses_open/258
EVALUATION OF EFFECT OF 17% EDTA AND 5.25% SODIUM HYPOCHLORIDE IRRIGATING SOLUTIONS ON SURFACE HARDNESS OF BRASSELER ENDOSEQUENCE ROOT REPAIR MATERIAL

by

Himanshu Sharma, DDS

A Thesis submitted to the Faculty of the Graduate School, Marquette University, in Partial Fulfillment of the Requirements for the Degree of Master of Science.

Milwaukee, Wisconsin

May 2014
ABSTRACT
EVALUATION OF EFFECT OF 17% EDTA AND 5.25% SODIUM HYPOCHLORIDE IRRIGATING SOLUTIONS ON SURFACE HARDNESS OF BRASSELER ENDOSEQUENCE ROOT REPAIR MATERIAL

Himanshu Sharma
Marquette University, 2014

Introduction:
Root Perforation is an artificial communication between the root canal system and supporting tissue. Various endodontic researchers have published that sealing the perforation immediately has the best prognosis but this predisposes the repair material to come in contact with various root canal irrigants during the course of treatment. The aim of this study was to evaluate the effect of 17% EDTA and 5.25% sodium hypochlorite irrigating solutions on surface hardness of Endosequence Root Repair Material Putty (ERRM).

Method:
ERRM, 5.25% Sodium Hypochlorite, 17% EDTA and Deionized water were used. 42 samples were prepared and divided into 2 groups. Each group was divided in three sub groups. Sub Groups in Group I were stored in water, 5.25% NaOCl and 17% EDTA for 7 days and were subjected to hardness testing.
After 7 days Group II samples were exposed to water, 5.25% NaOCl, 17% EDTA for 10 minutes and 7 days and were subjected to Vickers microhardness tester for hardness testing. Non-Parametric tests were used due to lack of normalcy of the data.

Results:
Exposure of ERRM to water, 17% EDTA, and 5.2% NaOCl during setting over 7 days had no significant effect on the microhardness of ERRM. NaOCl exposed samples were significantly harder than samples exposed to water for 10 minutes and 7 days. Exposure to EDTA resulted in significantly lower microhardness.

Conclusion:
1. Exposure of ERRM to water, 17% EDTA, and 5.2% NaOCl during setting over 7 days had no significant effect on the microhardness of ERRM.
2. Additional exposure to Water or 17% EDTA for 10 minutes reduced the microhardness possibly due to excessive hydration by water resulting in a porous matrix and acidic nature plus calcium depletion by EDTA interfering with the C-S-H gel structure of ERRM.
3. NaOCl (5.25%) increased the microhardness possibly due to non-inhibition of calcium hydroxide formation on the surface and increasing the number and size of the surface crystal.
4. Exposure to extended period of 17% EDTA had detrimental effects on ERRM and samples lacked structural integrity.
ACKNOWLEDGMENTS

Himanshu Sharma

I would like to gratefully acknowledge the help extended to me at various stages of my study and my two years of Endodontic Education by the following:

It is my privilege to express my gratitude and appreciation to Dr. Sheila Stover for her constant support, encouragement and Guidance. Thank you for your diligent editing and suggestions that went into completion of this Thesis.

I wish to Thank Dr. David Berzins, for all his help and for making this study possible.

I am grateful to Dr. Lance Hashimoto for his teachings, support and encouragement.

A special thank you to my co-residents, juniors, all the Endodontic Faculty and Staff who made these past two years enjoyable and helped me directly or indirectly throughout the course.

Lastly I would like to thank my parents, in laws and family for their co-operation and constant support. Most importantly, my wife and daughter for their patience, love and understanding.
**TABLE OF CONTENTS**

ACKNOWLEDGMENT.............................................................................i

LIST OF TABLES..............................................................................iii

LIST OF FIGURES............................................................................iv

CHAPTER

I. INTRODUCTION.........................................................................1

II. REVIEW OF LITERATURE..........................................................4

   A. Canal Preparation and Procedural Errors.........................4

   B. History of Perforation Repair Material.........................6

   C. MTA: As a Perforation Repair Material.........................9

   D. Endosequence Root Repair Material (ERRM):

      A newer material..............................................................14

   E. Irrigating Agents and ERRM.............................................20

II. MATERIALS & METHODS.........................................................22

   A. Preparation of samples....................................................26

III. RESULTS..................................................................................30

IV. DISCUSSION............................................................................36

V. CONCLUSIONS.........................................................................42

VI. BIBLIOGRAPHY.......................................................................43
LIST OF TABLES

TABLE 1. Microhardness values of all groups in kg/mm$^2$ ..........................30
TABLE 2. Comparison between different groups......................................31
TABLE 3. Pairwise comparisons at 10 minutes of exposure......................35
LIST OF FIGURES

FIGURE.1- ERRM cylinders prepared using plastic tubes.......................27
FIGURE.2- ERRM cylinder in polypropylene centrifuge tube...............27
FIGURE.3- ERRM cylinders mounted sideways in Acrylic Resin.....................28
FIGURE.4- ERRM cylinders mounted vertically in Acrylic Resin.......................28
FIGURE.5- Vickers microhardness tester .............................................29
FIGURE.6- Indenter & Optics for Vickers microhardness tester ...............29
FIGURE 7- ERRM exposed to EDTA (17%) for 7 days (Group II)...........33
FIGURE 8- ERRM exposed to NaOCl (5.25%) for 7 days (Group II)........34
INTRODUCTION

The root canals of the teeth with necrotic pulps and periapical pathology contain decomposed pulp and a diverse reservoir of microorganisms as well as products from pulpal necrosis and bacterial metabolism (1). To remove the organic material, microorganism and their toxins, numerous types of irrigating solutions in conjunction with the mechanical action of instruments have been proposed. This Chemo-Mechanical action have achieved a satisfactory debridement and antisepsis of root canals (2).

Historically, various irrigating solutions at different concentrations and irrigation times have been tested. Sodium hypochlorite (NaOCl) has been widely accepted as the endodontic irrigant of choice because of its antimicrobial and tissue dissolving properties (3). Ethylenediaminetetraacetic acid (EDTA) is also commonly used as an irrigant because of its ability to form complexes with calcium ions and removal of smear layer (4, 5).

Endodontic therapy, which is a last attempt to maintain the tooth’s functionality and esthetics, may become compromised if artificial opening in the root canal wall is created by instrumentation, resorption and caries (6).

Several studies have also shown that perforation predisposes a tooth to peri-radicular disruption and the eventual loss of periodontal attachment, which in most instances can be beyond repair and frequently leads to loss of the tooth (7, 8). Ingle reported that perforations were the second greatest cause of endodontic failure and accounts for 9.6% of all unsuccessful cases (9).
In the United States, it is estimated that more than 24 million endodontic procedures are performed annually, and up to 5.5% of these procedures are apical surgery, perforation repair, and apexification procedures (10, 11).

Various endodontic researchers have published that sealing the perforation immediately has the best prognosis (12) but this predisposes the repair material to come in contact with various root canal irrigants and medicament during the course of treatment.

An ideal endodontic root repair material should be biocompatible, radiopaque, antibacterial, dimensionally stable, easy to manipulate and unaffected by root canal irrigants and blood contaminations (13). Mineral trioxide aggregate (MTA) is considered to be a potentially ideal material for perforation repair, retrograde filling, apexification and vital pulp therapy (14). Several in vitro and in vivo studies have demonstrated that the sealing ability and biocompatibility of MTA are superior to other perforation repair materials like amalgam, IRM and super EBA (15, 16).

In addition MTA is not easy to handle and obtaining consistent results during the clinical application can be difficult. Particle size, powder to liquid ratio, temperature and the presence of air in the mixture may all influence the physical properties of MTA (17). Another possible disadvantage of MTA is the fact that it takes a long time to set (18). Furthermore an acidic environment due to various irrigants has been shown to influence the hydration of MTA, resulting in a weakening of the materials microstructure (19).

Recently, a new root repair material has become available for clinical use: Endosequence Root Repair Material Putty (ERRM Putty; Brasseler USA, Savannah,GA)
is ready to use, premixed bio ceramic material recommended for perforation repair, apical surgery, apical plug and pulp capping (20).

According to the manufacturer, ERRM has excellent physical and biological properties with easy handling characteristics compared to MTA.

Early repair of perforation by repair materials predisposes the material to come in contact with various endodontic irrigants. Literature has documented that routinely used irrigants like EDTA and sodium hypochlorite have influenced the physical properties of MTA. After the final flushing with a chemical irrigants, some amount of the irrigating solution may remain in the root canal space, which may affect the properties of the repair material (21-23).

The current literature does not show any studies related to effect of EDTA and NaOCl on the surface hardness of ERRM. The purpose of this study was to evaluate the effect of 17% EDTA and 5.25% sodium hypochlorite irrigating solutions on surface hardness of Endosequence Root Repair Material.
REVIEW OF LITERATURE

A. CANAL PREPARATION AND PROCEDURAL ERRORS

Endodontic treatment is based on the principle of endodontic triad consisting of biomechanical preparation, microbial control and complete obturation of the root canal space. These principles help to create an ideal environment in which the body can heal itself.

Herbert Schilder in 1974 described cleaning and shaping as “Removal of all the organic substrate from the root canal system and the development of purposeful form within each canal for reception of a dense and permanent root canal filling” (24).

Various authors have documented ‘chemomechanical debridement’ as one of the important steps in removal of root canal content before and during root canal preparation. Chemomechanical preparation of the root canal system includes a combination of both mechanical instrumentation and antibacterial irrigation that is principally directed towards the elimination of microorganisms and disinfection of the root canal system (19).

During root canal preparation an artificial communication between the root canal system and supporting tissue can occur which is termed as ‘Root Perforation’. Perforations can occur during access preparation, post space preparation and during rotary or conventional endodontic instrumentations. In addition, factors not related to operator mishaps like root resorption or caries may also result in root perforations (6).

Advancements in root canal instruments and techniques like rotary niti instrumentation have allowed the endodontist to deal with more complex cases than before but treatment and prognosis of canals with an immature open apex, which sometimes cannot be treated
by newer regenerative procedures and iatrogenic furcal perforation depends on a variety of factors.

In 1970 Seltzer et al identified that prognosis of perforation repair depends on the location of the perforation, time delay before perforation repair and the ability of the material to seal the defect (7).

Various endodontic researchers have also documented in the past about the success of perforation repair if it was done in early stages of the root canal treatment. Alhadainy in 1994 (25) documented in his review of literature that prognosis of an endodontically treated tooth with a small perforation is fair when the perforation occurs away from gingival sulcus or the furcation site and when the perforation is sealed immediately.

Meister et al. (26) and various other researchers (27, 28) found that delay of perforation repair can cause microbial contamination of the defect and breakdown of the periodontium resulting in endodontic-periodontal lesions that are difficult to manage and these perforation defects should be repaired before proceeding with any definitive endodontic treatment.

Fuss and Trope in 1996 (12) published classification and treatment choices based on prognostic factors and also concluded that the immediate sealing of perforation increases success and prevents infection. According to their published literature time of occurrence, size and location of perforation played an important role in achieving these goals.

Treatment of root perforations is presently undertaken by sealing the perforation in the early stage of root canal preparation.
**B. HISTORY OF PERFORATION REPAIR MATERIAL**

Ingel (29) has documented perforation as the second most common reason for endodontic treatment failure. According to Ingle (29) and Seltzer (30) there is a 3% to 10% frequency of root perforation.

A wide variety of root repair material has been used to seal the perforative defects surgically and non surgically. An ideal endodontic perforation repair material should be biocompatible, radiopaque, antibacterial, dimensionally stable, easy to manipulate, unaffected by blood contamination, tissue fluid and root canal irrigants.

Nicholls (31) filled non surgical accessible perforated teeth with zinc oxide eugenol and surgically accessible perforated areas with amalgam but failed to show the results of the treatment.

Stromberg et al in 1972 (32) sealed the perforation with a mixture of gutta percha, resin and chloroform and recalled patients from one to eight years and documented that 18 treatment were successful and 2 failed. In 1957 Grossman (33) recommended that root canals with perforations should be filled following routine protocol but using excessive sealer so that the sealer can be forced into the perforation defect.

William Harris in 1976 (34) presented a two step simplified approach to seal endodontic perforation by using Cavit via an intra-coronal approach. He recommended the use of Cavit at the perforation site with minimal pressure and delaying the conventional root canal filling till the next appointment to allow the setting of the soft Cavit. His paper presented a 75% successful response from 245 patients in a recall period of six months to ten years.
Frank and Weine (35) recommended that perforative resorptive defects should be filled with calcium hydroxide until the adjacent lesion is remineralized. The root canal should be filled with conventional filling material once newly mineralized bone is formed adjacent to the perforation defect. This newly formed bone will act as a matrix against which root canal filling material is placed.

Other endodontist and researchers from that period also recommended sealing the surgically accessible perforation with more rigid material. Taatz and Stiefel (36) recommended amalgam as a material of choice to repair surgically accessible perforation areas and calcium hydroxide followed by root canal filling for all other type of perforations.

Constant developments in new techniques to manage endodontic mishaps and new researches related to dental materials has documented that amalgam, gutta percha, calcium hydroxide and Cavit were used for the non-surgical repair of perforation defect with varying degree of success. One of the biggest challenges was to control the repair material extrusion into the periodontal space. Using bio-inert matrices before the placement of the repair material controlled the problem of extrusion.

In 1969 Auslander et al. (37) described the use of indium foil matrices to prevent the extrusion of amalgam and assumed that indium foil will coalesce with amalgam to produce a satisfactory seal but other researcher criticized their findings.

In 1991 and 1992 use of hydroxyapatite and tri-calcium phosphate was suggested as a matrix below the amalgam or glass inomer to prevent their extrusion in the periodontal space (38, 39).
Plaster of Paris use was first evaluated in 1993 (40) as a matrix below the repair material but its use was first recommended by Bahn in 1966 (41) as a readily available material which was stable, biocompatible, sterilizable with rapid rate of resorption coinciding with the rate of new bone formation. Others (42) have documented Plaster of Paris as a ready source of calcium ions for early mineralization that also excludes the epithelial tissue from site of the bone formation.

Perforation repair material seals the dentin by chemical bonding or by simple mechanical retention. Different irrigating solutions due to their chemical nature could potentially initiate the reaction that would degrade and subsequently predisposes the material to lose its seal.

Literature (28, 43) from 1993 and 1996 has documented that perforation repair material was not able to fulfill all the criteria of ideal repair material including a watertight seal, convenience of use, biocompatibility and adequate strength to withstand the condensation forces of intra coronal restorations.

Introduction of mineral trioxide aggregate widely known as MTA in 1993 by Mahmoud Torabinejad has changed the field of endodontics from perforation repairs to regenerative procedures and has created a new dimension for the success of complicated clinical procedure.
1993 saw the introduction of ‘MTA’ as a newer promising material in the field of endodontics by Torabinejad.

Several reviews (11, 43, 44) and literature has been published today about the chemical properties, biocompatibility and clinical applications of MTA. It has been recognized as bioactive (45), hard tissue conductive (46), hard tissue inductive and biocompatible.

According to the US Patent (47) and review of literature by Roberts et al. (11) MTA contains a mixture of dicalcium silicate, tricalcium silicate, tricalcium aluminate, gypsum, tetracalcium aluminoferrite and traces of bismuth oxide. Dammmaschke et al. in 2005 (48) documented that setting of MTA is more dependent on gypsum and lesser on tetracalcium aluminoferrite.

MTA was initially developed as a gray MTA (GMTA) but due to the discoloration potential, it was modified by lowering the iron, aluminum and magnesium content and is marketed as white MTA (WMTA)(49). MTA is supplied in a powder form and is mixed with water although different solutions like saline, local anesthetics etc. have been used to prepare a usable consistency(43).

According to Camilleri (50-52), when water is mixed with MTA, calcium hydroxide and calcium silicate hydrate is initially formed and later transforms into a poorly crystalline and porous solid gel. The ratio of calcium silicate is low due to the formation of a calcium precipitate. This precipitated calcium produces calcium hydroxide and produces the high alkalinity of MTA after hydration.
Kogan et al. in 2006 (53) studied the setting time and compressive strength of MTA when mixed with different liquids and additives. The setting time of MTA was lower when mixed with 3-5% calcium chloride solutions, lubricant (water based), sodium hypochlorite but the final compressive strength was significantly lower in comparison of MTA mixed with sterile water. MTA mixed with saline and 2% Lidocaine had increased setting time but no effect on the compressive strength was observed, whereas MTA mixed with chlorhexidine did not show any setting reaction.

MTA has a longer setting time in comparison to other restorative materials used in endodontics for perforation repair. According to Torabinejad et al. (17, 54, 55), MTA is prepared by mixing its powder with the sterile water in 3:1 ratio with a mean setting time of 165 mins. Dammaschke et al in 2005 (48) said that WMTA had a longer setting time in comparison to the Portland cement due to the lower levels of sulfur and tricalcium aluminate.

Walker et al.(56) and Chogle et al. (57) recommendations from their in vitro experiments included that MTA has longer setting time and MTA setting time and bacterial leakage is influenced if the samples are stored in dry conditions so 2 sided hydration was recommended for more flexural strength and a moist cotton pellet should remain in place for 24 hours.

Researchers have shown that MTA gains its physical properties such as flexural strength, compressive strength and push out strength when it is exposed to enough moisture. Push out strength is important for perforation repair material, as these materials will get dislodged under function.
Dammmaschek et al. in 2005 (48) also showed that the gypsum content of MTA is half compared to Portland cement, which prolongs the setting time as sulfur provided from gypsum shortens the setting time and another reduction of setting time is caused by tri calcium aluminate but MTA contains reduced Al-species so the setting time is prolonged. Their experiment showed a complex slower hydration reaction for dicalcium silicate than tricalcium silicate in wet environment, which is responsible for delayed push out strength of the material.

Microhardness is another important factor for perforation repair materials, as they will be subjected to different irrigating solutions and medicaments during the completion of the root canal treatment. Microhardness of MTA can be influenced by several factors like pH of the environment, thickness of the material, condensation pressure, amount of entrapped air in the mixture and temperature (19, 48, 54, 58).

Lee et al. 2004 (19) hydrated MTA samples in distilled water and normal saline at pH 7 and pH 5. They found that hydrated MTA consists of cubic and needle like crystals. The cubic like crystals are the principal structures of MTA, whereas the needle like structures are less prominent and are inter grained structures formed in between the cubic like structures. The final conclusion was that no cubic like structure is present in acidic pH (pH 5) and acidic pH affects the physical properties and hydration behavior of MTA.

Namazikhah et al. (58) also evaluated the surface microhardness after exposure to different acidic environment during hydration. It was found that there was no distinct morphological difference in internal microstructure between the groups but surface hardness was impaired in an acidic environment due to extensive porosity of the specimen.
Chemomechanical preparation during endodontic treatment involves the use of different irrigating solutions for varied durations. These chemical solutions may affect the setting reaction of MTA.

Aggarwal et al. (22) studied the effect of 5.25% sodium hypochlorite, 2% chlorhexidine, 17% ethylenediaminetetraacetic acid solution (EDTA) and BioPure MTAD on the surface microhardness and flexural strength of white MTA. It was found that EDTA and BioPure MTAD negatively affected the physical properties of MTA and this effect was more pronounced in comparison with sodium hypochlorite and chlorhexidine. It was hypothesized that EDTA may chelate the calcium ions released from MTA during hydration and disturbs the precipitation of calcium silicate hydrate (C-S-H) gel whereas BioPure MTA is also calcium depleting in nature and has a pH of 2. The former causes disruption of C-S-H formation and later is related to disruption of hydration due to acidic pH.

Smith et al in 2007 (23) examined the effects of calcium-depleting endodontic irrigants, 17% EDTA, 1.3% NaOCl and BioPure MTAD on the surface of white MTA. In their experiment MTA powder was mixed with water in a 0.35 water-cement ratio and was allowed to harden completely. The set samples were subjected to solutions for different time periods and results indicated that BioPure MTA caused higher surface roughness of MTA due to more calcium extraction than EDTA. This has increased the surface roughness and decomposition of particle binding hydration phase in MTA, this phase is responsible for strength and barrier properties of MTA.

Lee et al. also studied the effect of EDTA on hydration of MTA in 2007 (21) and proposed the following:
1. EDTA due to its calcium chelating ability disturbs the hydration of MTA by chelating calcium ions released from the principal ingredient of MTA i.e the tricalcium complex.

2. EDTA exposed samples had no crystalline structure.

3. Samples had poor cell adhesion, poor biocompatibility and reduced micro hardness.

The paper proposed that EDTA solution was detrimental to MTA, so the endodontist should ensure that EDTA is completely removed from the root canal system before placing MTA by flushing the area with copious amount of distilled water.
ENDOSEQUENCE ROOT REPAIR MATERIAL: A NEWER MATERIAL

MTA is one of the most popular materials worldwide because of its biocompatibility, good sealing capability, antibacterial properties and other improvements over prior materials. MTA has also been criticized in the past due to its longer setting time and difficult handling properties.

Recently bioceramic technology in endodontics has provided a useful alternative to MTA. Brasseler USA (Savannah, GA) has introduced EndoSequence Root Repair Material (ERRM) as a clinical replacement for MTA. ERRM has a faster setting time and superior handling characteristics. According to its Material Safety Data Sheet (59) it is a bioceramic material delivered as a pre mixed moldable putty (ESP) or as a preloaded syringe-able paste (ESS) and is composed of calcium silicate (tri and di variant), zirconium oxide, tantalum pentoxide and calcium sulfate with an alkaline pH of >12.

Introduction of the bioceramic material in endodontics has generated a new wave of material studies comparing it to MTA or other endodontic repair material. Enterococcus faecalis is the most frequently recovered microorganism from refractory periapical periodontitis and has the ability to survive conventional root canal therapy because of its resistance to few medicaments. The antibacterial effectiveness of root canal repair material against E. faecalis increases the success rate of endodontic treatment by eliminating the residual microorganism that has survived the chemomechanical instrumentation (60).

iRootSP (Innovative Bioceramix, Vancouver, Canada) also known as EndoSequence BC sealer (Brasseler USA, Savannah, GA) has been studied by Zhang et al. (60) and found that...
iRootSP, AH Plus and EndoRez were effective against E.faecalis. iRootSP was effective for 3 and 7 days after mixing whereas Sealapex and EndRez were effective even at 7 days after mixing. This study showed that iRootSP absorbs moisture from dentin that facilitates the hydration reaction of calcium silicate and produces calcium silicate hydrogel and calcium hydroxide. Calcium hydroxide reacts with the phosphates to form hydroxyapatite, water and increases the pH. Increased pH, hydrophilicity and active calcium hydroxide diffusion are considered important factors towards its antibacterial potential.

Lovato and Sedgley (61) studied the antibacterial activity of ERRM and ProRoot MTA against Enterococcus faecalis by direct contact test. ERRM has similar antibacterial efficacy like MTA against clinical strains of E.faecalis. This efficacy was attributed to ERRM’s high pH, hydrophilicity and active calcium hydroxide diffusion. Biocompatibility influences the clinician’s choice of endodontic repair material as these materials are placed in contact with the periapical tissues. Tissue response to these materials might influence the outcome of the endodontic repair.

Ma and Shen (20) compared the biocompatibility of the ERRM putty, ERRM paste and gray MTA with IRM and Cavit. Biocompatibility was tested by cytotoxicity assay using gingival fibroblast. ERRM materials are bio ceramic materials with the ability to form hydroxyapatite or apatite-like layer on its surface during contact with phosphate containing fluids resulting in biomineralization. ERRM and MTA were found by Ma & Shen to show similar biomineralization whereas IRM shows cytotoxic effect due to release of free eugenol causing hydrolysis. Cavit has cytotoxicity due to zinc oxide.
Studies (62, 63) on MTA have documented formation of cementum and periodontal ligament fibers when it was used as a root end filling material. AlAnezi et al. (64) compared ERRM with Gray and White MTA by using the MTT assay, which is a standard assay to evaluate the cytotoxicity of the material. ERRM showed a cell viability similar to GMTA and WMTA in freshly mixed and set conditions.

In 2011 Damas et al.’s. (65) experiment showed the results of ERRM, white MTA and MTA-Angelus cytotoxicity similar to the study done by AlAnezi in 2010.

The ability of biomaterials to promote mineralization can be also evaluated through the expression of different cellular biochemical markers like alkaline phosphatase (ALP). ALP is a biochemical marker of osteoclastic activity and is present on the plasma membrane fragments of the osteoblast. ALP presence is indicative of the cellular differentiation after an injury (66).

In 2012 Modareszadeh et al. evaluated the cytotoxicity and effects on ALP activity of ERRM, MTA and Geristore using human osteosarcoma cell line. Human osteosarcoma cell line is a widely used model for osteoblast like cells. Results of this study indicated that elutes of ERRM significantly reduced the bioactivity and ALP activity of human osteoblast like cells whereas MTA had no affect on cells bioactivity/ALP activity whereas Geristore at higher concentration decreased the bioactivity without any adverse effect on ALP activity.

A Bioactive material on interacting with the living tissues results in formation of an apatite layer and bio mineralization at the material tissue interface. In vivo hard tissue bioactivity is examined by evaluation of this apatite when the material is exposed to the body fluid (67, 68). Bioactivity of MTA has been reported by formation of
hydroxyapatite or carbonated apatite during interaction of MTA with phosphate containing fluids (69-74) whereas Shokouhinejad et al. (75) evaluated the bioactivity of ERRM, MTA and Bio aggregate (BA) by exposing the roots containing these materials to phosphate buffered saline (PBS). They found that there was precipitation of apatite crystals which became larger with increasing immersion times. It was found that all materials tested in this study were bioactive. The precipitation of the apatite crystals was a result of hydration leading to Ca and OH ions from tricalcium/dicalcium silicate into the surrounding environment resulting in formation of calcium hydroxide precipitate and calcium silicate hydrate (CSH) gel. Morphology of ERRM surface was different as it contains calcium phosphate that is not present in MTA, filler and thickening agents for maintaining the putty consistency that eventually affects its hydration.

Root canal repair materials should be able to establish a hermetic seal in order to prevent the egress of irritant into the peri radicular tissues from the root canal system. One of the methods to evaluate the sealing ability is bacterial leakage method as shown by previous studies (76).

Hirschberg et al. (76) compared the sealing ability of ProRoot MTA to ERRM using a bacterial leakage model and found out that there was significantly more leakage in the ERRM group than the MTA group. The results of this study were based on the study by Loushine et al. (77) which recommended that an increase in amount of water during setting of BC sealer (which is similar to the composition of ERRM) shows an increase in initial setting time from 72 hrs to 180 hrs and decrease in final setting time from 240 hrs to 168 hrs. It was also noted that when set sealer was exposed to additional water the microhardness of BC sealer decreased significantly and resulted in a more
porous matrix releasing tissue irritants from the set cement. This study explains that the presence or absence of excessive moisture may affect the sealing ability and leakage of ERRM.

The main advantages of bio ceramic materials in dentistry are related to their physical and biological property, which includes high alkaline pH, antibacterial activity, radiopacity and biocompatibility. Other advantages of the material are formation of hydroxyapatite during setting and a bond between the dentine and filling material (60, 77).

In 2012 Canderio et al. (78) presented the comparison and results of physiochemical properties of BC sealer and AH Plus. BC sealer showed less radiopacity than AH Plus because it was observed that cement can be more radiopaque if bismuth oxide, zirconium oxide, calcium tungstate, barium sulfate and zinc oxide are added in decreasing orders. BC sealer contains only zirconium oxide whereas AH Plus has zirconium oxide and calcium tungstate.

The pH analysis in the Canderio et al. study showed that BC sealer showed pH and calcium release greater than AH Plus. An alkaline pH promotes the elimination of Enterococcus faecalis and combined with calcium release helps in repair stimulation by deposition of mineralized tissue. The presence of moisture during the setting of Bioceramic based material facilitates the hydration reaction of calcium silicates and produces calcium silicate hydrogel and calcium hydroxide, which partially reacts with phosphate to form hydroxyapatite and water.
Brasseler has reported the working time of ERRM as 30 minutes compared with 5-15 minutes of MTA whereas the setting time of ERRM is 4 hrs compared with 4-6 hrs of MTA.

Charland (79) compared the abilities of MTA and ERRM to set in the presence of human blood and Minimal Essential Media (MEM). The results of the study showed that setting of both materials were much longer than those reported by their manufacturers. MTA took 36 hrs whereas ERRM was not completely set by 48 hrs so it is prudent to wait at least 36 hrs for MTA to set and even longer to allow ERRM before continuing the endodontic procedure.

The introduction of bioceramic based materials into endodontics has led to the repetition of original benchmark studies about antibacterial properties, cytotoxicity, pH, setting time but there is no study showing the effect of routinely used irrigants on the hardness of ERRM.
IRRIGATING AGENTS AND ERRM

After perforation repair, endodontic treatment is performed with various irrigating solutions to clean the root canal system. This procedure causes inevitable contact of endodontic irrigants with the repair material. Studies (21-23, 58) as mentioned in the review of literature section for MTA has shown that acidic environments of these routinely used irrigants affected the surface hardness of MTA.

Nandini et al. (80) tested the effect of carbonic acid, 2% chlorhexidine gluconate, 17% EDTA and saline on set white MTA (WMTA) on 1 day and 21 days after setting. Carbonic acid was found to be effective in dissolving WMTA even after 21 days because carbonic acid with a pH of 5.48 releases ion that act on calcium silicate and calcium hydroxide in WMTA, causing dissociation of calcium hydroxide into calcium and hydroxyl ions. The study failed to explain the reasoning behind reduced surface hardness of WMTA after 1 day of setting by chlorhexidine. EDTA was shown to cause minimal reduction in hardness after 1 and 21 days. Conclusions drawn from Nandini’s study recommended that carbonic acid could be used as an adjunct to dissolve the WMTA even after 21 days of setting, whereas chlorhexidine gluconate solution should be avoided as a root canal irrigants when WMTA is used.

Acidic pH of the routinely used irrigants such as EDTA has shown to cause the increase in the solubility of these repair material as mentioned earlier. In the light of these observation Uyanik et al. (81) studied the effect of 5.25% NaOCl, 5.25% NaOCl combined with EDTA and MTAD on the sealing ability of WMTA and Super-EBA-
repaired furcal perforation. Pulp chambers of the experimental teeth were exposed to different irrigation solutions after the furcal repair of the perforation and fluid transport method was used to check the micro leakage around the restorations. According to this study EDTA and MTAD are calcium-depleting irrigants and produce the detrimental effect on the seal of WMTA and Super-EBA and increase the micro leakage. One of the reasons for calcium depleting irrigants to interfere with the solubility and sealing of repair material was that they were capable of removing the smear layer on the surface of root canal and infiltrated into the interfacial layer where they also interfered with the chemical adhesion between repair material and dentin and as previously mentioned in other studied also interferes with the hydration of these materials.

The above findings were in accordance with Smith 2007 (23) who identified that hydration phases are responsible for the strength and barrier properties of MTA. According to Uyanik et al (81) NaOCl produces statistically insignificant improvement in micro leakage and this modest improvement was because of NaOCl being a halogenated compound can cause mineral accumulation in human dentine and exposes inorganic material which unlike EDTA and MTA may prevent dentin dissolution or may leave a smear layer of mineralized tissue that could increase the Ca/P ratio of the dentin surface.

Various studies have shown the effect of irrigating agents on widely used perforation repair material like MTA but there are no published studies demonstrating the effect of various root canal irrigants on the newer bioceramic material like Endosequence Root Repair Material. The purpose of this study was to identify the effect of routinely used irrigants like 5.25% NaOCl and 17% EDTA on the surface hardness of ERRM.
MATERIAL & METHODS

Materials used were Endosequence Root Repair Material putty (ERRM Putty; Brasseler USA, Savannah, GA), 5.25 % Sodium Hypochlorite (NaOCl) (Chlorox; The Chlorox Company, Okaland, CA), 17% EDTA (Vista; Inter-Med Inc, Racine, WI) solution and deionized water.

In order to check the effect of solutions on the material, forty-two prepared samples were divided into two groups. Group I was exposed to solution during setting and Group II were exposed to solution after the setting.

Group I (twenty one samples) was again divided in three sub groups (Sub Group I-A, I-B and I-C). ERRM cylinders in Sub Group I-A were stored in Deionized water for 7 days; Sub Group I-B were stored in 5.25% NaOCl and Sub Group I-C were stored in 17% EDTA immediately (Fig 2). In Group II (twenty one samples) all ERRM cylinders were stored immediately in Deionized water for 7 days

After 7 days for each group, the cylinders were mounted in acrylic and ground/polished to half of the diameter. After this, Group I was subjected to hardness testing. Whereas after 7 days ERRM cylinders in Group II were mounted in acrylic and ground/polished to half height and placed in Sub Group II-A, II-B and III-C (Seven samples each subgroup).

Sub Group II-A samples were exposed to Deionized water, Sub Group II-B was exposed to 5.25% NaOCl and Sub Group II-C was exposed to 17% EDTA for 10 minutes. After this exposure samples were subjected to hardness testing.
After hardness testing the samples in Group II, they were again ground/polished and stored for 7 days in Deionized water, 5.25% NaOCl and 17% EDTA using a glass container kept at 37°C. All the samples in Group II were subjected to hardness testing again after 7 days.

All the samples were subjected to hardness testing using a Vickers microhardness tester (Kentron; Torsion Balance Co., Clifton, NJ) with a 600 gm. load and dwell time of 15 seconds. Three indents were made at the polished surface of ERRM at different areas and then the measurements were averaged. Vickers microhardness number was calculated via the formula:

\[ VHN = \frac{2 \times F \times \sin (136^\circ / 2)}{d^2} \]

Where F is the force applied in kilograms and d is the calculated average of indentations in millimeters.
ERRM Cylinders
(42 prepared Cylinders)

Group-I
(21 cylinders)
(Divided in 3 Sub Groups
Of 7 each)

Sub Group-I A
(7 cylinders)
(Stored in Deionized Water)
(Fig A)

Sub Group-I B
(7 cylinders)
(Stored in 5.25% NaOCl)
(Fig A)

Sub Group-I A
(7 cylinders)
(Stored in 17% EDTA)
(Fig A)

Acrylic Resin Mounted
Samples
(After 7 days of initially
setting all the samples were
mounted sideways in resin
and ground/polished)
(Fig B)

HARDNESS TESTING
(21 Samples)
(Fig C)
ERRM Cylinders
(42 prepared Cylinders)

Group-II
(21 cylinders)
(All the cylinder were stored in water for 7 days)

Group-I

Acrylic Resin Mounted Samples
(After 7 days of initially setting all the Cylinders were mounted vertically in resin and ground/polished)
(Prepared samples were divided in 3 sub Groups)

Sub Group-II A
(7 Samples)
(Stored in water -10 mins)

Sub Group-II B
(7 Samples)
(Stored in 5.25% NaOCl-10 mins)

Sub Group-II C
(7 cylinders)
(Stored in 17% EDTA-7 days)

HARDNESS TESTING
(21 Samples)

Sub Group-II C
(7 cylinders)
(Stored in 17% EDTA-7 days)

Sub Group-II B
(7 Samples)
(Stored in 5.25% NaOCl-7 days)

Sub Group-II A
(7 Samples)
(Stored in water -7 days)

HARDNESS TESTING
(21 Samples)

After hardness testing each sub group is ground/polished and exposed for 7 days
**Preparation of the samples:**

7 mm X 3mm (height X diameter) ERRM cylinders were prepared by placing the material in plastic tubes of the same dimensions (Fig 1). ERRM cylinders with plastic tubes were placed in the different solutions according to the previously mentioned groups and were stored in a polypropylene centrifuge tube (Corning Inc. Corning, NY) containing 5ml of solution (Water, NaOCl, EDTA for 7days)(Fig 2). All the tubes were stored in an incubator at 37°C.

After 7 days, the cylinders were removed from their plastic tube by using a No.15 surgical scalpel. In Group 1, cylinders were mounted side ways in acrylic resin (Sampl-Kwick; Buehler Ltd, Lake Bluff, IL.)(Fig 3).

In Group 2, ERRM cylinders were mounted vertically in acrylic resin (Fig 4). In each group, samples were ground/polished using 180, 320, 400 and 600-grit SiC paper (CarbiMet 2 Discs; Buehler Ltd).

All the results were tabulated and non-parametric test were used due to lack of the normalcy of the data.
Fig: 1 ERRM cylinders prepared using plastic tubes

Fig: 2 ERRM Cylinder in polypropylene centrifuge tube (Corning Inc. Corning, NY) containing 5ml of solution.
Fig: 3 ERRM cylinders mounted sideways in acrylic resin

Fig: 4 ERRM cylinders mounted vertically in acrylic resin
Fig: 5 Vickers microhardness tester (Kentron; Torsion Balance Co., Clifton, NJ)

Fig: 6 Indenter & Optics for Vickers microhardness tester
RESULTS

The mean microhardness (SD) of ERRM samples stored in the deionized water (Control), 5.25% NaOCl, and 17% EDTA at different time periods is listed in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial Mean (SD)</th>
<th>10 min Exposure Mean (SD)</th>
<th>Exposed 7 days Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (Control)</td>
<td>28.8 (7.5)</td>
<td>30.1 (3.1)</td>
<td>7.3 (2.7)</td>
</tr>
<tr>
<td>17% EDTA</td>
<td>27.8 (6.4)</td>
<td>27.5 (7.4)</td>
<td></td>
</tr>
<tr>
<td>5.25% NaOCl</td>
<td>30.2 (9.4)</td>
<td>37.8 (2.7)</td>
<td>37.3 (6.8)</td>
</tr>
</tbody>
</table>

Table 1 Microhardness values of all groups in kg/mm²

Non-parametric tests were used due to lack of the normalcy of data. The Kruskal-Wallis test was used to compare the three groups at initial & 10 minutes exposure.

The Wilcoxon Test was used to compare the samples from Group II (Sub Group II-A & Sub Group II-B) at 7 days exposure to water and NaOCl.

The Signed Rank Test was used to compare samples from Group II (Sub Group II-A & Sub Group II-B) at an exposure of 10 minutes and 7 days to water and NaOCl.
Comparison between the different groups is presented in Table 2.

If $p > 0.05$ then there is no significant difference in the groups and following results can be interpreted from Table 2.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comparing three groups (Water, NaOCl, EDTA) at Initial (Kruskal-Wallis Test)</td>
<td>0.7082</td>
</tr>
<tr>
<td>Comparing three groups (Water, NaOCl, EDTA) at 10 minutes exposure (Kruskal-Wallis Test)</td>
<td>0.0042</td>
</tr>
<tr>
<td>Comparing two groups (Water, NaOCl) at 7 day exposure (Wilcoxon Test)</td>
<td>0.0022</td>
</tr>
<tr>
<td>Comparing 10 minutes exposure and 7 day exposure for Control (Signed Rank Test)</td>
<td>0.0156</td>
</tr>
<tr>
<td></td>
<td>Test Statistic = 14</td>
</tr>
<tr>
<td>Comparing 10 minutes exposure and 7 day exposure for NaOCl (Signed Rank Test)</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>Test Statistic = 0</td>
</tr>
</tbody>
</table>

**Table 2: Comparison between different groups.**

1. Mean surface microhardness (SD) of Water, 17% EDTA and 5.25% NaOCl samples in Group I after 7 days of storage time was 28.8 (7.5), 27.8 (6.4) & 30.2 (9.4). The Kruskal-Wallis Test showed no statistically significant difference among the three groups ($P=0.7082$).

2. Mean surface microhardness (SD) of samples in Group II at 10 minutes exposure to Water, 17% EDTA and 5.25% NaOCl was 30.1(3.1), 27.5(7.4) & 37.8 (2.7). Mean micro-hardness values via the Kruskal-Wallis Test showed a significant difference among the three groups ($P=0.0042$).
3. Mean surface microhardness of samples in Group II A (Water) and Group II-B (5.25% NaOCl) after 7 days final storage in Water and NaOCl was 7.3 (2.7) & 37.3 (6.8). The Wilcoxon Test showed a statistically significant difference between the two groups (P=0.002).

4. There were no measurements recorded for Group II-C (17% EDTA) samples after 7 days of final exposure to EDTA solution as these samples did not withstand the force produced by indenter and were non readable (Fig:7).

5. The Signed Rank test was used to compare the mean microhardness (SD) for samples stored in water for 7 days and samples exposed to water for 10 minutes. Test results showed values for samples stored for 7 days in water had lower values than samples exposed for 10 minutes and the difference was statistically significant (P=0.015).

6. Comparison of samples exposed to 10 minutes and stored for 7 days in NaOCl was done using the Signed Rank Test. The mean microhardness (SD) values were not significantly different (P=1.000).

Table 3 shows pairwise comparison for Group II samples exposed to Water, 17% EDTA and 5.25% NaOCl for 10 minutes. The following results can be drawn by this comparison.

1. The Mann-Whitney U Test showed the microhardness (SD) values of Group II samples exposed to Water and 5.25% NaOCl for 10 minutes were significantly different (P=.009). Samples exposed to water showed lower values compared to NaOCl exposed samples.
Fig. 7 ERRM exposed to EDTA (17%) for 7 days (Group II)
Fig: 8 ERRM exposed to NaOCl (5.25%) for 7 days (Group II)
2. Microhardness (SD) values for Group II samples exposed to Water and 17% EDTA for 10 minutes showed no significant difference using the Mann-Whitney U Test (P=0.387).

3. Microhardness (SD) values for samples exposed to 17% EDTA for 10 minutes are lower compared to samples subjected to 5.25% NaOCl for the same time. The Mann-Whitney U Test showed a significant difference (P=0.0305).

<table>
<thead>
<tr>
<th>Pairwise comparisons</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comparing Water and NaOCl groups at 10 minutes exposure</td>
<td>0.0090</td>
</tr>
<tr>
<td>Using Mann-Whitney Test</td>
<td></td>
</tr>
<tr>
<td>Comparing Water and EDTA groups at 10 minutes exposure</td>
<td>0.3874</td>
</tr>
<tr>
<td>Using Mann-Whitney Test</td>
<td></td>
</tr>
<tr>
<td>Comparing NaOCl and EDTA groups at 10 minutes exposure</td>
<td>0.0305</td>
</tr>
<tr>
<td>Using Mann-Whitney Test</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 Pairwise comparisons at 10 minutes of exposure.
DISCUSSION

Ceramic products or components employed in medical or dental applications that have osteoinductive properties are referred to as Bio ceramic materials(82). EndoSequence Root Repair Materia is a bioceramic material delivered as a premixed moldable putty or as a syringable paste (material). Both materials are of similar chemical composition.

ERRM has been manufactured as an alternative to MTA and its difficult handling characteristics. ERRM is supplied as a ready to use material whereas MTA needs to be mixed with a sterile liquid to achieve a desirable consistency.

According to the manufacturer, ERRM is composed of calcium silicate (tricalcium silicate and dicalcium silicate), calcium phosphate monobasic, zirconium oxide, tantalum oxide and filler agents(59). The material is hydrophilic, insoluble, radiopaque, aluminum free and has a high pH (>12). The working time is more than 30 minutes and setting time is 4 hours in normal conditions.

Presence of moisture is required for the material to set; moisture naturally present in the root canal and dentinal tubules initiates and completes its setting reaction(83). In the present study samples were prepared and stored in different solutions for 7 days (168 hrs.) to achieve complete setting because according to the manufacturers directions, ERRM takes 12 hours of direct contact with moisture for the material to completely set. However Damas et al. (65) observed that ERRM material did not set within the 12-hour time period when placed in 100% humidity at 37°C and partially set samples were found
at 72-hours and at the 120-hour mark. Only after an incubation of 168 hours was a completely set sample of the material obtained.

Similarly, Loushine et al. (77) also reported that EndoSequence BC sealer required 108-hrs (4.5 days) to achieve an initial set when mixed with water and the final setting occurred at 168-hrs (7 days). Both ERRM and BC sealer have similar compositions except the thickening agent that they contain.

Recently in 2013, Charland et al. (79) observed much longer setting times for MTA and ERRM materials than those reported by their respective manufacturers in the presence of human blood. Results indicated that MTA samples set within 36-hrs whereas ERRM were not completely set by 48 hrs.

In the light of the above mentioned studies, the decision of leaving the ERRM samples for 7 days to achieve complete setting before hardness testing seems appropriate. The Vickers hardness test, which was first developed by Robert Smith and George Sandland at Vickers Ltd in 1921(84) is used to measure the hardness of almost all the materials because the same indenter can be used for them irrespective of their hardness. This test was used in this study as it has been extensively used in the past to check the hardness of various dental materials.

This study showed no significant differences in mean microhardness of Water, 17% EDTA and 5.25% NaOCl samples in Group I after 7 days of storage time, but the microhardness of samples in Group II exposed to Water, 17% EDTA and 5.25% NaOCl for 10 minutes showed significant difference amongst the three groups.

Group II samples stored in Water and 5.25% NaOCl for 7 days also showed significant difference in the mean microhardness.
This study showed significant lower microhardness for Group II samples exposed to water for 10 minutes and 7 days when compared to samples exposed to the same amount of time to NaOCl, the possible explanation for this finding requires one to understand the setting reaction of ERRM. According to the manufacturer, moisture initiates the setting reaction by contacting the calcium silicate portion of the material; this reaction of moisture produces calcium silicate hydrate gel and calcium hydroxide. Calcium hydroxide then interacts with phosphate ions to form hydroxyapatite and water. The water produced continues to react with the calcium silicates to precipitate additional gel like calcium silicate hydrate. The manufacturer has also stated that water formed through this reaction is an important factor in controlling the hydration rate and setting time of the ERRM.

Loushine et al. (77) observed that samples of EndoSequence BC sealer stored in 100% humidity showed an initial setting time of 72 hrs and a final setting time of 240 hrs (10 days). The experiment also showed that by increasing the amount of water there was an increase in initial setting time (180 hrs) and a decrease in the final setting time (168 hrs). The important finding, which was noted in the Loushine et al. study and has a direct relation to this experiment, was a significant decrease in microhardness of BC sealer was observed when it was exposed to additional water due to the formation of more porous matrix.

Hirschberg et al (76) compared the sealing properties of MTA and ERRM putty and concluded that ERRM putty is very sensitive to the presence or absence of water and this affects the sealing properties of ERRM putty. Their finding was based on the similarity in the composition of ERRM putty and BC sealer.
In this experiment significant reduction in microhardness of water treated samples can be explained by the finding of Loushine and Hirschberg et al. (76, 77).

NaOCl and EDTA are the most commonly used endodontic irrigants. The pH of sodium hypochlorite is alkaline and is between 9-10.5 (85, 86). Literature has indicated that a lower pH environment may negatively affect various physical and chemical properties of MTA (87-89). It was documented by Kogan et al. (53) in 2006 that 3.0% NaOCl mixed with MTA improves the setting time but reduces its strength.

Hong et al. (90) have demonstrated that NaOCl did not interfere with the hardening of accelerated MTA (MTA+10% CaCl\textsubscript{2}) and quickened its setting mechanism. SEM analysis of the prepared samples showed that NaOCl did not inhibit calcium hydroxide formation on the surface of MTA and there was an increase in the number and the size of the surface crystal rendering improved physical properties even in the presence of NaOCl.

ERRM putty and White MTA are similar in composition except that ERRM is aluminum free (91) and contains calcium phosphate monobasic and tantalum pentoxide. In this experiment, microhardness was significantly more for 5.25% NaOCl samples exposed for 10 minutes and 7 days in comparison to samples exposed to water and this finding is in accordance to the reasons given by Hong et al.

Ethylenediaminetetraacetic acid is most commonly used as a chelating agent to remove the smear layer from the root canal walls (92). The chemical structure of EDTA suggests it has six potential sites i.e. four carboxyl groups and two amino groups available to bond with calcium to form highly stable bonds (21). EDTA is used as an
irrigant in non-surgical root canal therapy due to its ability to form complexes with calcium ions, which facilitates the removal of the smear layer.

Nandini et al. showed that White MTA (WMTA) can be dissolved by carbonic acid effectively even after 21 days of its setting and 2% chlorhexidine gluconate solution will dissolve MTA only in the first 24 hours, but EDTA solution had no effect on the surface hardness of WMTA.

The effect of EDTA on MTA have been identified and published by various authors from time to time. Lee et al (21) has identified by their experiment that residual EDTA remained after the irrigation in root canal system and could chelate the calcium ions released from MTA during hydration and disturb the precipitation of C-S-H gel (Calcium-Silicate-Hydrate gel) resulting in lower hardness value due to poor crystallization.

Aggarwal et al. (22) also found that EDTA treated MTA samples had decreased microhardness related to poorly formed C-S-H and recommended a copious rinse of distilled water to remove any remnant of chemical irrigants before MTA was placed in the perforation area.

It was also noted that EDTA is a calcium depleting irrigant with an acidic pH causing decomposition of particle binding hydration phases resulting in a change in strength and sealing properties of MTA (81).

In this experiment it was noted that there was a significant difference in microhardness between 17% EDTA and 5.25% NaOCl treated samples for 10 mins and the EDTA group had a lower microhardness, but when these samples were exposed for an extended period i.e 7 days to 17% EDTA it was not possible to record the microhardness
because the samples lacked structural integrity. These results are possibly due to poor formation of the calcium-silicate–hydrate gel due to the acidic and calcium depleting nature of EDTA as mentioned in previous studies (21, 22, 81) on MTA, and ERRM while being chemically similar to MTA would possibly reproduce the similar results.

It was also noted in this study that the Water and 17% EDTA groups did not show any significant difference in microhardness when ERRM was exposed for 10 minutes to these irrigants possibly because none of the irrigant had shown to increase the microhardness by extending the exposure time.
CONCLUSION

Within the experimental condition of this laboratory investigation the following conclusions were drawn:

1. Exposure of ERRM to water, 17% EDTA, and 5.2% NaOCl during setting over 7 days had no significant effect on the microhardness of ERRM.

2. After allowing ERRM to set for 7 days, additional exposure to Water or 17% EDTA for 10 minutes reduces the microhardness possibly due to excessive hydration by water resulting in a porous matrix and acidic nature plus calcium depletion by EDTA interfering with C-S-H gel structure of ERRM.

3. NaOCl (5.25%) increased the microhardness possibly due to non-inhibition of calcium hydroxide formation on the surface and increasing the number and size of the surface crystal.

4. Exposure to extended period of 17% EDTA has detrimental effects on ERRM and samples lacked structural integrity.

Based on this research it is recommended that one should not leave any traces of EDTA and should avoid excessive water exposure after 7 days.


