EFFECT OF THREE DIFFERENT ANTIOXIDANTS ON THE DENTINAL TUBULAR PENETRATION OF RESILON AND REALSEAL SE ON SODIUM HYPOCHLORITE TREATED ROOT CANAL DENTIN: AN IN VITRO SCANNING ELECTRON MICROSCOPIC STUDY

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In partial fulfillment of the degree of

MASTER OF DENTAL SURGERY

BRANCH IV

CONSERVATIVE DENTISTRY

AND ENDODONTICS

2012-2015

CERTIFICATE

This is to certify that this dissertation titled ***"Effect of three different antioxidants on the dentinal tubular penetration of Resilon and RealSeal SE on sodium hypochlorite treated root canal dentin: An in vitro Scanning Electron Microscopic study "*** *is a bonafide record of the work done by Dr. Sarah R. Christopher under our guidance during her post graduate study during the period of 2012-2015 under* **THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY, CHENNAI,** *in partial fulfillment for the degree of* **MASTER OF DENTAL SURGERY IN CONSERVATIVE DENTISTRY & ENDODONTICS, BRANCH IV.** *It has not been submitted (partial or full) for the award of any other degree or diploma.*

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This is to certify that the dissertation titled "**EFFECT OF THREE DIFFERENT ANTIOXIDANTS ON THE DENTINAL TUBULAR PENETRATION OF RESILON AND REALSEAL SE ON SODIUM HYPOCHLORITE TREATED ROOT CANAL DENTIN: AN IN VITRO SCANNING ELECTRON MICROSCOPIC STUDY"** is a bonafide research work done by **DR. SARAH R. CHRISTOPHER** under the guidance of **DR. VIJAY MATHAI**; *M.D.S.* Professor , Department of Conservative Dentistry and Endodontics, Sree Mookambika Institute Of Dental Sciences, Kulasekharam.

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This is to certify that the research project protocol, ***Ref no: 03/03/2014*** entitled, ***"The Effect of three different antioxidants on the dentinal tubular penetration of Resilon and Real seal SE on sodium hypochlorite treated root canal dentin : An Invitro SEM study"*** submitted by ***Dr. Sarah R. Christopher. II Year MDS, Department of Conservative dentistry & Endodontics*** *has* been approved by the Institutional Research Committee at its meeting held on ***18th March 2014.***

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This is to certify that the Research Protocol Ref. No. **SMIMS/IHEC/2014/A/24,** entitled "Effect of Three Different Antioxidants on the Dentinal Tubular Penetration of Resilon™ and RealSeal SE™ on Sodium Hypochlorite Treated Root Canal Dentin: An *In Vitro* Scanning Electron Microscope Study" submitted by Dr. Sarah R. Christopher, Postgraduate of Department of Conservative Dentistry and Endodontics, SMIDS has been approved by the Institutional Human Ethics Committee at its meeting held on 6th of May 2014.

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CONTENTS

LIST OF ABBREVIATIONS

NaOCl - Sodium Hypochlorite	SPSS - Statistical Package for Social Sciences
CHX - Chlorhexideinedigluconate	4-META - 4 - methacryloyloxyethyl trimellitate anhydride
EDTA - Ethylene di-amine tetra acetic acid	AMPS - 2-acrylamido-2-methyl-propanesulfonic acid
FDA - Food and Drugs Administration	Calcium hydroxyapatite - $Ca_{10}(PO_4)_6OH_2$
GSE - Grape seed extract	$HOCl^-$ - hypochlorous acid
SE – self etch	OCl^- - hypochlorite ions
EGCG - Epigallaocatechin gallate	NH group - amino group
MMP – Matrix metalloproteinase	SH group - sulphydryl group
ROS - reactive oxygen species	*E. faecalis – Enterococcus faecalis*
DNA-Deoxyribonucleic acid	BS – Bond strength
µm –Micrometer	Ni-Ti - Nickel Titanium
SEM - Scanning Electron Microscopy	Etc. – etcetera
PCL – Polycaprolactone	i.e. – that is
ANOVA - Analysis of Variance	Fig.- Figure

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Abstract

Introduction

A correct diagnosis, access opening, chemo-mechanical preparation and seal of the root canal space with a core material and a root canal sealer to achieve disinfection as well as a hermetic seal is essential for the success of endodontic treatment. For years gutta-percha has been the core material for sealing the root canal space along with a sealer. One of the main drawbacks of this material was that it did not adhere to the root canal wall. In 2004, Resilon, a resin based obturating material was introduced in the market which along with methacrylate based sealers like Epiphany, RealSeal and RealSeal SE created the "monoblock" concept.

Chemical irrigants like sodium hypochlorite adversely affect the adhesion of these resin based obturating materials to the root canal dentin as a result of residual oxygen generated. The use of antioxidants like ascorbic acid, tannic acid, gallic acid on sodium hypochlorite treated dentin can, not only reverse bond strength but also increase the dentinal tubular penetration of the sealer ensuring better seal.

Aims And Objectives

To observe the effect of antioxidants 10% ascorbic acid, 10% tannic acid and 10% gallic acid on the dentinal tubular penetration of Resilon and RealSeal SE on sodium hypochlorite treated root canal dentin .

Methodology

Fifty non carious, single rooted mandibular premolars freshly extracted for orthodontic purposes were collected. The teeth were decoronated to achieve 14 mm. root length and divided into five groups of ten teeth each. Initial apical file was no. 10 K file. Biomechanical preparation of the teeth were done with HERO Shaper Ni-Ti rotary instruments. Irrigation of group I was done with saline and 17% ethylenediaminetetraacetic acid. Groups II, III, IV and V were irrigated with 5.25% sodium hypochlorite and 17% ethylenediaminetetraacetic acid. Additional irrigation of the root canals of groups III, IV and V were done with the antioxidants 10% ascorbic acid, 10% tannic acid and 10% gallic acid respectively. The teeth in all the groups were obturated with Resilon and RealSeal SE. Longitudinal sectioning of the samples were done after 24 hours. This was followed by dehydration in alcohol. The specimens were then gold sputtered and analysed by scanning electron microscopy. The tubular penetration of the sealer was evaluated by observing the maximum tubular penetration in micrometers at the cervical, middle and apical third of each specimen in every group. Statistical analysis was done using computer software SPSS (16.0) version. The data was expressed in it's mean and standard deviation.

Results and Observations

Group I (negative control) showed intermediate penetration of the sealer at cervical, middle and apical third. Group II (positive control) had the least penetration of the sealer at cervical, middle and apical third. All the groups irrigated with antioxidants showed good penetration of the sealer at the cervical, middle and apical

third. The group in which irrigation was done with gallic acid showed maximum penetration at all the three levels.

Conclusion

In the present study it is well demonstrated that application of antioxidants in sodium hypochlorite treated root canal dentin increased the dentinal tubular penetration of Resilon and RealSeal SE in the cervical, middle and apical third. The use of gallic acid as an antioxidant showed the maximum tubular penetration at all the three levels.

Clinical Significance

The adhesiveness of resin based obturating materials Resilon and RealSeal SE to root canal dentin is adversely affected by the irrigants like sodium hypochlorite as a result of residual oxygen. Antioxidants with its radical scavenging activity can reverse the effect of sodium hypochlorite on root dentin thus enabling better dentinal tubular penetration of the sealer and better seal of the root canal space.

Introduction

Successful endodontic treatment outcome depends on the proper diagnosis, access cavity preparation, complete chemo mechanical preparation, debridement and three dimensional hermetic seal of the root canal system from the crown to the root tip. Leakage via any portals of entry can have disastrous consequences.[1,2]

Since its introduction in 1848, gutta-percha has been the most commonly used root canal obturating material. Over the years, various studies have proved that gutta-percha along with a sealer can provide long term and favourable prognosis for a root canal treated teeth. However, just like two sides of a coin, gutta-percha has its pros and cons.[2] One of the main disadvantages of gutta-percha is the inability to adhere to the dentinal wall which can lead to microleakage and failure of the endodontic treatment. To overcome this drawback, a quest for a new material as an alternative to the gold standard of obturating materials, i.e. gutta-percha began.[3]

In the past decade root canal filling materials have used the strategy of adhesive dentistry just like adhesion to coronal dentin using both total etch and self etch principles.[4,5]

In 2004, a thermoplastic, biodegradable, synthetic polyester root canal obturating material called polycapronolactone was introduced in the market as Resilon which was to be used with its particular sealer namely Epiphany and was marketed by Pentron Clinical Technologies, Wallingford.[3] Resilon along with Epiphany (third generation methacrylate based root canal sealer) was said to create what is called as a "Monoblock effect", referring to a scenario where different materials and interfaces create a gap-free solid mass in the root canal space with the purpose and advantage of improving the seal and fracture resistance.[5]

Predictable results have been reported with non-bondable root canal sealers, but with adhesive dentistry gaining popularity, a root canal sealer which bonds to the core material as well as root canal dentin was a welcome choice. Thus we have the functionally analogous low viscosity methacrylate based root canal sealers. Four generations of methacrylate based root canal sealers are commercially available out of which three were developed in the past decade. The fourth generation of methacrylate based self-etch bondable root canal sealers (Epiphany SE, RealSeal SE, MetaSEAL) have the added advantage of reduced application steps and is operator friendly.[5,6] Thus in this study we have selected Resilon and Real Seal SE, a bondable root canal obturation core material and methacrylate based sealer.

Irrigation during chemomechanical preparation in endodontic treatment is just as essential as any other step.[7] But these irrigation solutions cause changes in physical, mechanical and chemical properties of dentin.[8] Sodium hypochlorite (0.5% to 6%), is an irreplaceable irrigant which not only eliminates microorganisms but also dissolves proteins and is relatively cheap with a reasonable shelf life.[9] 5.25% concentration of sodium hypochlorite has been proved to be the most effective in removing the endodontic biofilm.[10] One of the disadvantages of this irrigant is that it interferes with the polymerisation of bonding resin including root canal sealers because of residual oxygen left in the dentinal tubules after irrigation.[11]

Studies have proved that irrigation with sodium hypochlorite decreases the bond strength of resin obturating materials with root canal dentin and this decrease in bond strength can be reversed with the use of antioxidants such as 10% ascorbic acid which works on the principle of oxidation-reduction or redox reactions.[11-13]

According to research, the use of ascorbic acid or sodium salts of ascorbic acid can reverse the decreased bond strength and improve the marginal seal of resin-dentin interface and also increase the degree of polymerisation of the resin. Ascorbic acid has also been approved by the Food and Drugs Administration(FDA) as a food preservative. [14]

Tannic acid is also a known antioxidant with related dental research to prove it's ability to increase the mechanical properties of dentin and increase the resin–dentin bond strength, thus ensuring better bonding and marginal seal of the resin with dentin.[15]

Gallic acid is a naturally occurring phenolic acid found in grape seed, gallnuts, sumac, witch hazel, tea leaves, oak bark and other plants. It's radical scavenging activity is proved to be the highest among phenolic acids.[16] It is medically proven to have anti-inflammatory, antimutagenic, anticarcinogenic and antioxidant activity along with antifungal, antiviral and antibacterial properties.[17] Grape seed extract (GSE) has excellent redox potential on sodium hypochlorite treated dentin.[12] Gallic acid is one of the main components in GSE.[18]

Sodium hypochlorite remnants in the dentinal tubules in the form of residual oxygen hinders the bonding, polymerization and dentinal tubular penetration of Resilon as well as RealSeal SE. The use of antioxidants with their radical scavenging activity may undergo redox reaction which in turn may enhance the bonding, polymerization and dentinal tubular penetration. This could provide better seal of the root canal space.

Hence in this study three different antioxidants have been used to analyse whether they would affect the dentinal tubular penetration of Resilon and RealSeal SE on sodium hypochlorite treated root canal dentin by means of their antioxidant activity. The study hypothesis was that an antioxidant application on sodium hypochlorite treated root canal dentin enhanced the dentinal tubular penetration of Resilon and RealSeal SE.

Aims and objectives

AIM

1. To observe the effect of antioxidants 10% ascorbic acid, 10% tannic acid and 10% gallic acid on the dentinal tubular penetration of Resilon and RealSeal SE on sodium hypochlorite treated root canal dentin.

OBJECTIVES

1. To analyze which among the three antioxidants usage gives better depth of dentinal penetration of Resilon and RealSeal SE on sodium hypochlorite treated root canal dentin.
2. To compare the above with dentinal tubular penetration of Resilon and RealSeal SE on sodium hypochlorite and saline treated root canal dentin.

Review of literature

Türkün et al, 1997 [19] in an in vitro study re-established the fact that 5% sodium hypochlorite was more effective in dissolving necrotic tissue than 0.5% sodium hypochlorite and that calcium hydroxide was more effective in paste form than in solution. Pre-treatment with calcium hydroxide increased the efficacy of 0.5% sodium hypochlorite. Scanning electron microscope analysis of debris and smear layer showed that passive ultrasonic irrigation with 5% sodium hypochlorite cleaned root canals in the cervical, middle and apical third compared to 0.5 % sodium hypochlorite, which was effective only in the cervical third.

Clarkson et al, 1998 [9] has reviewed the chemical properties and production of commercial sodium hypochlorite. Production of domestic bleaches and infant sanitizer was compared in terms of cost and ease of use. Brief guidelines for clinical use, storage, handling and disposal are as follows : Always use freshly prepared solutions, use only demineralized water for dilution, store solutions in opaque glass, or coated polyethylene containers which are tightly sealed, use Luer-Lok plastic syringes to prevent dislodgement of the needle, other accidents. Do not inject forcibly, or allow needle to bind in canal, always use rubber dam during endodontic treatment and ensure that it maintains a tight seal against the tooth and gingiva. Discard syringes and unused solutions at the end of an appointment, flush drains with copious quantities of water.

Morris et al, 2001[20] in an in vitro study evaluated the effect of sodium hypochlorite and RC Prep on the bond strength of resin cement to root canal dentin.

The results led to the conclusion that sodium hypochlorite and RC Prep treated specimens showed a significantly reduced bond strength and this could be completely reversed by application of 10% ascorbic acid or 10% sodium ascorbate.

Zuo et al, 2002 [21] used simple and fast HPLC method using photodiode array detector to determine catechins, caffeine, gallic acid in green, Oolong, black, pu-erh teas. It was concluded that green teas contains a higher level of the antioxidants and fermentation greatly increased the amount of gallic acid in pu-erh and black teas.

Slutzky-Goldberg et al, 2004 [8] in an in vitro study designed to evaluate the micro hardness of dentin when irrigated with 2.5% and 6% sodium hypochlorite for 5, 10 or 20 minutes. The results showed that decrease in micro hardness was seen in specimens irrigated with 6% than 2.5% sodium hypochlorite and there was a marked decrease in the microhardness when irrigated for 20 minutes than those for 10 minutes.

Fiuza et al, 2004 [22] studied the antiproliferative and cytotoxic properties of polyphenolic acid derivatives, structurally related with the natural models caffeic and gallic acids, that were tested in human cervix adenocarcinoma cells (HeLa). The results of the study showed that a significant growth-inhibition was observed for some of these compounds, clearly dependent on their structural characteristics.

Vongphan et al, 2005 [13] in an in vitro study determined the microtensile bond strength of total etch adhesive system (Single bond) when treated with various irrigants including sodium hypochlorite which significantly reduced the bond strength

of the total etch adhesive system. The reversal of this effect was seen in the group treated with sodium ascorbate before application of the bonding system.

Karamaæ et al, 2005 [16] compared the radical scavenging activity of certain phenolic acids (*i.e.* gallic, salicylic, *p*-hydroxybenzoic, gentisic, protocatechuic, vanillic, syringic, *o*-, *m*-, *p*-coumaric, caffeic, ferulic, isoferulic, and sinapic acids) through "stable" free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and thus evaluated the above using classical assay. The results showed that gallic acid and genticic acid showed the highest radical scavenging activity compared to all the other phenolic acids .

Teixeira et al, 2005 [23] in an in vitro study to evaluate through scanning electron microscope the time taken to remove smear layer by 1% sodium hypochlorite and 15% EDTA for 1, 3, and 5 minutes in the cervical, middle and apical third. Results showed that the above irrigants were effective in removal of the smear layer from the root canal walls of straight canals.

Schwartz et al, 2006 [24] in a review article summarized how the principles of bonding and adhesive dentistry was applied to root canal obturating materials. He describes the unique and complex environment of the root canal system and the challenges faced by bonded obturating materials like glass ionomer cements, resin sealers, resilon and methacrylate based sealers. Since adhesive dentistry is fast gaining popularity it would be a matter of time before most of these hurdles are overcome.

Santos et al, 2006 [25] evaluated the effect of chemical irrigants on the bond strength of self etch adhesive to pulp chamber dentin. The results showed there was a clear decrease in the bond strength associated with sodium hypochlorite irrigation whereas chlorhexidiene irrigation showed no effects on the bond strength.

Kim et al, 2006 [26]in a study on the antiallergic effect of gallic acid states that the anti-oxidant, anti-inflammatory, anti-microbial, and radical scavenging activities effect of gallic acid (3,4,5-trihydroxybenzoic acid), a polyphenyl natural products from gallnut and green tea is already proved. The study provides evidence that gallic acid inhibits mast cell-derived inflammatory allergic reactions by blocking histamine release and pro-inflammatory cytokine expression. Thus the in vivo and in vitro anti-allergic effect of gallic acid could lead to it's therapeutic application in inflammatory allergic diseases.

Bouillaguet et al, 2007 [27] in an in-vitro study analysed that bonding of resin core and sealer to root canal dentin can be optimised using indirect dentin bonding procedures to compensate for polymerisation stresses and reported that this technique had greater pushout bond strength compared to EndoREZ and Epiphany used alone. New techniques to overcome the challenges faced by adhesive obturating materials would always be researched.

Wilkinson et al, 2007 [28]conducted a study to evaluate the fracture resistance of immature teeth root canals filled with Resilon, gutta-percha, a self-curing flowable composite resin (BisFil 2B), or a self-curing hybrid composite resin (BisFil II). The

teeth were prepared with LightSpeed LSX and the immature apices of all the teeth were filled with Mineral trioxide aggregate (MTA). The access opening was filled with BisFil II. According to the result it was concluded that hybrid composite resin (BisFil II) was the only material significantly more fracture resistant.

Paque et al, 2007 [29] stated in a study evaluating immediate and sixteen month seal of gutta-percha –AH Plus and Resilon-Epiphany and concluded that both obturating materials prevented fluid movement immediately after obturation but after sixteen months of water storage gutta-percha – AH Plus showed more resistance to fluid movement analysed by nonparametric tests .

Conner et al, 2007 [30] evaluated the clinical outcome of endodontic treatment obturated with Resilon in private practice and immediate postoperative radiographs were compared with one year follow up radiographs. It was concluded that treatment with Resilon were well within success rates when compared to obturation with the gold standard of obturating materials i.e. gutta-percha.

Cunha et al, 2007 [31] in an in-vitro study assessed the removal of obturation and reinstrumentation time of Resilon/RealSeal and gutta-percha/AHPlus. The obturation material was removed using chloroform and irrigated with 2.5% sodium hypochlorite and finally analysed via radiographs and SEM and concluded that Resilon/RealSeal was removed better than gutta-percha/AHPlus from the root canals.

Tanomaru-Filho et al , 2007 [32] in an in vitro study evaluated the thermo plasticity of conventional and thermoplastic gutta-percha and Resilon. According to

their research it was concluded that Resilon had good thermoplastic behaviour and in comparable with gutta-percha, endorsing its use as a thermoplastic root filling material.

Gulsahi et al , 2007 [33] in an in vitro analysis of the voids in obturating with gutta-percha, Resilon with Epiphany sealer. Horizontal sections of the specimens were obtained at every 1mm, till 5mm from the apex. Digital photographs that were taken proved that there was not much difference in the sealer void area in both Resilon and gutta-percha obturations.

Siqueira Jr et al, 2007 [34] conducted a clinical study to assess the microbial efficacy of 2.5% sodium hypochlorite as irrigant calcium hydroxide as intracanal medicament. It was concluded that in all the treatment regimens used i.e. irrigation with 2.5% sodium hypochlorite and after seven day calcium hydroxide medicament bacteria and their toxins were reduced and there were no resistant species.

Schafer et al, 2007 [35] in a review article about action and interactions of root canal irrigants and different irrigation protocols that could be used clinically. Example 2-5 ml. of sodium hypochlorite between instruments, 5-10 ml. of sodium hypochlorite and 5ml. of EDTA for a minute after cleaning and shaping. The use of at least two irrigating solutions has been advocated. NaOCl dissolves organic components of dentin, pulpal remnants. 17% EDTA can remove the smear layer effectively. Combination of the above i.e. 5.25% NaOCl and 17% EDTA is recommended.

Economides et al, 2008 [36]assessed the cytotoxicity of Resilon and two commercially available gutta-percha cones (Roeko, Dentsply). Two well established cell lines L929 and RPC-C2A were used for this study. According to this research it was inferred that Resilon showed maximum cytotoxicity followed by Roeko gutta-percha and Dentsply gutta-percha. The cytotoxicity of Resilon increased from 24 hours to forty eight hours in both cell lines. The explanation to this could be that Resilon compared to gutta-percha has resin content . Resilon being biodegradeable is susceptible to alkaline and enzymatic hydrolysis thus the cytotoxic changes.

Pasqualini et al, 2008 [2] in an in vitro study compared the microbial leakage of Resilon vs gutta-percha against *Enterococcus faecalis* using a microleakage model and a new sequence detection assay One Cut Event Amplification (OCEAN^{TM}). DNA from the specimens were extracted, amplified by Polymerase Chain Reaction and identified by OCEAN. The conclusion drawn was that gutta-percha provided a better seal compared to Resilon.

Mohammadi et al , 2008 [11] in a review about sodium hypochlorite talks about the history of chlorine releasing agents, mechanism of action, its efficacy as antibacterial, antifungal agent, biofilm removal quoting both in vivo and in vitro studies.It's tissue solubility, decontamination of operating field, effect on instruments. Sodium hypochlorite may effect the microhardness of dentin by degradation of it's organic components. Bonding to dentin is also adversely affected. It has a good hemostatic property. It's limitations are that it is toxic, damage clothing, eyes, has adverse effects when pushed beyond the foramen (hypochlorite accident) and allergic

reactions. But despite its limitations there is no replacement for sodium hypochlorite as an irrigant in endodontics.

Garcia et al, 2009 [37] in an in-vitro study comparing the quality of apical obturation of polycapronolactone based Resilon- Epiphany and gutta-percha–sealer 26 using scanning electron microscope and reported that both the systems had a similar behaviour of apical obturation. Since apical seal is vital for successful endodontic therapy it is vital for any obturating material to attain maximum seal.

De-Deus et al, 2009 [38] compared the bond strength of Epiphany and EpiphanySE to root canal dentin. The conclusions drawn from the study proved that AHplus and gutta-percha showed higher bond strength than both Epiphany and EpiphanySE but there was no significant between the methacrylate based sealers. The root canal anatomy and other reasons greatly reduce the adhesiveness of Epiphany sealers to root dentin.

Bedran-Russo et al, 2009 [15] evaluated the effects of tannic acid on dentin matrix mainly the modulus of elasticity and enzymatic degradation. Hence the effect on pre treatment with tannic acid on resin-dentin bond strength of two bonding sysems. Results showed that 10% and 20% tannic acid treatment inhibited the effect of collagenase digestion on dentin matrix . The tannic acid-dentin matrix complex improved bond strength for both adhesive systems. Emphasising the fact that tannic acid treated dentin can reverse the effect of oxidising agents on dentin.

Kim et al, 2010 [5] in a review summarised the four generations of methacrylate based resin sealers that are commercially available. The first generation Hydron (Hydron Technologies, Inc, Pompano Beach, Florida) appeared in the mid-1970s, when dentin bonding were at their initial stage of development. The use of poly-2-hydroxyethyl methacrylate (poly HEMA) as the main constituent made the sealer very hydrophilic. The second generation example is EndoREZ. It is nonetching, hydrophilic methacrylate based resin sealer which does not require the use of a dentin adhesive. EndoREZ (Ultradent Products Inc, South Jordan, UT) is a dual cured radiopaque sealer that might be used in the wet environment of the root canal system and is effective in penetrating dentinal tubules and adapting closely to the canal walls. It can be used with gutta-percha or specific EndoREZ points. FibreFill R.C.S. (Pentron Clinical Technologies, Wallingford, Connecticut) is a third generation methacrylate resin–based sealer aimed for filling root canals with fiber reinforced obturators attached to thermoplastic root filling material tip. Other third generation methacrylate resin–based sealers introduced with Resilon incorporates the use of self-etching primers. They are commercially available as Epiphany (Pentron Clinical Technologies), RealSeal (SybronEndo, Orange, CA), Resinate (Obtura Spartan Corp, Fenton, MO), and Smart (Discus Dental, Culver City, CA) systems. The self-etching primers were further reduced from a 2-bottle system to a single-bottle system (self etch primer and adhesive) . The fourth generation methacrylate resin–based sealers (eg, Meta-SEAL, Parkell Inc; RealSeal SE, SybronEndo) functionally analogous to a similar class of self-adhesive resin luting cements where the separate etching/ bonding step was eliminated. Acidic resin monomers originally present in dentin adhesive

primers were incorporated into the resin-based sealer to render them self-adhesive to dentin substrates. The combination of an etchant, a primer, and a sealer into an all-in-one selfetching, self-adhesive sealer is advantageous in that it reduces the application time as well as errors that might occur during the procedure .

Ardana et al, 2010 [39] in a review article emphasises the need for a proper seal of the root canal space during obturation. Earlier dentin bonding agents along with the gutta-percha cones and resin sealers were used for proper adhesion to dentin. With the introduction of self etch materials with higher pH values than the etch and rinse used in adhesive systems in which the self etching layers smear layer is incorporated into the bonding layers i.e hybrid layer there is better adhesion to the root canal dentin, decrease in clinical steps and ease of operator use. But more clinical studies are neede in this regard.

Shokouhinejad et al, 2010 [40] evaluated the push out bond strength(BS) of obturating systems Resilon/Epiphany self-etch (SE) and guttapercha/ AH26 after different irrigation protocols in an in –vitro study. The results showed that irrigation with 5.25% sodium hypochlorite and EDTA and obturation with guttapercha/ AH26 showed higher values of BS compared to Resilon/Epiphany self-etch(SE) . The BS of Resilon/EpiphanySE did not show any significant difference when irrigation protocol was 5.25% sodium hypochlorite + EDTA or 1.3% sodium hypochlorite + MTAD. The decrease in BS of the resin based obturating system could be because of the effect of chemical irrigants such as sodium hypochlorite which could adversely affect it.

Shokouhinejad et al , 2010 [41] in an ex vivo study comparing the sealing ability of Resilon/ new EpiphanySE sealer to that of gutta-percha/AH-Plus following smear layer removal with EDTA or mixture of tetracycline acid and detergent (MTAD) demonstrated that the resin obturating system is as effective in preventing saliva leakage as gutta-percha/AH-Plus and that MTAD did not adversely effect the sealing ability of Resilon/EpiphanySE.

Shrestha et al, 2010 [4] in a review article on Resilon and the methacrylate based obturating system a replacement to the gutta-percha- sealer obturating system by quoting various in vivo and in vitro studies states that the sealing ability of Resilon- Epiphany system depends on the ability of the resin to penetrate the hybrid layer and incomplete penetration would allow nanoleakage to occur which would cause failure of the resin- dentin interface. Bond strength/ root strengthening of this new material is still a controversy and more research is needed to substantiate Resilons effectiveness as an ideal obturating material.

Pameijer et al 2010 [42] in a article on resin materials for root canal obturation discusses about the oxygen inhibited layer formed during irrigation with sodium hypochlorite and the drawbacks of this layer specially concerning the polymerization of resin sealers. Thus care should be taken to use ethelenediamintetraacetic acid as the final rinse before obturation.

Haapasalo et al, 2010 [43] in a review on endodontic irrigants summarises the goals of irrigation, ideal endodontic irrigants, and details on various endodontic

irrigating solutions their chemistry, method of action, use, interactions, advantages and disadvantages. About sodium hypochlorite commonly used concentrations are 0.5%-6%. It effectively dissolves collagen, pulpal remnants and organic content of dentin. Although it does not remove the smear layer it aids its removal by dissolving the organic components of the smear layer and then EDTA or citric acid can be used. Sodium hypochlorite has a pH of 11. It's antibacterial efficacy and ability to remove biofilm has been proved and quoting various studies it was stated that higher concentrations of sodium hypochlorite was more effective than lower concentrations. Regarding limitations it has an unpleasant taste and smell, toxicity, cannot remove smear layer by itself. Poorer in vivo performance as compared to in vitro could be attributed to complexities of the root canal system. Long term exposure to sodium hypochlorite adversely effects the dentin elasticity and flexural strength.

Retamozo et al, 2010 [44] conducted an in vitro study to determine the concentration and time required for sodium hypochlorite irrigation to eliminate *Enterococcus faecalis*. According to this study, the most effective regimen was 5.25% sodium hypochlorite for forty minutes to remove *E. Faecalis* contaminated within the root canal dentin.

Kandaswamy et al, 2010 [10] in a review on endodontic irrigants, on sodium hypochlorite (NaOCl) apart from the chemistry, setting reaction microbial efficacy, pH, temperature, biofilm the article also reviews the mechanical properties stating through various referral studies that NaOCl does not significantly corrode nickel-titanium instruments. On bond strength studies have proved that resin sealers and

cements bond strength is decreased with irrigation with NaOCl and can be reversed with ascorbic acid or sodium ascorbate. EDTA a chelating agent helps to dissolve the smear layer and greater smear layer removal is seen with irrigation 17% EDTA for one minute.

Garcia et al, 2010 [45] in an in vitro study assessed the antioxidant activity of several substances believed to revert the problems caused by bleaching procedures by 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical assay and concluded that 10% ascorbic acid solution, 10% ascorbic acid gel, 10% sodium ascorbate solution, 10% sodium ascorbate gel and Vitamin E presented the highest antioxidant activity among substances tested in this study.

Chia et al, 2010 [46] in a study on *Toona sinensis* (*TS*) extracts has reported that it has various effects on cultured cell lines, including anti-proliferative activity in cancer cells. Gallic acid (3,4,5-trihydroxybenzoic acid) is one of the major bioactive compounds purified from *TS* extract is the responsible for the anti neoplastic activity of *TS* leaf.

Shashidhar et al, 2011 [47] in an in vitro study evaluated microbial leakage to *streptococcus mutans* in the roots filled with Resilon and gutta-percha using two filling techniques and inferred from the results that Resilon with Epiphany sealer in lateral as well as vertical condensation is superior to gutta-percha.

Nagas et al, 2011 [48] investigated the effect of different light emitting diodes (LED) photopolymerization modes on the bond strength of methacylate based sealer

RealSeal with gutta-percha and Resilon. Time and intensity of exposure was the standard (20 seconds of maximum intensity) and exponential (5 seconds of exponential power increase, followed by15 seconds of maximum intensity). The test samples were stored in 100% humidity at 37 ˚ C for a week. Push out bond strength was tested and the fracture mode was observed under a scanning electron microscope. Results showed that RealSeal and gutta-percha showed greater bond strength compared to Real Seal and Resilon. It was concluded that exponential photopolymerization modes had no advantage over standard regimen in terms of bond strength.

Shenoy et al , 2011 [3] in a review article on the Resilon-Epiphany obturating system focuses on the "monoblock concept" and its advantages over traditional gutta-percha . This resin based obturating system provides both coronal and apical seal comparable to gutta-percha . The weak link of this obturating systm is mainly the sealer-dentin interface and further research is needed in this area to overcome the disadvantages.

Tanomaru-Filho et al, 2011 [49] compared the effectiveness of gutta-percha and Resilon in filling lateral canals using Obtura II system. Artificial teeth were used in this study and lateral canals were made in the root canal in the cervical , middle and apical third according to standard protocol. The canals were obturated with Obtura gutta-percha, Endo-points flow gutta-percha, or Resilon by using the Obtura II system. The specimens were analysed by digitised radiographs Image Tool software.

Results showed that Resilon and EndoFlow gutta-percha were were effective in filling lateral canals using Obtura II .

Jaju et al , 2011 [50] in a review article on endodontic irrigants and newer irrigants has concluded that there is no replacement for sodium hypochlorite as a root canal irrigant in Endodontics till date. Despite the limitations of sodium hypochlorite the newer irrigants can be used as an adjunct to sodium hypochlorite not a replacement.

Manimaran et al, 2011 [12] assessed the application of proanthocyanidin agent to increase the decreased bond strength of root dentin treated with sodium hypochlorite. Naturally occurring antioxidants like grape seeds contain oligomeric proanthocyanidin complexes (OPC). Proanthocyanidins (PAs) have free radical scavenging activity, which is 20 times more potent than sodium ascorbate They also have antibacterial, antiviral, anti-carcinogenic, anti-inflammatory, anti-allergic, and vasodilatory actions. The results of the study led to the conclusion that sodium hypochlorite decreased adhesiveness of any resin ro root canal dentin . This decreased bond strength can be reversed with application of antioxidants and 5% PA significantly had more effect than the 10% sodium ascorbate.

V.U. Borde et al, 2011 [17] in a study to demonstrate the presence of gallic acid in Ayurvedic herbs through screening by the silica gel thin layer chromatograph quantitatively analyzed by ferric reducing antioxidant power (FRAP) assay. Amla 27.36mg/g ranking first followed by Triphala 18.24mg/g. The study suggested the presence of gallic acid in some of the Ayurvedic herbs and formulations.

Reblova et al, 2012 [51] in a study on the effect of temperature on the antioxidant activity of phenolic acids (gallic, gentisic, protocatechuic, syringic, vanillic, ferulic, caffeic, and sinapic; 0.5 mmol/kg). The results showed that gallic, gentisic, protocatechuic, and caffeic acids showed a significant antioxidant activity at 150°C and vanillic acid was active only at 90°C.

Cecchin et al, 2012 [52] compared the bond strength of Resilon-Epiphany and guttapercha with Sealer 26 and gutta-percha-Endo Fill in an in-vitro study and came to the conclusion that the bond strength of Resilon-Epiphany and guttapercha with Sealer 26 were similar but that of gutta-percha-Endo Fill was lesser.

Demiryürek et al, 2012 [53] in an in vitro study determined the microbial contamination of gutta-percha and Resilon cones. It was inferred that both gutta-percha and Resilon cones are contaminated once the seal of the box is opened for clinical use thus it is safer to decontaminate the cones before clinical use by the operator.

Rodrigues et al, 2012 [54] in an study comparing the cytotoxicity of various sealers namely RealSeal, Apexit Plus, Guttaflow, AH Plus after setting and accessing them at 24 hours, 7th day and 14th day and also compared the cytotoxicity of these sealers at different time intervals i.e. 24 hours, 7th day and 14th day. They concluded that according to increasing order of cytotoxicity the results were as follows Guttaflow <AH Plus <Apexit Plus <Realseal. The cytotoxicity of all the sealers showed maximum toxicity at 24 hours after which the toxicity decreased as the sealer set.

Vandekar et al, 2012 [55] in an in-vitro study compared the dentinal tubular penetration of sealers RealSeal and Tubliseal by using confocal microscopy and Rhodamine B dye and inferred from the results that RealSeal had better mean penetration within the dentinal tubules compared to Tubliseal. The difference in the penetration could be attributed to the fact that RealSeal – Resilon bonds to the root canal dentin unlike Tubliseal and gutta-percha which is cemented to the dentinal walls.

Zamin et al, 2012 [56] assessed the fracture resistance of root canal treated teeth, prepared with different tapers no preparation, #30/.08, #30/.10 and #70/.12. The root canals were then obturated as unfilled, Endofill/gutta-percha, AH Plus/gutta-percha, EpiphanySE/Resilon . The results showed that there was no significant difference in the fracture resistance with different filling materials. Increase in taper or increase in cervical preparations did decrease the fracture resistance of endodontically treated teeth.

Rahimi et al, 2012 [57] evaluated the application of a total-etch/separate adhesive layer to enhance the bond of a Urethane dimethacrylate based sealer to dentin in an in vitro study. Various methods have been advocated to enhance the bond strength of resin based sealers to root dentin and it was concluded from the study that application of a total-etch/ separate adhesive layer enhanced bond.

Chadha et al, 2012 [58] in an in vitro study compared the dentinal tubular penetration of three resin based root canal sealers namely EndoREZ , Epiphany and AH Plus. Scanning electron microscope images showed that the highest tubular penetration was by EndoREZ followed by Epiphany and the least penetration by AH Plus sealer.

Said et al, 2012 [59] assessed the sealing ability of sealer when placed with different techniques in an in vitro study. Teeth were obturated with cold lateral compaction technique using gutta-percha and the sealer used was AH26. The sealer was placed with rotary lentulo spiral, manual lentulo spiral and master gutta-percha coating. There was no significant difference in the microleakage values but sealer placement by master gutta-percha coating technique has ease of use when compared to others.

Amaravani et al, 2012 [60] in a study evaluated the COX-2(cyclooxygenase) structural analysis and docking studies with gallic acid structural analogues. COX-1 and COX-2 are two distinct isoforms of cyclooxygenase, which play an important role in the conversion of arachidonic acid to prostaglandins . Prostaglandins (PGs) are involved in various pathophysiological processes like inflammatory responses, carcinogenesis and cardiovascular events. *Emblica officinalis* is an ayurvedic herbal plant. Gallic acid (GA) isolated from this plant has the highest inhibitory effect against cyclooxygenase-2 (COX-2). The study proves that gallic acid and it's structural analogues have this effect.

Marion et al, 2012 [61] in a literature review on the ideal concentration of sodium hypochorite used as an endodontic irrigant stated that higher the concentration of sodium hypochlorite used, the greater the toxicity and irritation of periapical tissues but lower concentrations like 0.5% and 1 % needs more time to dissolve tissue and organic debris.

Hegde et al, 2012 [62] in an in vitro study on the amount of sodium thiosulphate that was required to neutralize the effect of various concentrations of sodium hypochlorite demonstrated that 1.4 ml, 2.4ml, and 3.5ml of 5% sodium thiosulphate is required to neutralize 2%, 3% and 5% of sodium hypochlorite respectively.

Glassman et al, 2013 [7] in a review on endodontic irrigants and irrigant delivery systems emphasises on the need of irrigation for proper decontamination of the root canal system. Irrigants such as sodium hypochlorite the best in removing endodontic biofilms and can be coupled with other irrigating solutions such as EDTA. But precautions like the use of rubber dam should be taken while using the above. Irrigant delivery systems from manual agitation of the irrigants to machine assisted agitation such as sonics and ultrasonics, as well as newer systems such as the EndoVac (SybronEndo), which delivers apical negative-pressure irrigation, plastic rotary F File (Plastic Endo),Vibringe (Vibringe), the Rinsendo (Air Techniques), and the EndoActivator (DENTSPLY Tulsa Dental Specialties) have all been discussed.

Thakur et al, 2013 [6] in an overview on resin based root canal sealers emphasises the advantages of bondable root canal filling material, including monoblock concept, superior sealing ability , newer self etching technology thus reducing the multiple steps associated with bonded materials makingthe material popular in the twenty first century. The properties of methacrylate based root canal sealers and their limitations in clinical practice have been discussed specially regarding it being technique sensitivity and long term follow up after retreatment.

Mahdi et al, 2013 [63] investigated in an in-vitro study the bond strength and seal of AHPlus/ guttapercha , EndoREZ and RealSeal systems to human root canal dentin and ultimately proved that Push out bond strength values and sealing ability of all three root canal sealers were not significantly different. Thus again emphasising the fact that the Resilon is comparable gutta-percha in terms of bond strength

Patil et al, 2013 [64] in an in–vitro study compared the push out bond strength of three obturating systems namely gutta-percha/AH Plus, Resilon/Epiphany self-etch (SE) and EndoREZ obturation system to root canal dentin and the results showed Gutta-percha/AH Plus with the highest bond strength. They concluded that the bond strength of newer bondable obturating materials were compromised due to the unique environment of the root canal system.

Al-Katani et al, 2013 [65] in a review article focuses on carrier based obturating materials Thermafil and Resilon (Real Seal 1) and concluded on the basis of various studies that both did not provide excellent apical seal and research needs to be done in this area to overcome this disadvantage.

Lotfi et al, 2013 [66] has reviewed Resilon a thermoplastic synthetic polymer-based root canal filling material composed of a parent polymer, polycaprolactone or Tone, a biode-gradable aliphatic polyester, with filler particles consisting of bioactive glass, bismuth oxychloride and barium sulfate. Resilon polymeric matrix consists of 25-40% polycaprolactone(PCL) and 3-10% di-methacrylates**.** It's physical, mechanical, thermal properties reviewed has established Resilon as a material which

can be used as an alternative to gutta-percha. One of the mentioned disadvantages of Resilon is the reduced bond strength(BS) to root canal dentin compared to gutta-percha and that sodium hypochlorite when used as a final irrigant decreased the BS, degree of conversion of the resin sealer.

Bhandi et al, 2013 [67] comparatively evaluated in an in-vitro study the sealing ability of three root canal obturating materials Guttaflow, Thermafil and Resilon using silver nitrate dye and observed the samples under a stereomicroscope. Results demonstrated that the sealing ability was maximum in Resilon followed by Thermafil and finally GuttaFlow.

Mozayeni et al, 2013 [68] compared the apical microleakage with three different obturating techniques Thermafil/Adseal, lateral condensation with gutta percha/Adseal and lateral condensation with Resilon/Epiphany using dye penetration method. They concluded from the results that Resilon/Epiphany showed the most, Thermafil/Adseal ranked second , gutta percha/Adseal showed the least leakage.

Tanomaru-Filho et al, 2013 [69] did a study to evaluate the filling of artificially created lateral canals by Resilon and gutta-percha using thermomechanical compaction . The teeth were analysed by digitised radiographs the authors concluded that Resilon compared to gutta-percha had better effectiveness as a filling material specially in filling lateral canals in the apical third.

Gupta et al, 2013 [70] evaluated the effect of precipitate formed with the interaction of endodontic irrigants sodium hypochlorite and chlorhexidiene on the

bond strength of gutta-percha/AHPlus and Resilon/Epiphany. The push out bond strength was assessed and it was concluded that precipitate formation does effect the bond strength. Gutta-percha/AHPlus showed greater push out bond strength than Resilon/epiphany.

Shrestha et al, 2013 [14] in an vitro study assessed the effect of sodium ascobate on the degree of conversion and bond strength of RealSeal SE to root canal dentin . The teeth were bulk filled with RealSeal SE and analyzed by Micro-Raman Spectroscopy and universal testing machine for degree of conversion(DC) and bond strength(BS) respectively. Results showed that teeth treated with sodium hypochlorite and then filled with RealSeal SE had the least DC and BS and the group treated with 10% sodium ascorbate just before obturation showed maximum values of DC and BS. Thus the authors concluded that the decreased DC and BS of RealSeal SE because of sodium hypochlorite irrigation can be reversed by 10 % sodium ascorbate.

Liu et al , 2013 [18] in an study suggested that gallic acid the main component in grape seed extract can prevent Alzheimer's, Parkinson's and Huntington's diseases which are characterised by the accumulation of protein aggregates in an amyloid fibrillar form. Among the various constituents of grape seed extract, gallic acid was the most active component and helped inhibit j-CN fibril formation, by stabilizing j-CN to prevent its aggregation. Furthermore gallic acid significantly reduced the toxicity of j-CN to pheochromocytoma cells. Therefore it could be concluded that gallic acid effectively inhibited fibril formation by the amyloid-beta peptide, the putative causative agent in Alzheimer's disease.

Afzal et al, 2013[71] in an in vitro study establishes once again that 5.25% sodium hypochlorite is the most effective root canal irrigant in eliminating E. faecalis biofilm and despite its drawbacks and side effects an alternative to this irrigating solution is yet to be found.

Gunalol et al, 2013[72] evaluated the effect of 10% sodium ascorbate on dentin bonding after being treated with various oxygen releasing root canal irrigants, 5.25% sodium hypochlorite and 10% hydrogen peroxide. Results showed that 10% sodium ascorbate increased the bond strength of dentin with adhesive system.

Kumar et al, 2014 [73] in an in vitro study which assessed the push-out bond strength of Resilon – Ephiphany obturated teeth with final rinse as NaOCl (5.25%), chlorhexidine CHX (2%), EDTA solution (17%), and BioPure MTAD solutions before obturation concluded that the irrigants did not effect the bond strength of the Resilon – Epiphany system.

Vikram M et al, 2014 [74] assessed the voids present in root canal after obturating with gutta-percha with sealers Epiphany, AHplus and Guttaflow and Resilon with Epiphany . Results showed that Epiphany sealer with both gutta-percha and Resilon showed least voids leading to the conclusion that the methacrylate based sealers and adhesive sealers have better prospects in obturation.

Khoroushi et al , 2014 [75] in an in vitro study evaluated the effect of three antioxidants namely, 10% rosmarinic acid (RA, Baridge essence), 10% hesperidin (HPN, Sigma), and 10% sodium ascorbate hydrogel on sodium hypochlorite treated

root dentin cemented with a self-adhesive resin cement (Bifix SE, Voco Gmbh). Pull out bond strength was evaluated and the result showed that the group treated with sodium ascorbate after NaOCl treatment showed the highest values.

Chakraborthy et al, 2014[76] reviewed studies to elucidate the fact whether ascorbic acid is an antioxidant or a pro-oxidant. After reviewing ten articles the authors concluded that ascorbic acid has both these activities i.e. antioxidant and pro-oxidant properties by a plethora of mechanisms.

Materials and methods

MATERIALS USED IN THE STUDY:

a) 5.25% NaOCl - Novo Dental Products Pvt. Ltd. Mumbai, India.

b) 17% EDTA – Desmear. Anabond Stedman Pharma Research Ltd.Tamil Nadu.

c) Saline – Nirlife, Nirma Ltd. Gujarat, India.

d) Double distilled water – Nice Chemical Laboratory Supplies, Ltd. India.

e) Ascorbic acid – HiMedia. Mumbai, India.

f) Tannic acid – Sigma-Aldrich. Bangalore, India.

g) Gallic acid - Sigma-Aldrich. Bangalore , India.

h) Resilon – SybronEndo Corporation . Orange, CA, USA.

i) RealSeal SE – SybronEndo Corporation. Orange, CA, USA.

j) Sticky wax – Dental Product of India (DPI). Mumbai, India

k) Paper points – Dentsply Maillefer, Switzerland.

l) Absolute Ethanol – Chanshu Yangyuan Chemical, China.

EQUIPMENTS/ INSTRUMENTS USED IN THE STUDY:

a) Micromotor handpiece – Contra Angle Hand piece.NSK Korea

b) Micromotor handpiece – Straight Hand piece.NSK Korea.

c) K Files – Mani Inc. Tochigi, Japan.

d) Spreader - Mani Inc. Tochigi, Japan.

e) 28 gauge two side vented irrigation needle – RC Twents, Prime Dental Products Pvt. Ltd. Mumbai.

f) X smart endo rotary system – Dentsply Maillefer, Switzerland.

g) Rotary files – HERO Shaper - Micro Mega. Cedex FRANCE

h) Lentulo spiral - Mani Inc. Tochigi, Japan.

i) Composite curing light – Dentsply, Milford Detroit USA.

j) Electronic weighing balance – Infra Instruments Pvt. Ltd. Chennai, India

k) Diamond disc – SS White, USA

l) Chisel and mallet

m) Gold sputtering machine – No. E-1010 Ion sputter, Hitachi. Japan

n) Scanning electron microscope - No. S-2400, Scanning electron microscope Hitachi, Japan.

METHODOLOGY

Tooth preparation

Fifty single rooted mandibular premolars freshly extracted for orthodontic purpose were disinfected in 0.5% chloramine and stored in saline and used within three months of extraction. They were decoronated using a diamond disc (SS White) to attain a 14 mm. root length . The teeth were then randomly divided into five groups of ten teeth each. The apex of the teeth were sealed with sticky wax to prevent extrusion of irrigants. In all teeth root canal treatment was initiated with patency instrument no. 10 K file (Mani). Working length was established 1mm short of the apex and confirmed with radiographs. Crown down technique of instrumentation was done, using HERO Shaper (Micro Mega) Ni-Ti rotary instruments. The canals were enlarged till 30 size 6% taper.

Irrigation protocol for groups

Group I (negative control) - Irrigation with saline (Nirlife) and 17% ethylenediaminetetraacetic acid (Desmear). For groups II (positive control), III, IV and V the irrigation protocol followed was 5.25% sodium hypochlorite (Novo Dental products), 5ml. between each change of instrument and 5ml of 17% ethylenediaminetetraacetic acid for one minute after biomechanical preparation. Final irrigation and flushing of the canals was done using distilled water.

Antioxidant preparation

All three antioxidants were freshly prepared before application. 10% ascorbic acid (Himedia), 10% tannic acid (Sigma-Aldrich), 10% gallic acid (Sigma-Aldrich) were prepared by mixing 10 grams of powder of the respective antioxidants in 100 ml. distilled water.

Antioxidant application

Canals of Groups III, IV and V were further irrigated with 10% ascorbic acid, 10% tannic acid and 10% gallic acid respectively for ten minutes.

Group I – Irrigation with saline +17% EDTA

Group II – Irrigation with 5.25% NaOCl + 17% EDTA

Group III - Irrigation with 5.25% NaOCl + 17% EDTA+ 10% ascorbic acid

Group IV - Irrigation with 5.25% NaOCl + 17% EDTA+ 10% tannic acid

Group V - Irrigation with 5.25% NaOCl + 17% EDTA+ 10% gallic acid

Obturation with Resilon(SybronEndo) and sealer RealSeal SE(SybronEndo)

Following irrigation the canals of the specimens were dried with paper points (Dentsply). Resilon cones 30 size, 6% taper, were used to obturate the canals 1mm. short of the apex. RealSeal SE was automixed and applied to root canal with a lentulo spiral (Mani). The Resilon cones were coated with the sealer and placed into the canals. Lateral condensation was done with 2% taper, 25 size Resilon cones using 25 size spreader (Mani). Vertical heat condensation was done with a hot instrument and curing was done with a LED light (Dentsply), (intensity 500mW/cm²) for forty seconds. Radiographs were taken to assess the quality of obturation. The canal orifices were sealed with sticky wax. All the specimens were stored in 100% relative humidity at 37°C for 24 hours to ensure complete set of the root canal sealer.

Scanning electron microscope analysis

Samples were then sectioned in the bucco-lingual direction with the help of a diamond disc (SS White), in a slow speed hand piece under copious irrigation with distilled water in such a way that one portion retained the obturating material. The portion which retained the obturation material was chosen for examination under a scanning electron microscope (Hitachi). The tooth sections were soaked in 17% ethylenediaminetetraacetic acid solution for 10 minutes, followed by soaking in 5.25% sodium hypochlorite solution for 10 minutes, and then washed thoroughly with distilled water. The sections were dehydrated by placing them sequentially in 50%, 75% and 100% ethyl alcohol for a total of eight hours and left overnight in a drying chamber maintained at 60ºC. They were gold sputtered for SEM evaluation at the cervical, middle and apical third.

Evaluation of sealer penetration

Samples were evaluated for dentinal tubule penetration of sealers at three levels - cervical, middle and apical. Photographs showing the maximum penetration at each level were taken. The maximum tubular penetration at each level was recorded in micrometers (μm). At each level, five points were marked corresponding to the maximum sealer penetration. The distance of the points was measured from the sealer/Resilon - dentin interface and the depth of penetration was calculated. A mean of the five readings was taken at each level and tabulated.

Statistical analysis

Data entry was done in Microsoft Excel. The values obtained were tabulated and statistically analyzed using computer software SPSS (16.0) version. The data was expressed in it's mean and standard deviation.

One way ANOVA was applied for statistical analysis. Post hoc followed by Dunnett's test was used to find the statistical significance between the groups. P value less than 0.05 ($P<0.05$) was considered statistical significant at 95% confidence interval.

Results & Observations

RESULTS

Table 1 – shows the maximum dentinal tubular penetration of Resilon and RealSeal SE in micrometers in group I, in the cervical, middle and apical region of the specimens at five points in each region. The irrigants used were saline and 17% EDTA.

Table 2 - shows the maximum dentinal tubular penetration of Resilon and RealSeal SE in micrometers in group II, in the cervical, middle and apical region of the specimens at five points in each region. The irrigants used were 5.25% sodium hypochlorite and 17% EDTA.

Table 3 - shows the maximum dentinal tubular penetration of Resilon and RealSeal SE in micrometers in group III, in the cervical, middle and apical region of the specimens at five points in each region. The irrigants used were 5.25% sodium hypochlorite, 17% EDTA and 10 % ascorbic acid.

Table 4 - shows the maximum dentinal tubular penetration of Resilon and RealSeal SE in micrometers in group IV in the cervical, middle and apical region of the specimens at five points in each region. The irrigants used were 5.25% sodium hypochlorite, 17% EDTA and 10 % tannic acid.

Table 5 - shows the maximum dentinal tubular penetration of Resilon and RealSeal SE in micrometers in group V, in the cervical, middle and apical region of the specimens at five points in each region. The irrigants used were 5.25% sodium hypochlorite, 17% EDTA and 10 % gallic acid.

Table 6 – shows the mean and standard deviation values of the dentinal tubular penetration in micrometers of five different groups in the cervical, middle and apical regions.

Cervical level dentinal tubular penetration mean values of group I was observed as 466.66±9.18, group II was 370.96±1.37, group III value was 601.69±1.14, group IV was 617.67±1.66 and group V was observed as 671.62±1.63.

Middle level dentinal tubular penetration mean values of group I was 292.60±8.65, group II was 223.55±1.35, group III was 414.32±1.19, group IV was 503.22±1.81 and group V was 542.51±6.42.

Apical level dentinal tubular penetration mean values of group I was observed as 108.09±7.61, group II was 62.14±8.24, group III was 324.21±8.92, group IV was 325.80±1.57 and group V was observed as 378.29±2.03.

According to the above values group V, i.e. 5.25% NaOCl, 17% EDTA and 10% gallic acid irrigated specimens show the maximum dentinal tubular penetration at cervical, middle and apical regions and group II, i.e. 5.25% sodium hypochlorite and 17% EDTA irrigated specimen without the use of an antioxidant show least penetration at all three levels. Among the antioxidants, 10% gallic acid irrigated specimens show maximum penetration at all the three levels, followed by 10% tannic acid and 10% ascorbic acid irrigated teeth.

Table 7 – shows the significance of each group with respect to the P values. P value less than 0.05 ($P<0.05$) was considered statistical significant at 95% confidence interval.

In group I where the irrigants used were saline and 17% EDTA, P value is significant compared to other groups. In group II where the irrigants used were 5.25% NaOCl and 17% EDTA, even though mean penetration at all three levels is least compared to all the groups, P value is significant. In group III where irrigants used were 5.25% NaOCl , 17% EDTA and 10% ascorbic acid , P value is significant compared to all the groups except group IV. In group IV where the irrigants used were 5.25% NaOCl , 17% EDTA and 10% tannic acid, the P value at cervical, middle and apical third is significant compared to all the groups except group III. In group V, where the irrigants used were 5.25% NaOCl , 17% EDTA and 10% gallic acid, the recorded readings showed the maximum dentinal tubular penetration at all three levels compared to the other groups and where the P value is significant compared to all the other groups.

OBSERVATIONS

Dentinal tubular penetration of Resilon and RealSeal SE – in the cervical third [Fig. 7(a), Fig. 8(a), Fig. 9(a), Fig. 10(a), Fig. 11(a)]

Maximum penetration at cervical third was seen in group V specimens irrigated with 5.25% NaOCl, 17% EDTA and 10% gallic acid and minimum penetration was seen in Group II where the specimens were irrigated with 5.25% NaOCl and 17% EDTA. Among the antioxidants gallic acid (groupV) irrigated specimens showed maximum penetration followed by 10% tannic acid(group IV) and 10% ascorbic acid (group III) which showed similar mean penetrations at cervical region.

Dentinal tubular penetration of Resilon and RealSeal SE – in the middle third [Fig. 7(b), Fig. 8(b), Fig. 9(b), Fig. 10(b), Fig. 11(b)]

Maximum penetration at middle third was seen in group V specimens irrigated with 5.25% NaOCl, 17% EDTA and 10% gallic acid and minimum penetration was seen in group II where the specimens were irrigated with 5.25% NaOCl and 17% EDTA. Among the antioxidants irrigated groups, group V i.e. 10% gallic acid treated specimens showed maximum penetration followed by Group IV (10% tannic acid) and Group III (10% ascorbic acid.)

Dentinal tubular penetration of Resilon and RealSeal SE – in apical third [Fig. 7(c), Fig. 8(c), Fig. 9(c), Fig. 10(c), Fig. 11(c)]

Maximum penetration at apical third was seen in group V specimens irrigated with 5.25% NaOCl, 17% EDTA and 10 % gallic acid and minimum penetration was seen in group II where the specimens were irrigated with 5.25% NaOCl and 17% EDTA. Among the antioxidants, 10% gallic acid treated specimens i.e. group V showed maximum tubular penetration whereas group IV (10% tannic acid) and group III (10% ascorbic acid) showed almost similar mean values for dentinal tubular penetration at the apical third.

Discussion

The teeth specimens treated with antioxidants 10% ascorbic acid, 10% tannic acid and 10% gallic acid showed deeper tubular penetration of the sealer at all three levels, i.e. cervical, middle and apical levels of the root canal dentin. This confirmed the study hypothesis that an antioxidant application on sodium hypochlorite treated root canal dentin can enhance dentinal tubular penetration of Resilon and RealSeal SE.

Obturation of the root canal space in order to obtain a hermetic seal is of prime importance. [2] The word "hermetic seal" literally means airtight seal. But in reality such a condition cannot be achieved as there are always micro channels through which leakage can occur.[80]

In 1891 , Miller's " Theory of Focal Infection" describes how bacteria roving through the blood stream can cause generalised or localised infection. Thus in the 19th and 20th centuries dentists and physicians advocated the extraction of pulpless and endodontically treated teeth. In 1965 Kakehashi et al proved that bacteria was the root cause of endodontic disease and therefore advocated that the root canal should be sterilized by eliminating vital and necrosed pulp tissue, bacteria and their toxins. This could be done by thorough instrumentation, irrigation, intracanal medications and finally obturation. Obturation aims at eliminating leakage pathways, entombing any remnant bacteria and sterilizing the canals. Different obturating materials have been used over the years which include solids, semisolids and pastes. An ideal obturating material should seal the root canal apically, coronally and laterally. It should have minimum shrinkage and be nonirritating to periradicular tissues. It should be easily introduced into the canal and be easily retrievable. It should be radiopaque, sterile, antimicrobial, impervious to moisture and should not discolour the tooth. Since 1848

guttapercha has been used along with a sealer like zinc oxide, calcium hydroxide, glass ionomer, resin based sealers to obturate the root canal and has encountered immense success as an obturating material. It mainly consists of 20% gutta-percha, 66% zinc oxide, 11% barium sulphate, 3% resin or wax .[65] The draw back of gutta-percha was that it did not provide a hermetic seal as it did not adhere to the root canal dentin wall[2,3]. Thus a quest for a new obturating material began.[3]

In 2004 Resilon, a resinous thermoplastic root filling material based on a biodegradable synthetic polyester called Polycaprolactone was marketed and was to be used with a resin based dual cure sealer called Epiphany (Pentron Clinical Technologies, Wallingford Connecticut, USA) .[3,65]

There are four generations of methacrylate based sealers and Epiphany, RealSeal (SybronEndo), Resinate (Obtura Spartan Corp) and Smart (Discus Dental) belong to the third generation. In these sealers, self-etching primers are further reduced from a two-bottle system to a single-bottle system. These self-etching primers/adhesives mostly contain 2-acrylamido-2-methyl-propanesulfonic acid (AMPS) as the functional acidic monomer. They are similar to the so-called one-component type all-in-one adhesives. By combining self-etching adhesives and methacrylate resin–based sealers with Resilon, the manufacturers introduced the fourth generation of methacrylate based root canal sealers example Meta-SEAL, Parkell Inc; RealSeal SE, SybronEndo. It is an all-in-one selfetching, self-adhesive sealer and it's advantageous in that it reduces the application time as well as errors that might occur during each bonding step.[5]

The composition of Resilon include polymeric matrix which consists of 25-40% polycaprolactone (PCL) and 3-10% di-methacrylates, booactive glass,

radiopaque fillers (65% of the matrix) of bismuth oxychloride and barium sulphate.[3,66] Resilon was initially introduced with third generation methacrylate sealer Epiphany, which bonds to both the root dentin and Resilon cones to form a single unit, termed a "monoblock" . These have been developed on the foundation of dentin adhesive technology borrowed from restorative dentistry .[4]

In this study RealSeal SE was chosen as the sealer of choice which was used to obturate the tooth specimens along with Resilon cones. Meta-SEAL, (Parkell Inc.) and RealSeal SE (SybronEndo) are fourth generation methacrylate resin–based sealers. These are functionally comparable to a similar group of recently introduced self-adhesive resin luting composites which have the advantage of reduced steps of application due to the elimination of the etching and bonding steps. Initially it was the dentin adhesive primers that contained the acid monomers which were now incorporated into the resin based sealer/composite to render them self-adhesive to the root canal dentin. Reduction in the number of application steps as well as procedure time thus minimising the potential for error are it's advantages. The addition of 4-META i.e. 4 - methacryloyloxyethyl trimellitate anhydride makes this fourth generation based methacrylate sealer self-etching, hydrophilic, and promotes monomer diffusion into the underlying dentin tubules to produce a hybrid layer after polymerization. This also bonds to the resin root filling material as well as root canal dentin via creation of hybrid layers.[5]

Root canal dentin has a unique structure and is comprised of 70% mineral, 20% organic components and 10% water by weight . It constitutes the maximum volume of the tooth structure and has a complex three dimensional framework composed of dentinal tubules that extend from the pulp to the dentino-enamel

junction, intertubular and peritubular dentin. This particular structure of dentin forms the basis of restorative dentistry especially adhesive dentistry.[15] The main inonorganic component consists of calcium hydroxyapatite, $Ca_{10}(PO_4)_6OH_2$ and the organic component consists of type I collagen and some minor type V collagen.[81]

As the major component of dentin, type I collagen plays an important role in the mechanical properties of dentin including viscoelasticity, forming a rigid and strong space-filling biomaterial.[15] The complex root canal anatomy, the dentinal tubules which harbour various microorganisms and their toxins, the smear layer formed during instrumentation are all obstacles for cleaning and shaping during endodontic treatment. This makes irrigation all the more important.[76]

A single irrigant cannot successfully fulfil all the requirements needed during the process of irrigation during root canal treatment . A minimum of two irrigation solutions are required and a specific sequence needs to be followed to optimize the effect of irrigation. Conventional irrigants used during instrumentation are sodium hypochlorite, EDTA, citric acid, chlorhexidine digluconate etc. These are used in various concentrations for proper effective cleaning and disinfection of the root canal system .[43]In the study 5.25% sodium hypochlorite and 17% EDTA have been used as routine root canal irrigants.

Sodium hypochlorite has been used as an endodontic irrigant since 1920. It is an oxidising as well as a hydrolyzing agent and has bactericidal, virucidal as well as proteolytic properties and has a reasonable shelf life.[9] Root canal irrigation is done with sodium hypochlorite with concentrations ranging from 0.5% to 6 %. It is an ideal irrigant to remove the organic matter in the pulp chamber including vital as well as

necrosed pulp and is successful in removing the endodontic biofilm especially *Enterococcus faecalis.* [43]

The mechanism of action of sodium hypochlorite is as follows:

$$NaOCl + H_2O \leftrightarrow NaOH + HOCl \leftrightarrow Na^+ + OH^- + H^+ + OCl^-$$

Sodium hypochlorite works as an organic and fat solvent, degrading fatty acids and transforming them into fatty acid salts (soap) and glycerol (alcohol) and thus reduces the surface tension of the solution (saponification reaction). Next is the amino acid neutralization reaction in which sodium hypochlorite neutralizes amino acids forming water and salt and there is the removal of hydroxyl ions causing a decrease in the pH. The amino acid neutralization reaction also occurs when hypochlorous acid ($HOCl^-$) present in sodium hypochlorite solution contacts organic tissue and acts as a solvent releasing chlorine, which in turn combines with the protein of the amino group to form chloramines. The hypochlorous acid and hypochlorite ions (OCl^-) cause amino acid degradation and hydrolysis. The chloramination reaction between chlorine and the amino group (NH) leads to the formation of chloramines that interfere in cell metabolism. Chlorine is a strong oxidizing agent that has an antimicrobial action, inhibiting bacterial enzymes and toxins leading to an irreversible oxidation of sulphydryl group (SH groups) of essential bacterial enzymes. Thus, the saponification, amino acid neutralization, and chloramination reactions that occur in the presence of microorganisms and organic tissue lead to the antimicrobial effect and tissue dissolution process. [9-11]

According to various research, 5.25% of sodium hypochlorite is the ideal concentration in eliminating the *E. faecalis* endodontic biofilm. Despite the various

irrigants in the market today, there is yet an alternative to be found for this root canal irrigant .[71]

During root canal instrumentation, dentin chips along with organic debris and irrigating solution forms a smear layer which primarily consists of two layers 1) 1–2 μm of thick layer of organic matter and dentin particles; 2) extending into dentinal tubules to a depth of 40 μm (smear plugs) which is formed largely of dentine chips. [23]

Neutral ethylenediaminetetraaceticacid (EDTA) solutions in concentrations ranging from 15–17% , are effective in demineralizing dentin and can be used to remove the smear layer but cannot remove organic debris when used as a sole irrigant and when used in combination with sodium hypochlorite effectively removes organic as well the inorganic debris. [23]

EDTA reacts with the calcium ions in dentin to form soluble calcium chelates. Studies have reported that EDTA can demineralize dentin upto a depth of 20-30 μm in five minutes.[10] Irrigation with 17% EDTA enhances the permeability of dentin enabling deeper dentinal tubular penetration of root canal irrigants.[7]

Therefore a combination of 5.25% NaOCl and 17 % EDTA was the choice of irrigants used in this study which would enable the deeper penetration of the obturating material with the removal of the smear layer.

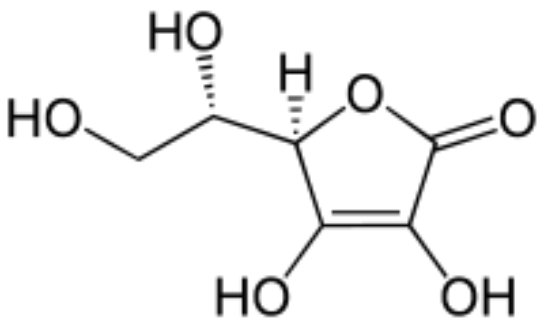
Sodium hypochlorite also has it's demerits when used as a root canal irrigant. Hypochlorite solutions are said to adversely affect the mechanical properties of dentin due to dissolution of the dentinal organic components. NaOCl can cause a concentration-dependent reduction in the elastic modulus and flexural strength in human root canal dentin. This could altogether reduce the carbon and nitrogen content

of the dentin matrix . Furthermore dissolution of dentinal collagen causes degradation of dentin. Bonding as well as polymerization of any kind of resin dental material is considerably reduced as a result of the residual NaOCl left in the dentinal tubules after irrigation which generates residual oxygen.[11]

The use of 5.25% NaOCl and 17% EDTA as irrigants resulted in the removal of the organic as well as inorganic components of the dentin and caused collapse of the dentinal tubules [Fig. 8 (a),(b) and (c)].

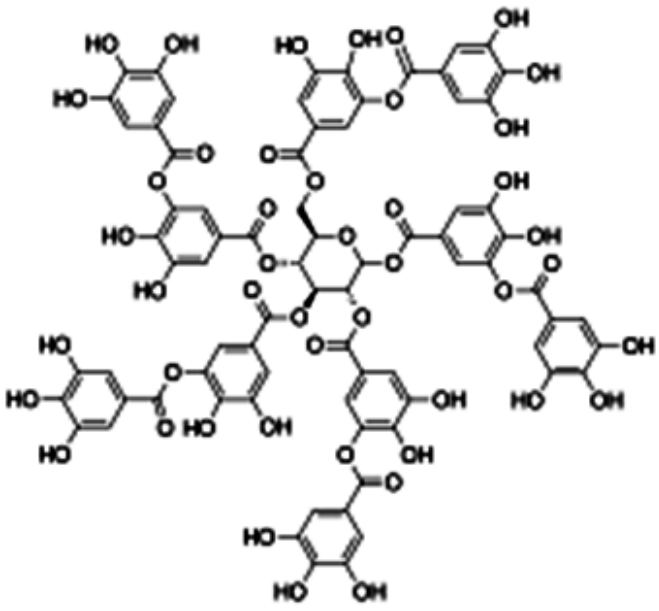
Dental literature has recorded various ways to counter the adverse effect of sodium hypochlorite on root dentin especially with respect to the bond strength as well as polymerisation of resin.[75] Studies have reported that a hybrid layer is formed by resin tags and root canal dentinal tubules. Degeneration of this layer or defective or incomplete hybrid layer could result in the failure of the adhesive – dentin bond. Disintegration of the hybrid layer is due to the resultant hydrolytic degradation of both the resin and collagen fibrils, thus exposing some collagen fibrils, to enzymatic degradation of the host's dentin matrix metalloproteinase (MMPs). The dentin MMPs are activated by the acidic nature of present adhesive technology.

Ascorbic acid is a naturally produced water soluble organic compound.[76] It has a acidic pH of 1.8 and it is a potent antioxidant capable of quenching reactive free radicals in biological systems.[12,45]



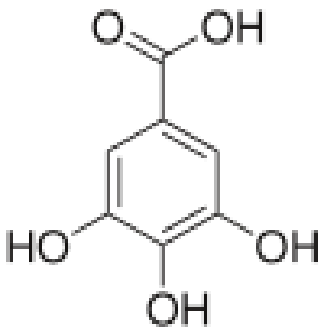
The use of 10% ascorbic acid ((5*R*)-[(1*S*)-1,2-Dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)-one) or sodium salt of ascorbic acid can reverse the compromised bond strength of sodium hypochlorite treated root canal dentin.[20] Sodium hypochlorite is a strong oxidising agent that leaves behind a thick layer of oxygen free radicals in the dentinal tubules after it's use as an endodontic irrigant. It removes the organic matrix and dentin debris to leave behind the inorganic components of dentin which results in reduced bond strength with any resin material (resin cement, resin obturating material).[12,24] The bond strength as well as the polymerization of resin based materials is also retarded probably due to premature chain termination and thus incomplete polymerization. Vitamin C and its salts like sodium ascorbate can neutralize the adverse effects of oxidative solutions via redox reactions. These are non-toxic and used in food industry, and their use on dentin does not cause any adverse biological effects .[14]

Tannic acid is a polyphenol and a weak acid. Is used as an astringent, desensitizing agent, surface treatment for removal of smear layer.



According to Bedran-Russo et al, it significantly effects the mechanical properties of dentin by increasing the modulus of elasticity of demineralised dentin and has a positive effect on the dentin-adhesive interface. Tannic acid is a naturally occurring cross linking agent comprising of a complex mixture of polygalloylglucose esters. Medical science has even reported it's use in increasing the mechanical properties of collagen film in the aortic wall, causing structural and dynamic changes in collagen-tannic acid interactions in tannic acid stabilized pericardial tissue assessed by infra-red spectroscopy. Hydrogen bonds which are formed by the interaction of amide NH groups from collagen and hydroxyl groups from tannic acid causes changes in the dentin matrix which would be similar to other type I collagen-based tissues; therefore, the formation of hydrogen bonds by tannic acid is the reason for the changes in the dentin modulus of elasticity. Tannic acid also has the capacity to inhibit the activity of collagenase enzyme and also increase the bond strength of resin-dentin interface .[15,18]

Gallic acid (3,4,5-trihydroxybenzoic acid) is a naturally occurring phenolic acid found in green tea, red wine , grape seed gallnuts, sumac, witch hazel, tea leaves, oak bark, and other plants. Salts and esters of gallic acid are termed 'gallates'. This phenolic acid has proved it's worth as an antioxidant, antimicrobial, anti-cancer, antiinflammatory and various other uses in medical science. [18,26]



It has a primary antioxidant activity and also displays a wide variety of biological functions like anticarcinogenic potential, antimicrobial activity, cholesterol reduction, protection from cardiovascular disease, decreases hypertension, inhibits amyloid fibril formation and has anti-inflammatory and antiallergic effect. [18,21, 22,26, 46] Gallic acid and its esters are hydroxybenzoic derivatives which are used as antioxidant additives in both food and pharmaceutical industry i.e. E-310 (propyl gallate) and E-311 (octyl gallate). These are known to protect against oxidative damage induced by reactive oxygen species (ROS). [22] Numerous plants and herbs are a rich source of gallic acid either in free form or as a part of tannin molecule. It is present in tea, red wine, fruits, beverages and various medicinal plants. Pharmacologically it is of much importance as it is a known matrix metalloproteinase inhibitor.[17] According to Reblova et al, the antioxidant potential of gallic acid is significantly present at temperatures as high as 150˚C. [51] Karamac et al evaluated the antioxidant activity of phenolic acids including gallic acid using classical assay and found that gallic acid along with gentisic acid, among the fourteen phenolic acids tested showed the highest antiradical property or redox potential.[16] Moreover gallic acid is approved by the FDA and is used in the food preservation industry.

In this in vitro study, fifty single rooted mandibular premolars freshly extracted for orthodontic purposes were chosen. Teeth with cracks, caries, restorations, resorptive defects and open apices were avoided. [40]

The teeth were disinfected by immersion in a 0.5% chloramine solution and were then washed under running water to eliminate traces of the chloramine solution and stored in saline till use.[37,41,58] The teeth were then decoronated with a diamond disk (SS White) in a slow speed handpiece (NSK) under copious water irrigation to

attain a 14 mm. length from the apex. This was done to standardize the root length. Then they were randomly divided into five groups of ten teeth each. The working length was established one mm. short of the apex and confirmed by using radiographs. No. 10 K file (Mani) was used as the initial patency instrument. The apex was sealed with sticky wax to prevent extrusion of irrigants.[58] Each canal was instrumented with 6% tapered Hero-Shaper Nickel-Titanium rotary file number 30 up to D13 (1.08 mm in diameter).[14]

The protocol followed for irrigation was as follows. Group I (negative control) in which irrigation was done with saline and 17% ethylenediaminetetraacetic acid. For Groups II (positive control), III, IV and V, the irrigation protocol followed was 5.25% sodium hypochlorite, 5ml. between each change of instrument and 5ml of 17% ethylenediaminetetraacetic acid for one minute after biomechanical preparation. Finally distilled water was used to flush the canals of all the specimens. The irrigation protocol followed for group II, III, IV and V were according to protocol by Schafer et al, 2007. [35] EDTA was used as a final rinse as recommended by the manufacturer for obturation with Resilon, followed by irrigation with distilled water.[58]

10% ascorbic acid, 10% tannic acid and 10% gallic acid were freshly prepared by mixing 10gm. of the respective powders in 100 ml. of distilled water.[12] The powder was weighed in an electronic weighing balance and then dissolved in 100ml. of distilled water. In studies conducted by Manimaran et al and Shreshta et al, 10% ascorbic acid and 10% sodium ascorbate irrigation for ten minutes have been used to counter the effects of sodium hypochlorite on root canal dentin, thereby improving polymerization as well as bonding of resin.[12,14] Bedran-Russo et al in an in vitro study has also demonstrated that 10% tannic acid irrigation for ten minutes can improve the

elastic modulus and bond strength of deminerlized dentin.[15] No studies on gallic acid as an antioxidant irrigant for root canal treatment have been reported. Therefore 10% of the three antioxidants were used to irrigate the root canals of the respective groups for a time period of ten minutes.

The canals were then finally irrigated with 10 ml. of distilled water and dried with paper points to avoid any moisture contamination which may hinder the polymerization and bonding of Resilon and RealSeal SE. [12,14,]

Manufacturer's instructions were followed for the manipulation of RealSeal SE. Lateral compaction technique was applied for the obturation of samples using 6%, 30 size Resilon points as the master cone, followed by filling of any remaining space with accessory Resilon cones(2% taper, 25 size) by lateral condensation with 25 size spreader. Each cone was coated with the sealer before insertion. Vertical compaction was done with a hot instrument followed by curing with LED light (Dentsply) with intensity 500mW/cm² for 40 seconds as recommended by the manufacturer. Radiographs were taken to assess the obturation quality. The canal orifices of all the tooth specimens were sealed with sticky wax. All samples were stored in 100% relative humidity at 37°C for 24 hours to ensure complete set of the root canal sealers.[58]

After twenty four hours, longitudinal sections of all the tooth samples were made in the bucco-lingual direction with a diamond disc under copious irrigation to evaluate tubular penetration of Resilon and RealSealSE. Studies have reported that maximum tubular penetration is seen in the bucco-lingual direction, hence the longitudinal sections were obtained so that one half contained the obturating material. These were then soaked in 17% EDTA and 5.25% sodium hypochlorite for ten

minutes alternatively to remove the smear layer formed during sectioning and then they were washed thoroughly with distilled water.[58]

A scanning electron microscope study was chosen as it allows a highly descriptive and detailed observation of the dentinal tubules and the obturating material, and the penetration depth could be calculated with great accuracy throughout the section. Moreover it does not require any dye incorporation to assess the penetration depth. [58]

The teeth were dehydrated by placing them sequentially in 50%, 75% and 100% ethanol for a total of eight hours and left over night in a drying chamber. The specimens were then gold sputtered for SEM evaluation.[76]

Photomicrographs of each specimen in each of the five groups were taken at three levels - cervical, middle and apical. At each level, five points were marked corresponding to the maximum tubular penetration. The distance in micrometers (μm) was calculated from the dentin-obturating material interface to the point marked. A mean of the readings was calculated and tabulated. [58]

Data entry was done in Microsoft Excel. The values obtained were tabulated and statistically analyzed using computer software SPSS (16.0) version. The data was expressed in it's mean and standard deviation. One way ANOVA was applied for statistical analysis. Post hoc followed by Dunnett's test was used to find the statistical significance between the groups. P value less than 0.05 ($P<0.05$) was considered statistical significant at 95% confidence interval.

In this study three antioxidants 10% ascorbic acid, 10% tannic acid and 10% gallic acid were used to irrigate the tooth specimens in groups III, IV and V

respectively and their effect on the dentinal tubular penetration of resin based obturating materials Resilon and RealSeal SE on sodium hypochlorite treated root dentin was assessed. The SEM images and tabulated results of the mean penetration of the antioxidant irrigated groups compared to the groups not treated with antioxidants before obturation showed that antioxidant irrigated groups III, IV and V demonstrate greater tubular penetration of the obturating material compared to group I [figures 7(a),(b),(c)] where saline and 17% EDTA was used as an irrigant. The least penetration was seen in group II [figures 8(a),(b),(c)] where 5.25% NaOCl and 17% EDTA solutions were used as irrigating solutions without any antioxidant application at all three levels cervical, middle and apical third. Antioxidants with their radical scavenging activity have the ability to reverse the effect of NaOCl on root dentin leading to better sealer penetration into the tubules.[11]

Among the three antioxidant irrigated groups, group V specimens irrigated with 10% gallic acid[figures 11(a),(b),(c)] displayed maximum dentinal tubular penetration at all three levels compared to groups III specimens irrigated with 10% ascorbic acid [figures 9(a),(b),(c)] and group IV specimens irrigated with 10% tannic acid [figures 10(a),(b),(c)] . The re-dox potential of phenolic acids depends on the number of hydroxyl moieties that are attached to the aromatic ring of the benzoic acid molecule. Gallic acid with three hydroxyl groups was observed to be the most active phenolic acid and showed the highest radical scavenging activity among a group of phenolic acids. [16]

The 10% ascorbic acid(group III) and 10% tannic acid (group IV) irrigated specimens did not show significant difference in the SEM images and showed similar penetration depths(P value > 0.05). Morris et al (2001) has reported how 10%

ascorbic acid and 10% sodium ascorbate can reverse the action of 5% sodium hypochlorite and RC prep on the resin–dentin bond strength.[20] da Cunha et al (2010) has also proved the reversal of bond strength . Shrestha et al (2013) has demonstrated that not only bond strength but polymerization of resin increased with the use of 10% sodium ascorbate after sodium hypochlorite irrigation.[14] Vongphan et al (2005) in a study demonstrated how micro tensile bond strength of root dentin is affected by various irrigants and sodium hypochlorite reduces bond strength which can be reversed by using sodium ascorbate.[13] Gonulol et al (2013) in a study evaluated the effect of 10% sodium salt of ascorbic acid on different oxygen releasing irrgants like sodium hypochlorite and hydrogen peroxide on root dentin and results demonstrated that sodium ascorbate increased the bond strength to dentin after the use of an oxidising irrigant. [72]

Bedran Russo et al (2009) has justified how tannic acid can improve the mechanical properties (elastic modulus) of demineralised dentin as well as bond strength of resin-dentin interface.[15] Both these antioxidants have the ability to undo the damage caused by NaOCl when it comes to bonding and adhesive endodontics.

The present study demonstrates that gallic acid has a significant effect on the effect of sodium hypochlorite on root dentin when compared to ascorbic acid and tannic acid by expressing its redox potential and enhancing dentinal tubular penetration of resin obturating core material Resilon and fourth generation methacrylate based sealer RealSeal SE .

Since the results of this study has showed that irrigation with 10% gallic acid can improve tubular penetration of Resilon and RealSeal SE, this could be used as an antioxidant irrigant which could improve the bonding between resin obturating

material and root canal dentin, despite the use of sodium hypochlorite as an irrigant and it's negative effect on bonding, polymerization and sealing of the root canal space. However this being an in vitro study it cannot absolutely mimic in vivo conditions of the oral cavity. A number of factors affect the success of endodontic treatment especially when based on the technique sensitive adhesive procedures. Secondary tests and clinical trials are the ultimate in deciding the outcome of in vitro studies. Hence further clinical trials are necessary to substantiate the results and findings.