“A COMPARATIVE EVALUATION OF INTRA-RADICULAR SMEAR REMOVAL EFFICACY OF CHITOSAN, 17% EDTA AND 10% CITRIC ACID USED AS FINAL RINSE IN IRRIGATION PROTOCOLS – A FIELD EMISSION SCANNING ELECTRON MICROSCOPIC STUDY

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CERTIFICATE

This is to certify that **DR.M. PRAVEEN,** *post graduate student (2013-2016) from the Department Of Conservative Dentistry and Endodontics, J.K.K.Nataraja Dental College, Komarapalayam, Namakkal District–638183,Tamilnadu has done the dissertation titled* **"A COMPARATIVE EVALUATION OF INTRA-RADICULAR SMEAR REMOVAL EFFICACY OF CHITOSAN, 17% EDTA AND 10% CITRIC ACID USED AS FINAL RINSE IN IRRIGATION PROTOCOLS – A FIELD EMISSION SCANNING ELECTRON MICROSCOPIC STUDY"** *under my direct guidance and supervision in the partial fulfillment of the regulations laid down by* THE TAMIL NADU DR. M.G.R MEDICAL UNIVERSITY, CHENNAI, FOR M.D.S BRANCH – IV CONSERVATIVE DENTISTRY AND ENDODONTICS DEGREE EXAMINATION. It has not been submitted (partial or full) for the award of any other degree or diploma**.**

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CONTENTS

The successful outcome of endodontic therapy depends primarily on efficient mechanical preparation, debridement, cleansing and shaping procedures of the root canal system. The complex anatomical structure of the root canal space imposes limitations during bio-mechanical preparation. Effective removal of vital and necrotic pulp tissue, microbial organisms, their by-products, the smear and debris created during the instrumentation procedures is a pre-requsite for successful endodontic treatment outcomes. During canal preparation smear layer tends to be formed on the surface of the root canal. Smear layer removal can be fairly easy in the coronal and the middle one third whereas in the apical one third of the root canal it is relatively difficult.

Peters O.A., et al in 2001[49] observed that though instrumentation of canals increased the volume and surface area of the prepared canals, the use of nickel titanium files in all the instrumentation techniques left 35% or more of the canal surfaces unchanged. The irrigants and their usage sequence play an important role in debriding these areas. The copious use of irrigants helps to eliminate debris, smear and microbes from within the confines of the root canal space.

The curvature of the roots in the mandibular and maxillary posterior teeth pose a challenge with regard to the preparation of the canal space, the irrigant delivery and replacement in the apical one third of the root. A review of literature highlights the limited effectiveness of irrigating solution in the apical one-third of the root canal regardless of the instrumentation and irrigation techniques.

Smear layer is a thin, amorphous layer, consisting of both organic and inorganic substance that covers the prepared canal walls. **Eick et al in 1970**[21] first reported the layer of smear as being made up of particles of sizes ranging from less than 0.5 to 1.5μm, and encompasses a thin layer of grinding debris to achieve a

overall thickness of 2-5μm. It also extends a few micrometers into the dentinal tubules **(Brännström.M and Johnson.G et al in 1974)[9].** The layer of smear on the instrumented root canal surfaces was first described by **Mc Comb and Smith in 1975[44],** suggested that they consisted not only of dentin but also the fragments of odontoblastic processes, pulpal tissue remnants, microorganisms and their byproducts.

The layer of smear was described as consisting of two components, the superficial layer and a deeper second layer that was packed into the dentinal tubules for a depth of upto 40 micrometers **(Mader et al in 1984)[40]** . Smear layer occludes the orifices of the dentinal tubules within the canal system. It interferes with the adaptation of the obturating materials to the prepared root canal, prevents the penetration of irrigant solutions and intra-canal medicaments into dentinal tubules and ramifications of the root canal space. The root canal is a complex structure where the occlusal one third is highly accessible, middle one third is fairly accessible and the apical one third being the least accessible. The layer of smear thus formed is easily removed in the occlusal and the middle one third whereas in the apical one third it is relatively more difficult and even more so in curved canals. The authors concluded that the tubular packing phenomenon was primarily as a result of the cutting action of hand and rotary instrumention procedures, which can possibly force the components of the smear layer deeper into the tubular structure of dentin for varying distances and form smear plugs.

Aktener et al 1989[2] proposed the capillary action hypothesis which explains the phenomenon of penetration of smear into tubules upto depths of 110μm when using surface active agents within the canal during canal preparation**. Lussi et al in**

1993[39] found that canal preparations without the formation of smear layer may be possible by using a non-instrumental hydrodynamic technique.

Various studies have pointed out the presence of intraradicular microorganisms as a crucial factor in influencing the treatment outcomes during endodontic procedures. Microorganisms can not only remain viable but also multiply in the smear layer and subsequently penetrate into the tubular structure of dentin. Micorganisms in dentinal tubules have been reported as far as halfway through the root dentin of infected teeth (**Shovelton DS 1964)**[62] **.**

The presence of a smear layer can also prevent the penetration of intracanal medicaments into the dentinal tubules. They also interfere with the sealer adaptation to radicular dentin. Evaluation of the effect of smear layer on the apical and coronal seal suggest that smear layer being a loosely adherent structure should be completely removed from the surface of the root canal as it can harbour microorganisms and cause microleakage. The ability of sealer to penetrate into dentinal tubules is also enhanced by removal of the layer of smear **(White et al 1987)**[76]**. Safavi et al in 1990**[59] have reported that maintaining the smear layer may block the dentinal tubules by altering dentinal permeability. **Diamond & Carrel in 1984**[19] proposed that the smear layer acts as a barrier to bacterial metabolites preventing the bacterial invasion of the dentinal tubules**.** There has been an enormous amount of debate and discussion on whether to retain or remove the smear layer before root canal obturation. A mid pathway of modifying the smear in such a way that it becomes resistant to dissolution and disintegration resulting in blocking of the dentinal tubules has been visualised **(A.P. Tikku et al in 2011)** [70] .

Various irrigants have been used alone or in combination and various methodologies have been suggested for smear layer removal from within the

confines root canal space. Chemicals, Sonic technologies, Ultrasonics, and LASERS have been used either individually or in combination with appropriate canal preparation techniques to achieve this objective (**Violich D R et al 2010)**[73] **.** Efficient use of irrigant delivery and agitation methodologies are mandatory for successful outcome of endodontic procedures **(Gu et al in 2009)**[28]**.** Certain adjunctive therapies like the ozone delivery technique, photo sensitization technique and high electrical impulse technique aim to improve the elimination of viable microorganisms, smear and debris from the root canal space **(Pong-Yin Ng-B in 2004)** [51] .

Irrigant delivery systems have also evolved over the years. **Howard et al in 2011**[32] observed that the irrigant volume, type of delivery, the method of agitation and the depth of delivery are important parameters of which the depth and volume have been shown to be important for removal of smear, debris and microbes than the method used**.**

Chelating agents have played a important role as final rinses in irrigation protocols. Ethylenediaminetetra-acetic acid (EDTA) a chelating agent has been used for negotiating difficult and curved canals. More recently it has been used in gel form as a canal lubricant during Ni-Ti rotary instrumentation of the root canals as a protection against instrument separation. EDTA as an irrigating solution has been shown to effectively remove the smear layer when used as a final rinse solution **(Violich D R & Chandler N.P in 2010)** [73]**.** A number of other chelating agents like citric acid have been used for removal of smear as a final rinse solution. They have also been modified to improve the tubule penetration and anti-microbial properties of the irrigant solution.

The tetracycline antimicrobials which include tetracycline hydrochloride, minocycline and doxycycline also act as calcium chelators and cause demineralization of root dentin **(Bjorvatn et al in 1982)**[8]. Doxycycline hydrochloride was first used in a concentration of 100mg per millilitre and was effective in removing the layer of smear on intra-radicular dentin **(Barkhordar R.A in 1997)**[6]. Various irrigants based on tetracycline with the aim of effectively combating both the smear, debris and microorganisms from the confines of the canal space have been formulated. Tetracyclines have the property of substantivity which helps molecules to readily attach to dentin and are released without losing their antibacterial activity over a period of time. This creates a reservoir of an anti-microbial agent which subsequently is released from the radicular dentinal surface in a slow and sustained manner. Tetracycline based root canal formulations like Biopure MTAD and Tetraclean have been recommended for use as final rinse irrigant solution during bio-mechanical preparation of the root canal space.

Chitosan is a natural polysacchride molecule derived from the shells of crustaceans mainly shrimp, crabs and other sea crustaceans. It is found in abundance in nature. The molecule has got good bio-compatibility, bio-degradability, bio-adhesion and low toxicity. It has been tried for a variety of potential pharmacological applications such as anti-bacterial, anti-cancerous, anti-coagulant, haemostatic, immuno-stimulant, anti-oxidant, anti-viral and ani-inflammatory agents (**Gavahne Y.N et al in 2013)**[25]. Chitosan shows excellent chelation capacity for different metallic ions due to its acidic pH and has been used in different sectors for recovery of metals. This is commercially obtained by a process of de-acetylation of chitin. It has been evaluated in dentistry for improving dentin bonding as hydrogels, stimulating the cells of the apical dental papilla, for retrival of calcium hydroxide

medicament from within the canal space, as a root canal irrigant, incorporated into filling materials for modifiying properties, in tooth pastes and chewing gum, wound healing, inactivation of bacterial endotoxins and bone regeneration and repair **(Roymond K.A., et al in 2015)** [56].

This study aims to compare the smear layer removal ability of various available forms of the chelating agent chitosan, 10% citric acid and 17% EDTA when used in specific irrigant protocols.

Baumgartner et al in 1984[5] in his in-vitro study using a scanning electron microscope plus rank-ordered scoring system evaluated statistically the amount of superficial debris and the smeared layer that remained on the canal wall following root canal preparation with six different debridement regimens. Citric acid or a combination of NaOCl and citric acid for irrigation were better than NaOCl alone as an irrigant in smear removal from the prepared radicular dentinal walls.

The chemistry properties and applications of chitin and chitosan have been discussed in detail by **Dutta P.K et al in 2004**[20]. They observed that both are versatile and promising biomaterials. Chitosan, which is a de-acetylated form of chitin, is a more useful and interesting bioactive polymer. It has a number of reactive amino groups, which offers the possibility of chemical modification and formation of a large number of useful molecules. The authors focus on the contemporary research of chitin and chitosan towards applications in various industrial and biomedical fields.

The effect of EDTA (Ethylene Diamine Tetra Acetic acid), CDTA (1,2,CyclohexaneDiamine Tetra Acetic acid), EGTA (Ethylene Glycol Tetra Acetic acid) and citric acid on the de-mineralization of radicular dentin in a in-vitro setting studied by **Galvao et al in 2005**[23] and concluded that 1% citric acid solution was most effective for radicular dentin calcium ion extraction. They also observed that citric acid at neutral pH did not significantly change the calcium content of radicular dentin. Lower concentrations of EDTA and EGTA were found to be more effective than CDTA. 1% EDTA and 1% EGTA had a similar demineralization effect. They recommended the use of combinations of solutions of sodium hypochlorite and decalcifying agents because no single irrigating solution is capable of removing both the organic pulpal material and predentin as well as demineralizing the inorganic

portions of the radicular dentin. They suggest that the acidity of these solutions could be removed by final flushing with distilled water or saline, control of exposure time and subsequent use of calcium hydroxide sealers.

Giardino et al in 2006[26] studies the surface tension of two antibiotic based root canal irrigant solutions (MTAD and Tetraclean) with the commonly used root canal irrigants. (17% EDTA, Cetrexidin, cetrimide and chlorhexidine), smear clear (17% EDTA plus Tween 80) and 5.25%sodium hypochlorite. Distilled water was used as a control. They found that Tetraclean had the lowest surface tension and that both MTAD and Tetraclean were capable of removing the radicular smear layer due to the low surface tension which increases the surface area of contact of the irrigant solutions with the dentinal walls. This in turn permits deeper penetration of the irrigant in to the tubules increasing anti-microbial efficacy and removal of smear.

The properties and applications of chitin and chitosan were analysed by **Rinaudo M., et al in 2006**[53]**.** They note that chitin is the most important polymer in the world sourced from Marine crustaceans, shrimps and crabs. Chitosan is derived from chitin. When the degree of deacetylation of chitin reaches 50% it becomes soluble in acid aqueous media. They have reviewed the role of chitin and chitosan particularly the recent technical aspects and developments.

The antibacterial effect of chitosan containing gum was evaluated by **Hayashi Y., et al in 2007**[30] and they concluded that the chitosan containing gum has a greater antimicrobial effect and caused increased salivary secretion. The authors discuss the role in use of this technique in older people where salivary secretion is less. Chitosan provides gentle stimulus and is a bioactive material. They also furher suggest that the gum chewing could contribute to the mental health and stress relief. Chitiosan being a natural material could is very beneficial.

Henryka Bodek K et al in 2007[31] assessed the effect of temperature and chitosan form on the process of metal ions sorption. They observed that the influence of the surface dimensions associated with the form of chitosan on the ability of sorption as regards metal ions was confirmed. Chitosan flakes had the least effectiveness while hydrogel had the highest effectiveness. The rise in temperature affects the sorption of metallic ions by all forms of chitosan. The rise in temperature increases the sorption ability. Gel, flakes and powder forms of chitosan were tested in this study.

Sharavan et al in 2007[64] did a systematic review and meta analysis on whether the smear layer removal reduces the leakage of obturated human teeth invitro. They concluded that smear layer removal improves the fluid tight seal of the root canal system whereas other factors such as the obturation technique or the sealer did not produce any significant effects.

In a in-vitro study of the microporous, demineralised collagen matrices in radicular dentin as a result of the use of common calcium depleting endodontic irrigants, **Tay et al in 2007**[68] observed that it is difficult to simultaneously remove smear layer and render dentinal tubules patent without demineralising dentin. The presentation of a demineralised collagen matrix might be viewed as a consequence of use of calcium depleting irrigants as final rinses during endodontic therapy. Smear layer removing endodontic irrigants (EDTA and Biopure MTAD) were evaluated in this study. These collagen matrices have a role in the bonding of sealer to canal walls, and effective distribution of stresses. The authors also suggest the use of remineralising sealers like MTA.

Khedmat & Shokubinejad et al in 2008[37] in their study on the smear layer removal by three chelating agents observed that the protocols used in this study were not sufficient to completely remove the smear layer in the apical third of the radicular dentin. They also noted that there was no significant difference in smear removal by adding surfactants to the irrigant solutions

Gu et al in 2009[28] in a review of contemporary irrigant agitation techniques and devices emphasize the role and need of understanding fundamental issues in endodontic therapy. This is very important for clinical scientists as it paves way for better design and user-friendly newer generation systems in future as well as manufacturers whose contention that these systems play a pivotal role in contemporary endodontics. They observe that various technological advances in the past decade have seen the introduction of newer methods of irrigant agitation devices, which have improved on irrigant transfer removal of debris and smear and safety. They have employed cutting edge technologies, which result in overall canal cleanliness when compared to syringe irrigation. However there is a need for more studies, which are evidence based which co-relate the clinical efficacy of these devices with improved treatment outcomes. Whether these devices and technologies are really necessary and what the clinician perceive in terms of practicality and usage needs to be addressed.

In computational fluid dynamics study of irrigant flow within a prepared root canal using continuous flow rates **Boutisioukis et al in 2010**[10] found that irrigant needles should be placed 1mm from working length to ensure fluid exchange. The turbulent flow of irrigant leads to more efficient irrigant replacement in the canal system. Irrigant flow rate appears to be highly significant for determining the flow pattern within the root canal and impart displacement apical to the needle tip (side

vented). The apical displacement was not satisfactory for any of the flow rates of the irrigants investigated.

Boutsiokis et al in 2010[10] analyzed the effect of needle insertion depth on irrigant flow in the root canal using a unsteady computational fluid dynamics model and observed that needle insertion depth was found to affect the extent of irrigant replacement. Positioning the needle closer to the working length improved irrigant displacement at the apical part of the canal, but also increased the mean pressure at the apical foramen indicating a increased risk of extrusion. Variations in needle position and the canal taper had to be taken into account to decide the ideal needle position for each situation in the study.

Bronnec F et al in 2010[11] in a ex-vivo study evaluated the efficacy of irrigant penetration into curved canals. The authors concluded that the variables 'apical taper', 'volume of irrigant used', 'corono-apical level of needle tip placement', and 'needle tip design' influenced on outcome of irrigation penetration. The authors concluded that only active irrigation allowed total penetration and irrigant exchange. For syringe irrigation alone, the depth of placement of the needle tip in the root canal was the most dominating factor.

Caron et al in 2010[13] examined the effect of different final irrigation regimens and methods of activation on smear layer removal in curved canals after root canal instrumentation and concluded that root canal cleanliness benefits from irrigant solution activation especially sonic and manual dynamic activation in comparison with no activation during the final irrigation regimen. They also observed that a tapered tip which closely resembles the final canal preparation to be most effective.

Haapsaalo M et al in 2010[29] have reviewed various irrigation protocols in endodontic procedures and has discussed the irrigants, their interactions, and protocols for combining them effectively. They have dealt with the chelating agents used as irrigants and emphasised a detailed understanding of the mode of action of various solutions for effective and optimal irrigation. They also discussed various devices used for irrigation procedures which are safer and prevent the extrusion of the irrigant from within the confines of the canal system.

On the review of root canal irrigants by **Kandaswamy D., and Venkatesh Babu N., et al in 2010**[33] the authors observed that during root canal instrumentation the canals should be copiously irrigated with 5% sodium hypochlorite. Once the shaping procedure is completed they should be rinsed with EDTA or citric acid for a minimum of 1 minute with a 5 to 10ml of the chelating agent. After the smear layer removal procedure a final rinse with an antiseptic solution appears beneficial and chlorhexidine appears to be promising as it has the property of substantivity.

The effect of the influence of the final rinse technique on the ability of 17% EDTA (Ethylene Diamine Tetra acetic Acid) on the removal of smear layer was evaluated by **Mello et al in 2010**[43] in an in-vitro study. They concluded that a continuous three minute rinse of 5ml of 17% EDTA can effectively remove smear layer from all areas of root canals. They recommended the use of the decalcifying agent EDTA as a final rinse with the aim of effectively removing the radicular smear layer. The volume of EDTA and the duration of exposure used in this study did not cause significant undesired alteration in the radicular dentinal structure.

Pargolia et al in 2010[48] in a ex-vivo setting compared the smear layer removal ablility of four different final rinse protocols and concluded that the use of a chelating agent resulted in a higher amount of smear removal from the radicular

dentinal surface. The addition of a surfactant did not specifically improve the penetration of the irrigant in the case of Tetraclean particularly in the apical one third of the root. The authors made a null hypothesis that there was no difference between the four different final rinse protocols. They used a volume of 3 ml of the irrigant solution and note that different application times might yield different results. The irrigants solutions used for the final rinse were 5% sodium hypochlorite, 17% EDTA, and tetraclean with or without doxycycline. The results were analysed using a scanning electron microscope. The authors observe that the removal of the smear was more efficient in the coronal and middle thirds than in the apical third of the root. The volume of the irrigant reaching the apex might have a direct bearing on the effective removal of the smear and they recommend using activation devices for the apical third after sufficient enlargement. They also note that whenever a antibiotic is included as a part of a irrigant a possibility of developing resistance to the drug exists and there fore could be used with discretion.

Boutsioukis C et al in 2010[10] evaluated effects of needle tip design on the irrigant flow inside a prepared root canal during final irrigation with a syringe. A validated computational fluid dynamics model was used in this in vitro study. They concluded that the patterns of flow of irrigant in open ended needles was different from the close ended needles resulting in more irrigant replacement in front of the open ended needles but also higher apical pressure which indicates a increased risk of irrigant extrusion. From the clinician viewpoint the prevention of irrigant extrusion should be given more importance than adequate irrigant replacement and shear stress of the walls. They also observe that the effect of additional factors such as depth of needle placement taper of canal, size of root canal should be considered before suggesting supremacy of a particular needle type.

Tay F R et al in 2010[69] examined the effect of vapor lock on the canal debridement efficiency in an in vitro setting. They used a hypothesis that there is no difference between a closed and an open system design in smear and debris removal. They concluded that the presence of an apical vapor lock effect adversely affects the efficacy of debris removal. They observed that the current results are applicable only to side vent needle delivery and cannot be interpreted to other irrigant delivery or activation systems like ultrasonic, sonic or negative suction devices. They suggest that use of a manual dynamic activation could prevent vapor lock at the apex.

Violich D. R. & N. P. Chandler in 2010[73] in a review of smear layer observed that root canal instrumentation produces a layer of organic and inorganic material called the smear layer. It also contains microorganisms and their by-products. Penetration of intracanal medicaments into dentinal tubules is compromised and influences the adaptation of filling materials to canal walls. The authors reviewed 1277 articles, and for both smear layer dentine and smear layer root canal reviewed 1455 publications. A search on smear layer in endodontics revealed 408 papers. Potentially relevant material was also sought in contemporary endodontic texts, whilst older books revealed historic information and primary research not found electronically, such that this paper does not represent a 'classical' review. Data obtained suggests that smear layer removal should enhance canal disinfection. Current methods of smear removal, none of which are totally effective throughout the length of all canals or are universally accepted. If smear is to be removed, the method of choice seems to be the alternate use of ethylene diamine tetra acetic acid and sodium hypochlorite solutions. Conflict remains regarding the removal of the smear layer before filling root canals, with further investigations

required to ascertain the role of the smear layer in the outcomes of endodontic therapy.

Zou L et al in 2010[77] evaluated the effect of concentration, time of exposure and temperature on the penetration of sodium hypochlorite into radicular dentinal tubules in an in vitro study. This study was done in 4mm long blocks cut out of the root portion of anterior teeth and stained using crystal violet. The depth of penetration of sodium hypochlorite was determined by bleaching of the stain and measured by light microscopy. The authors concluded that temperature, time and concentration contribute to the penetration of sodium hypochlorite into the dentinal tubules. They also observed that the deepest penetration was achieved when all of these factors were present suggesting an additive effect. This study is the first in which hypochlorite penetration into dentin has been measured very accurately (micrometers). The staining procedure though can be postulated to alter the physical properties of dentin which might result in a altered pattern of fluid penetration both crystal violet and safanin produced similar results which is a pointer to the validity of the results obtained. This study also evaluated and established a method to quantify the depth of penetration of sodium hypochlorite into dentinal tubules one of the most commonly used irrigants used in endodontics.

Adcock et al in 2011[1] in a histological evaluation study compared side vented needle irrigation (SNI) with continuous ultrasonic irrigation (CUI) for debridement efficiency in canal and isthumus in mesial root of mandibular first molars. Within the limitations of this study they concluded that there is no difference between SNI and CUI at any root level from the apical third of the canal. However CUI produced significantly cleaner isthmuses than SNI. They also observed that both the irrigation techniques left a small but significant amount of debris at apical

1-1.4 mm of the canal when compared with other root levels. Regarding cleanliness of the isthmuses they observed that CUI was significantly more effective at 1-2.2 mm levels whereas both techniques produced equally clean isthmuses at 2.4-2.8mm root levels. Neither technique was capable of removing the debris completely from either the canal or the isthumus.

The effects and the role of chitosan on dental bone repair have been evaluated by **Ezoddini-Ardakani F., et al in 2011**[22]. The authors observe that chitosan has been shown to be one of the most promising biomaterials for orthopaedic and dental applications. It is suitable alternative for bone graft and improves bone regeneration in dental bone loss and that chitosan bone scaffold can also be used as a good mediator in bone regeneration.

Peeters and Suardita in 2011[49] compared the efficacy of LASER driven irrigation in removing smear layer and debriding the apical region of the root canal with that of ultrasonic irrigation, in a invitro setting and found that the use of a LASER with a plain fiber tip which produces cavitation in the irrigant and has potential to be an alternative method for removal of smear layer from the apical region of a straight canal.

Tikku et al in 2011[70] evaluated the role of Titanium tetra Fluoride as a root canal irrigant in endodontics and observed that there has been an enormous amount of research and debate on the advantages and disadvantages of removing smear layer before obturation and a mid pathway concept of modifying the smear layer in a way that it becomes completely resistant to dissolution and disintegration, which also blocks the dentinal tubules permanently. Such a promising biochemical and biomechanical change has been observed when treated with titanium tetra fluoride irrespective of the presence or absence of smear layer. The smeared surface showed

a thicker coating (1-5μm). It has also been shown that the interaction of titanium tetra fluoride and smear layer produces a stable, acid resistant structure indicating its potential role in reducing microleakage and improving apical seal of the root canal.

The efficacy of different final irrigation activation techniques on radicular smear layer was evaluated by **Saber S D et al in 2011**[58] in an in-vitro setting in straight canals. The irrigation techniques used were passive irrigation, apical negative pressure irrigation (Endovac), manual dynamic activation, and passive ultrasonic irrigation. Apical negative pressure irrigation presented with statistically significant least smear scores. They concluded that apical negative pressure and manual dynamic activation resulted in better removal of smear layer than with passive ultrasonic irrigation and passive irrigation. The evaluation was done in vitro using a scanning electron microscopic analysis. The irrigant solutions used were 2.5% sodium hypochlorite as initial rinse and 17% ethylene diamine tetra acetic acid (EDTA) as a final rinse solution.

Al-Ali M et al in 2012[3] evaluated the smear layer and debris removal effectiveness of four root canal irrigation protocols and efficiency in removing remaining soft tissues in curved root canals in a invitro study. They concluded that the use of sodium hypochlorite in conjunction with hydrogen peroxide was effective in removing soft tissue debris from the apical third of the canals and that canal brushes were as effective as PUI in removal of smear and debris. They further recommended studies taking the volume of the irrigant and the configuration of the canal systems using these irrigation regimens.

Lotfi et al in 2012[38] evaluated the effect of duration of irrigation with sodium hypochlorite in clinical use of MTAD on the removal of smear layer and dentinal erosion in root canals. This was an in vitro study and used 1.3% sodium

hypochlorite as initial rinse for varying times followed by a final rinse of MTAD. They observed and concluded that 1.3% sodium hypochlorite for 5 and 10 minutes in the MTAD protocol removed the smear layer in the coronal and middle third without erosion. They also observe that MTAD and 17% ethylene diamine tetra acetic acid did not have the ability to clean the apex in the closed canal system model. The smear layer and erosion evaluation was based on the methods used by Torabinejad et al.

The effect of various irrigating solutions on intra-radicular dentinal surface was evaluated in-vitro by **Karunakaran J V et al in 2012**[34] using scanning electron microscopy. Normal saline, de-ionised water, 17% EDTA, 5% sodium hypochlorite with and without ultrasonic agitation, 3% hydrogen peroxide, 2% chlorhexidine, MTAD with and without ultrasonic agitation were the irrigants with the respective agitation protocols. The authors observed that within the limitations of this study none of the irrigants were able to achieve a totally clean dentinal surface. The action of these irrigants on the dentinal surface was enhanced by ultrasonic agitation of the irrigant. They also observed that the pattern of surface alteration varies for each irrigant solution and these changes may have a negative or positive impact on the bonding characteristics of radicular dentinal surface.

Pimenta J A et al in 2012[50] evaluated the effect of 0.2% chitosan, 15% EDTA and 10% citric acid on the micro hardness of root dentin in an in vitro study. The authors concluded that the reduction in the micro hardness achieved by the three solutions were not of statistical significance. All the three solutions effectively removed smear from the middle thirds. Although chitosan 0.2% was prepared using acetic acid solution 1% the role of acetic acid in smear removal

has been shown to be negligible and the reduction in micro hardness is due to chitin alone. The exposure time in this study was 5 minutes.

Silva P V et al in 2012[65] evaluated the smear layer removal ability of chitosan on dentin for a 3 and 5 minutes exposure time. The initial rinse used during canal preparation was 1% sodium hypochlorite. Chitosan is a natural polysaccharide, which is obtained from shells of crab and shrimp after deacetylation of chitin, has acid PH, atoxic, biocompatible and biodegradable. This is one of the most abundantly available substances in nature. The PH used in this study was 3.2 and volume was 5ml with an exposure time of 3 minutes at concentration of .1, .2, and .37%. The authors concluded that .2% chitosan solution with an exposure time of 3 minutes removed the layer of smear adequately and caused less erosion than 17% ethylene diamine tetra acetic acid. The chelation can be explained by a bridge model or alternatively metal ion complex formation. The role of chitosan as an irrigant is very promising.

Andrabi et al in 2013[4] in an in vitro study compared the effectiveness of four different irrigation protocols on smear layer removal using a scanning electron microscope. The irrigants compared were 3% sodium hypochlorite, 17% ethylene diamine tetra acetic acid smear clear and bio pure MTAD. The authors concluded that Bio pure MTAD was the most effective for smear layer removal in the apical one third of the root canal system. The total irrigation time was 3 minutes for all solutions. They observe that smear layer was predominantly removed using chemical method of chelation using ethylene diamine tetra acetic acid. Smear clear was introduced for smear layer removal that has cationic cetrimide and anionic surfactant in addition to 17% ethylene diamine tetra acetic acid. Bio pure MTAD is a mixture of tetracycline acid and detergent and has been advocated for smear

removal, antibacterial action and substantivity. They observe that there is no single irrigation protocol that dictates the volume, time of exposure, mode of irrigant delivery or activation to achieve optimal results.

Cehreli ZC in 2013[15] studied the effect of different irrigation regimens on the elimination of smear and erosion in laboratory and clinical conditions. The investigators concluded regardless of the irrigation system, the use of NaOCl alone failed to remove radicular smear layer. Where a combination of sodium hypochlorite and 17% EDTA were used, smear was partially or completely eliminated but was not statistically significant. They also recommend the use of EDTA as a final rinse regardless of the technique used.

A review of literature of chitosan and its applications has been done by **Gavahne et al in 2013**[25]**.** They note that it is a abundantly available material sourced from crab and shrimp shells and other sea crustaeceans. Chitosan has received a great deal of attention as a pharmaceutical exicipient due to its low toxicity and bio-compatibility in both conventional and novel applications. Chitosan is used widely in many fields like agriculture, water treatment in food and beverage industries and pharmaceutical. It has a number of pharmacological properties due to which it is used in medicine.

Silva P V et al in 2013[66] evaluated the efficacy of smear layer removal using chitosan compared with different chelating agents. They later quantified by atomic absorption spectrophotometry with flame (AASF), the concentration of calcium ions in these solutions after irrigation protocol. The solutions used in the study were15% EDTA, 0.2% chitosan, 10% citric acid, 1% acetic acid and control (without final irrigation). They concluded that 15% EDTA and 0.2% chitosan were associated with the greatest effect on root dentine demineralization, followed by 10% citric acid and

1% acetic acid. The smear layer from the middle and apical thirds of the root canal was effectively removed by15% EDTA, 0.2% chitosan and 10% citric acid.

Many potential applications of chitosan as a pharmaceutical excipient have been discussed in detail by **Usman M R M., et al in 2013[72].** They observe that polymers have been used as a tool to control the drug release from the formulations. Chitosan being a natural polymer has distinct advantages and has many applications as a pharmaceutical agent. They have been tried for the long term release of hormones, vaccine delivery and have a very high safety and biocompatibility. They recommend chitosan as a biopolymer for the development of new derivatives.

The effectiveness of different final irrigation solutions on smear removal in intra-radicular dentin was analysed by **Darrag A M.,et al in 2014[17]** and concluded that .2% chitosan was effective at smear removal in intra-radicular dentin when compared to chelating agents like ethylenediamine tetraacetic acid, citric acid and MTAD. The removal of the smear was not complete at the apical levels. This was a invitro study and the authors recommend further studies to evaluate physical, chemical and biological properties of chitosan and to verify the use of chitosan as chelating agents for the root canal.

Grover C., et al in 2014[27] on the evaluation of calcium ion release and changes in pH on combining calcium hydroxide with different vehicles found that chitosan when used as a vehicle showed better controlled and sustained release of the calcium ions from calcium hydroxide for a period of one month and that chitosan can be used a a vehicle for calcium hydroxide inside the root canal system.

Shaheen V., et al in 2014[61] the role of irrigants used in endodontics and observed that there is no single irrigant which possesses all the requisites of a irrigant solution and they have to be used in sequence with the aim to achieve the goals.

The study of chitosan citrate as a root canal irrigant was evaluated by **Suzuki S et al in 2014**[67]**.** Chitosan which is abundantly available in nature has anti-bacterial, high chelating ability of metallic ions, good properties of bio-compatibiliy and bio-degradability. It is insoluble in water and therefore has to be dissolved in dilute acid solutions. Citric acid has also been used and tried as a root canal irrigant and found to have good chelating ability. When chitosan is dissolved in a citric acid solution it forms a citrate aolution which was evaluated for smear removal and antibacterial efficacy in this study. The results of this study indicate that this combination enabled antibacterial activity and removal of smear and has been indicated as a possible root canal irrigant.

The retriveability of calcium hydroxide and chitosan used as a intra canal medicament from the canals has been assessed by **Vineeta N., et al in 2014**[75] in a invitro setting and they have found that the addition of chitosan enabled the easier removal of the calcium hydroxide from the canal system. The type of vehicle used played a role in the retrival of the medicament.

Carpio Perochina et al in 2015[14] evaluated the chelating and anti-bacterial properties of bio-active Chitosan nanoparticles.They tested the ability of the chitosan nanoparticles to remove the smear and inhibit the bacterial re-colonoization of dentin. They concluded that chitosan nanoparticles had the potential to be used as a final rinse solution and acts effectively against bio-films and has good chelating property. This solution has the potential to be an alternative to ethylenediamine tetra

acetic acid during endodontic therapy. The anti-bacterial mechanism of chitosan has been attributed to is polycationic nature that alters cell wall permiabiility and inhibit bacterial enzymatic degradation.

Madhusudhana k., et al in 2015[41] compared the effect of chitosan and morinda citrifolia on smear layer removal in an invitro setting and observed that .2% of chitosan was effecive at smear removal and better than that of morinda cirtifolia juice. The overall smear removal was best at the coronal third of the root canal system followed by the middle and the apical third. Chitosan was a preferred material as it was biocompatible and biodegradable. They also discuss the bridge theory and amino group binding theory which possibly explains the binding effect of chitosan to metallic ions. They also observe that morinda citrifolia removed smear better than the control group.

Roymond K A., et al in 2015[56] discussed the role of chitosan in various specialities of dentistry. They noted that chitosan a linear poly sacchride is a sugar obtained from the hard outer skeleton of shell fish including crab, lobster and shrimps. In medicine it is used to reduce bleeding, as an anti-microbial, delivery of drugs, haemodialysis, cholesterol, hypertension control etc,. In dentisry it is used as a anti-plaque agent in tooth pastes, as an anti-microbial agent, chelating agent in endodontics, as a restorative material along with composite, glassionomer cement and as a hydrogel. It is also used for wound healing, bone regeneration and repair. In endodontics it has been used as a anti bacterial against enterococcus faecalis and S.Aureus. The chelating effect has allowed it to be used as a irrigant for removal of the inorganic portion of the tooth structure in different concentrations. It has also been found to be effective in smear removal during endodontic procedures. It has been combined with riboflavin to be used in the root canal to modifiy the dentin

collagen matrix and stabilises it to enhance resin penetration and hybrid layer formation. Chitosan nanoparticles have been shown to be effective against staphylococcus saphrophyticus and Escherichia coli.

The comparison of the anti microbial activity of two chelating agents chitosan and etidronate was done by **Vidya N., et al in 2015**[74] and they concluded that the anti microbial and chelating ability of 18% etidronate can be combined with sodium hypochlorite to yield a complete irrigation solution which would reduce the need for the concurrent use of other agents. They note that a ideal irrigating solution should have good tissue dissolving property, smear removal and anti microbial efficacy. The concentration of chitosan at .2% had a lesser anti bacterial effect compared to a 2% chitosan solution.

ARMAMENTARIUM

Collection of natural teeth:

1. Normal saline (Nirlife Health Care, Nirma Products, India)
2. 2% Thymol solution(Alpha Chemicals, Maharashtra, India)
3. Disposable gloves (Dispodent, Chennai)
4. Vented glass bottles
5. Tissue forceps

Preparation and selection of samples:

1. RadioVisuoGraphy Satelec RVG (Satelec X- Mind Ac / Dc radiography unit, Italy)
2. Diamond disc
3. Magnifying Lens with Illumination
4. Magnifying loupe
5. Modeling wax (Hiflex –Prevest Denpro Limited, Jammu, India)
6. Wax carvers
7. Spirit lamp
8. Small transparent plastic containers for sample placement
9. Polyvinyl siloxane impression material (Flexceed vinyl polysiloxane GC Dental products, Tokyo, Japan)
10. Indelible marker - bold and fine (Camlin, India)
11. DG-16 Endodontic probe(Hu-Friedy)
12. N 95 masks (3M products, USA)

ARMAMENTARIUM

Fig 1 : Instrument Tray

Fig 2 : High speed motor with diamond disc

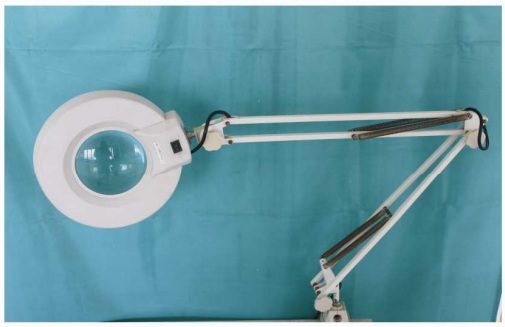


Fig 3 : Magnifying Loupe

Fig 4 : Radiovisuography Unit

Fig 5 : Ultraviolet chamber

Fig 6 : Irrigants

Fig 7 : Magnetic Stirrer

Fig 8 : Precision weighing scale

13. Marking pencil
14. Labelled storage boxes
15. Ultrasonic unit- (EMS)
16. Mc Intosh sheet
17. Illumination light
18. Goggles and Gloves

Root canal preparation:

1. Size 8,10,15 K file of 21mm length (Dentsply, Maillefer, Ballaigues, Switzerland)
2. Endo block (Dentsply Maillefer, Ballaigues, Switzerland)
3. 5ml syringe with leur-lock needle (Dispovan, Hindustan Syringes and Medical Devices Ltd, Faridabad, India)
4. 30 gauge side-vent Pro-rinse needle (Dentsply, Tulsa dental, Tennessee, USA)
5. 5ml, 10ml syringe unolock (Hindustan Syringes and Medical Devices Ltd, Faridabad, India)
6. Endomotor (X-smart plus with 1:16 reduction hand piece- Dentsply Maillefer, Ballaigues, Switzerland)
7. Protaper rotary file system (21mm- S1,S2,F1,F2,F3) –(Dentsply Maillefer, Ballaigues, Switzerland)
8. Gutta percha points F3 (Dentsply Maillefer, Ballaigues, Switzerland)

ARMAMENTARIUM

Fig 9 : Sputter Coating Machine

Fig 10 : Samples being sputter coated

Fig 11 : Field Emission SEM Unit

Fig 12 : Samples after sputter coating

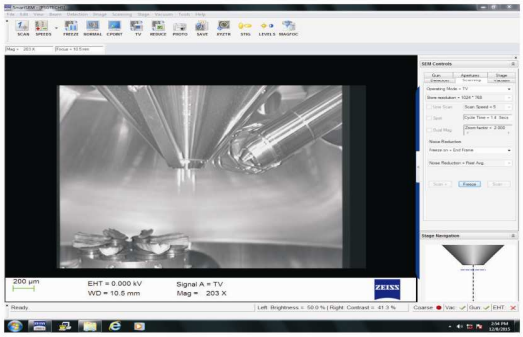


Fig 13 : Samples viewed under SEM

Irrigating solutions:

1. Magnetic stirrer (Remi equipment India pvt.Ltd)
2. Normal saline (Nirlife Health Care, Nirma Products, India)
3. 5% Sodium Hypochlorite solution (Nice chemicals Pvt Ltd, India)
4. 17% EDTA solution (pulpdent corporation, USA)
5. Sterile Distilled water (Ives drugs, Pvt Ltd, India)
6. 2 % Chitosan solution – low molecular weight (Sigma Aldrich, Iceland)
7. 1 % Chitosan solution – crab shells (Sigma Aldrich, Japan)
8. 1 % Chitosan solution – Shrimp shells(Sigma Aldrich, Japan)
9. 4 % Chitosan solution – oligosaccharide (Sigma Aldrich, Japan)
10. 4 % Chitosan solution – citrate
11. 10% Citric acid solution (Nice chemicals Pvt Ltd, India)
12. 100ml glass beakers (Borosil,India)
13. Glass pipette (Borosil,India)
14. Volumetric Beakers 100 ml (Borosil,India)

Sectioning of samples:

1. Diamond disc
2. High speed motor (KaVo Dental ,Charlotte, NC)
3. 0.5 inch Stainless Steel bibeveled chisel
4. Stainless steel mallet
5. Zip lock covers
6. Storage container
7. Stainless steel tray

Preparation for SEM analysis:

1. Ascending concentrations of Isopropyl alcohol (S.V. Drugs and chemicals, Faridabad, India)
2. Sterile self sealing coded sterilization pouches (Reach Global Pvt.Ltd, Pune, India)
3. U-V light chamber (Apex Industrial Electronics, Haryana, India)
4. Custom sample mount block for distance marking
5. Silica gel
6. Fused calcium hydroxide
7. Air tight containers
8. Glass bottles
9. Vaccum chamber

Scanning Eectron Microscopic Imaging:

1. Scanning Electron Microscope (Sigma 0336 FESEM, Ziess, Munchen, Germany)
2. Gold Plladium Sputter coating machine (Quorum, United Kingdom)
3. Carbon tape (Royal tapes Pvt Ltd., Chennai, India)
4. Storage media (SONY)
5. Data recording media (Seagate)
6. Storage boxes
7. Observation sheets
8. Tissue forceps

Image intrepretation:

1. Adobe Photoshop (CS3Extended)
2. Corel draw (X7)
3. Apple & Sony VIAO computing systems
4. Image analysis software (EDS software).
5. High resolution monitor(Samsung, India)

Statistical analysis and tabulation:

1. Statistical Analysis Software (SPSS)
2. Apple & Sony Viao computing systems
3. HP color laserjet high resolution printer

MATERIALS AND METHODS

1. Collection of teeth:

One hundred and fifty two extracted human permanent maxillary incisors and canines were collected and stored in isotonic saline solution in vented glass bottles for a maximum of 72 hours. Protocols for infection control as per OSHA and CDC guideline regulations in collection, storing, sterilization and handling were followed.

2. Selection of samples:

Teeth devoid of anomalies, defects, carious lesions, restorations and endodontic treatment were separated. They were then observed for cracks with the help of illumination and magnification and such teeth were excluded. Teeth with mature and intact root apices were selected for the purpose of the study. The selected teeth were then analyzed using digital radiography to ensure that they had a patent single canal and the root lengths were a minimum of 15mm (measured from the tip of the root to the cemento-enamel junction).The selected teeth were then stored in normal saline solution at 4°C until use. A total of one hundred and twenty teeth were selected for the purpose of the study.

3. Standardization of samples:

The working length was determined by passively placing a size 10K file (Dentsply Maillefer, Ballaigues, Switzerland) into the canal until the tip was visualized at the apical foramen using a magnifying loupe and was adjusted to the apical foramen. Then the actual canal length was measured and working length was calculated by subtracting 0.5mm from this measurement.

MATERIALS AND METHODS

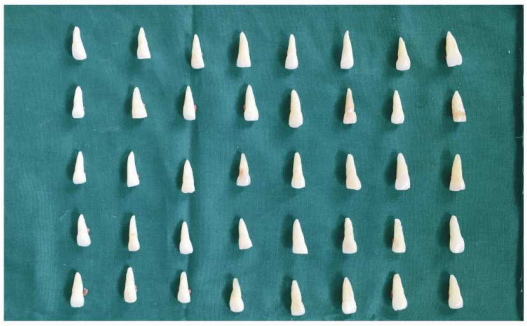


Fig 14 : Selected teeth

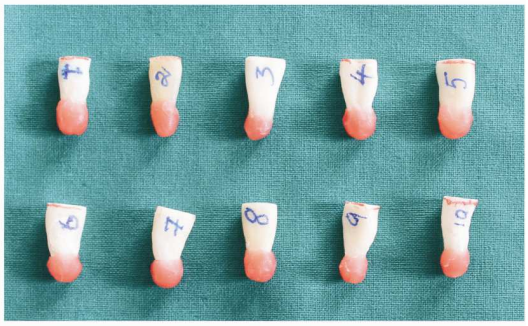


Fig 15 : Apices sealed with wax

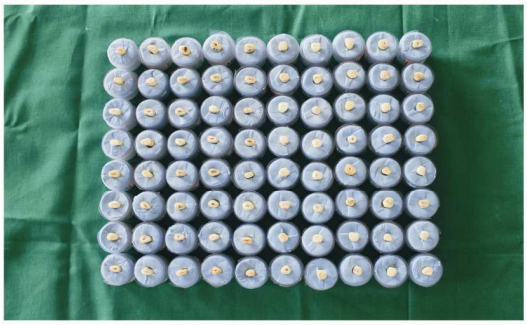


Fig 16 : Teeth placed in polyvinylsiloxane base

Fig 17 : Endomotor Unit

Fig 18 : Protaper Rotary files

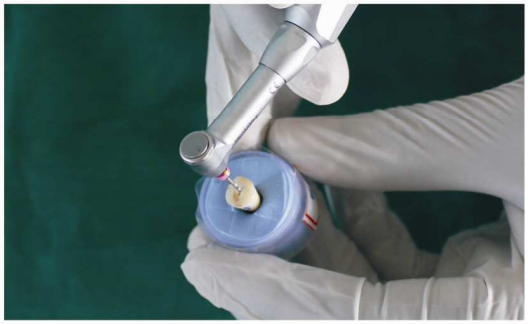


Fig 19 : Samples being prepared

Fig 20 : Chitosan solutions in magnetic stirrer

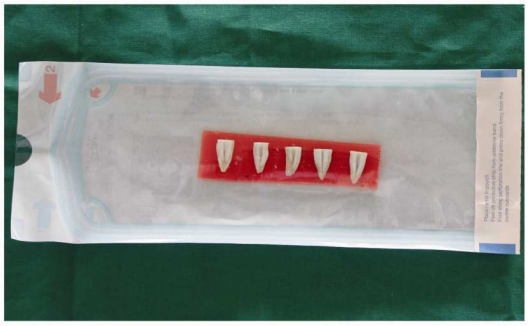


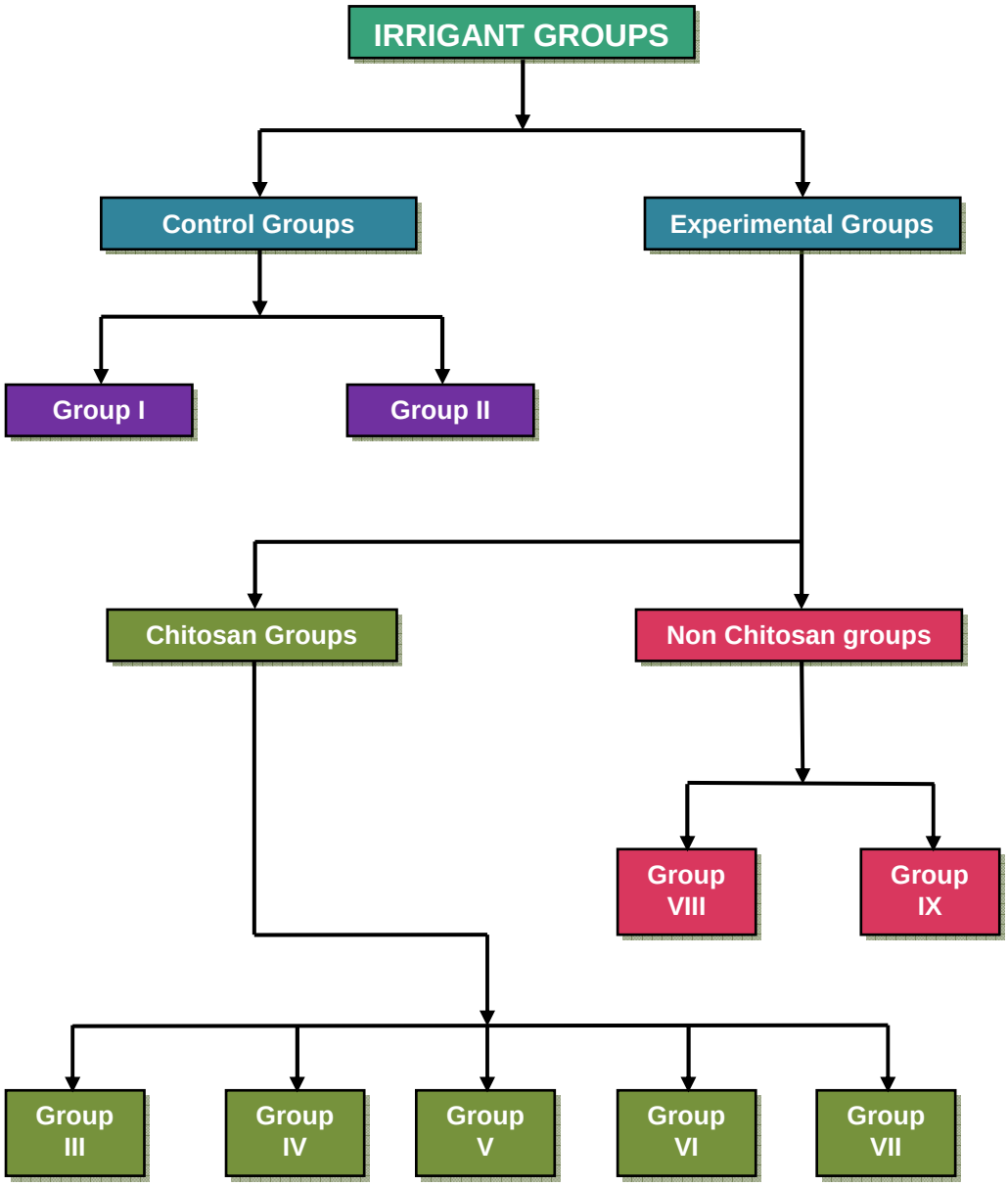
Fig 21 : Sectioned samples

4. Preparation of the Sample:

The selected samples were standardised by de-coronating them at a distance of 15mm from the apical foramen (working length) by sectioning with a water cooled diamond disc. The sectioned teeth were then rinsed with distilled water and stored in normal saline at 4ºC for further processing. The teeth were then dried, coded. Wax was applied at the apical third of the root. They were then placed in a transparent small plastic container into which a soft poly-vinyl siloxane impression material had been placed. The aim was to prevent the irrigants from extruding the apex in order to simulate in-vivo closed apex conditions. The samples were then randomly divided into two control groups (n=5) and seven experimental groups (n=10).

5. Root Canal Preparation Technique:

The instrumentation was initiated with hand files (Dentsply, Maillefer, Ballaigues, Switzerland) upto size 20 followed by protaper rotary files from size S1-F3.The root canals of the samples were prepared using protaper rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland) with X-smart plus endomotor (Dentsply Maillefer, Ballaigues, Switzerland) as per the manufacturer instructions. 1ml of the irrigant was used for canal irrigation after using each instrument and before proceeding to the next. A total of 8ml of the irrigant was used during the bio-mechanical preparation procedure. The irrigant was delivered using a 30- gauge side vent pro-rinse needle (Dentsply, Tulsa Dental) at the working length.


IRRIGANT GROUPS
Control Groups
Experimental Groups
Group I
Group II
Chitosan Groups
Non Chitosan groups
Group VIII
Group IX
Group III
Group IV
Group V
Group VI
Group VII

6. **Final Rinse of Samples**:

Subsequent to the canal preparation the samples were irrigated with a final rinse of 5ml of the irrigant solution as per the respective group. The Chitosan solutions at their respective concentrations were prepared using 1% acetic acid. Oligosacchride was prepared using distilled water and Chitosan citrate was prepared using 10% citirc acid. All the irrigating solutions were mixed in a magnetic stirrer for two hours. A total of 5 millilitre of the irrigant was delivered using a 30- gauge side vent pro-rinse needle (Dentsply, Tulsa dental) for duration of five minutes.

During the first minute delivery of the irrigant, the needle was withdrawn to 5mm inserted back to working length followed by rotation of the needle by 180° three times alternatively. During the second minute a F3 size gutta percha cone (Dentsply Maillefer, Ballaigues, Switzerland) was inserted to working length and withdrawn three times (Manual Dynamic Activation). This was done to improve the irrigant delivery and replacement to the apical third of the canal space. The remaining irrigant was left in the canal for three minutes. After the completion of five minutes a post-final rinse irrigation of 10ml of distilled water was done to flush out the remaining final rinse irrigant from within the canal. The samples were then stored safely.

7. Preparation of samples for SEM analysis:

The teeth after removal from the poly-vinyl siloxane base were covered with cotton wool at the canal orifice. Subsequently they were grooved longitudinally on the external surface in a bucco-lingual plane with a diamond disc with sufficient care

not to accidentally penetrate the root canals. The teeth were then carefully split longitudinally in a bucco-lingual plane dividing them into two halves using a mallet and a chisel. For each tooth the half containing the most visible part of the apex was selected, stored and coded. The teeth were then placed in a 10% neutral buffered formalin solution at 18°C for 24 hours. They were then post fixed in Osmium Tetroxide (1%w/v) for two hours before being dehydrated in graded solutions of Isopropyl alcohol (S.V. Drugs and chemicals, Faridabad, India).The teeth were then placed in a filter paper for 24 hours after which separation markings of 5mm made for the apical, middle and coronal thirds respectively on the split half of the root using a custom made former. The prepared samples were then irradiated with UV light in a UV light sterilization chamber and stored in sterile pouches. Each group was processed and stored separately for further analysis and examination.

8. SEM Examination:

The coded samples of each group were mounted on to aluminium stubs with carbon tape (Royal tapes Pvt Ltd., Chennai, India) with the entire root canal visible and facing upwards. Each of the specimens was coated with a 20-30nm thin layer of gold in a gold sputter coating machine (Quorum, United Kingdom). The samples were then examined using a field emission scanning electron microscope with a high resolution(SIGMA 0336 FESEM, ZIESS, MUNCHEN, GERMANY). The SEM photo micrographs were obtained at X2000 magnification using digital image analysis software and stored appropriately for subsequent analysis. The most representative micrographs were taken for each millimeter of the specimen and were recorded for apical, middle and coronal thirds respectively.

Table 1: IRRIGANT GROUPING

GROUPS (n=5-10)	INITIAL RINSE	FINAL RINSE
I-Positive control (n=5)	5%NaOCL	17%EDTA
II-Negative control (n=5)	Normal saline	Normal saline
III	5%NaOCL	2% Chitosan - LMV
IV	5%NaOCL	1% Chitosan - Shrimp shell
V	5%NaOCL	1% Chitosan – Crab shell
VI	5%NaOCL	4% Chitosan oligosaccharide
VII	5%NaOCL	4% Chitosan citrate
VIII	5%NaOCL	10% Citric Acid
IX	5%NaOCL	1% Acetic acid

9. Analysis of photomicrographs:

The photomicrographs were analyzed after coding based on the representative groups in a blind manner by two independent investigators for the presence of smear layer, debris and erosion in the apical,middle and coronal one thirds of each specimen using high resolution monitors using established assessment criteria.

The **smear layer** was analyzed using the following criteria **(Caron et al 2010).**

Score 1: No smear layer and dentinal tubules open.

Score 2: Small amounts of scattered smear layers and dentinal tubules open.

Score 3: Thin smear layer and dentinal tubules partly open. (Crescent shaped)

Score 4: Thick smear layer with partial covering of dentinal tubules.

Score 5: Total covering with thick smear layer.

The presence of **debris** was analyzed using the following criteria **(Dadresenfar et al in 2011)**

Score 1: Clean canal wall, few debris particles.

Score 2: Few conglomerations.

Score 3: Many conglomerations less than 50% of canal wall.

Score 4: More than 50% of canal wall with conglomerations.

Score 5: Complete or near complete covering of canal wall by debris.

The presence of **erosion** was analyzed by using the following criteria **(Torabinejad et al in 2003)**

Score 1: No erosion (All tubules normal in appearances)

Score 2: Moderate erosion (Peritubular dentin eroded)

Score 3: Severe erosion (Intertubular dentin destroyed and tubules connected to each other)

10. Tabulation of result and statistical analysis:

The results which were scored by the independent operators were compared and tabulated for their respective score values of smear layer, debris and erosion in the apical, middle and coronal thirds of the root canal. The results were then statistically analyzed and appropriate technical interpretations done.

TABLE 2: AVERAGE SMEAR SCORES

GROUP	CORONAL	MIDDLE	APICAL
I	1	1	1.7
II	5	5	5
III	2	2.2	3.0
IV	1.2	2.2	2.6
V	1.6	2.5	3.4
VI	1.8	2.0	2.2
VII	1.2	1.4	2.0
VIII	1.4	1.8	2.8
IX	3.0	3	3.4
MEAN	**2.02**	**2.34**	**2.90**
SD	**1.26**	**1.15**	**0.98**

CHART I: AVERAGE SMEAR SCORES

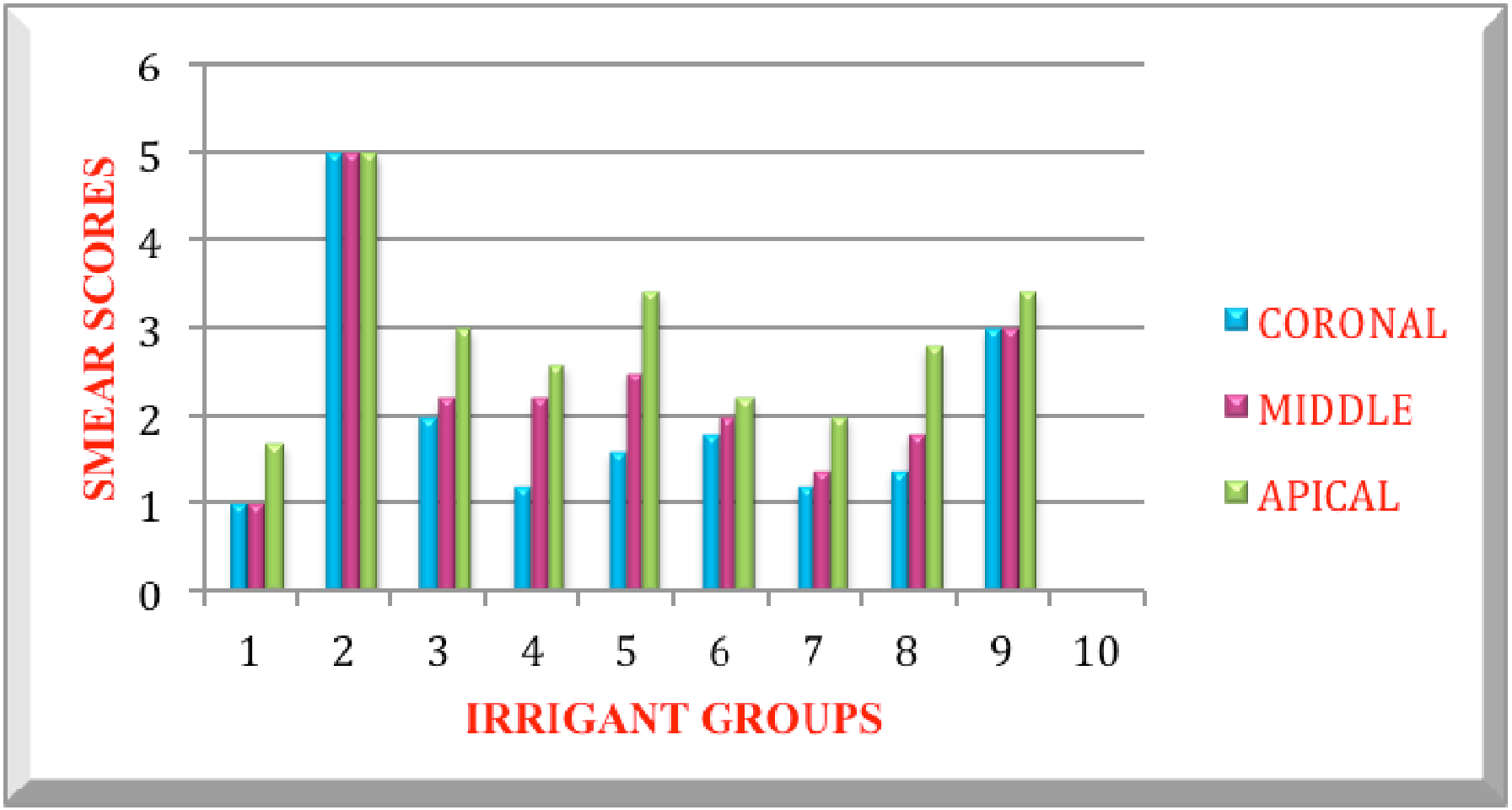


TABLE 3: AVERAGE DEBRIS SCORES

GROUP	CORONAL	MIDDLE	APICAL
I	1.0	1.0	1.8
II	4.6	5	5
III	1.4	1.6	2.0
IV	1.2	2	2.2
V	2.6	2.8	2.8
VI	2	2.4	2.4
VII	2.1	2.1	2.4
VIII	1	1.2	1.8
IX	2.6	2.8	3.7
MEAN	**2.06**	**2.32**	**2.68**
SD	**1.14**	**1.19**	**1.05**

CHART II: AVERAGE DEBRIS SCORES

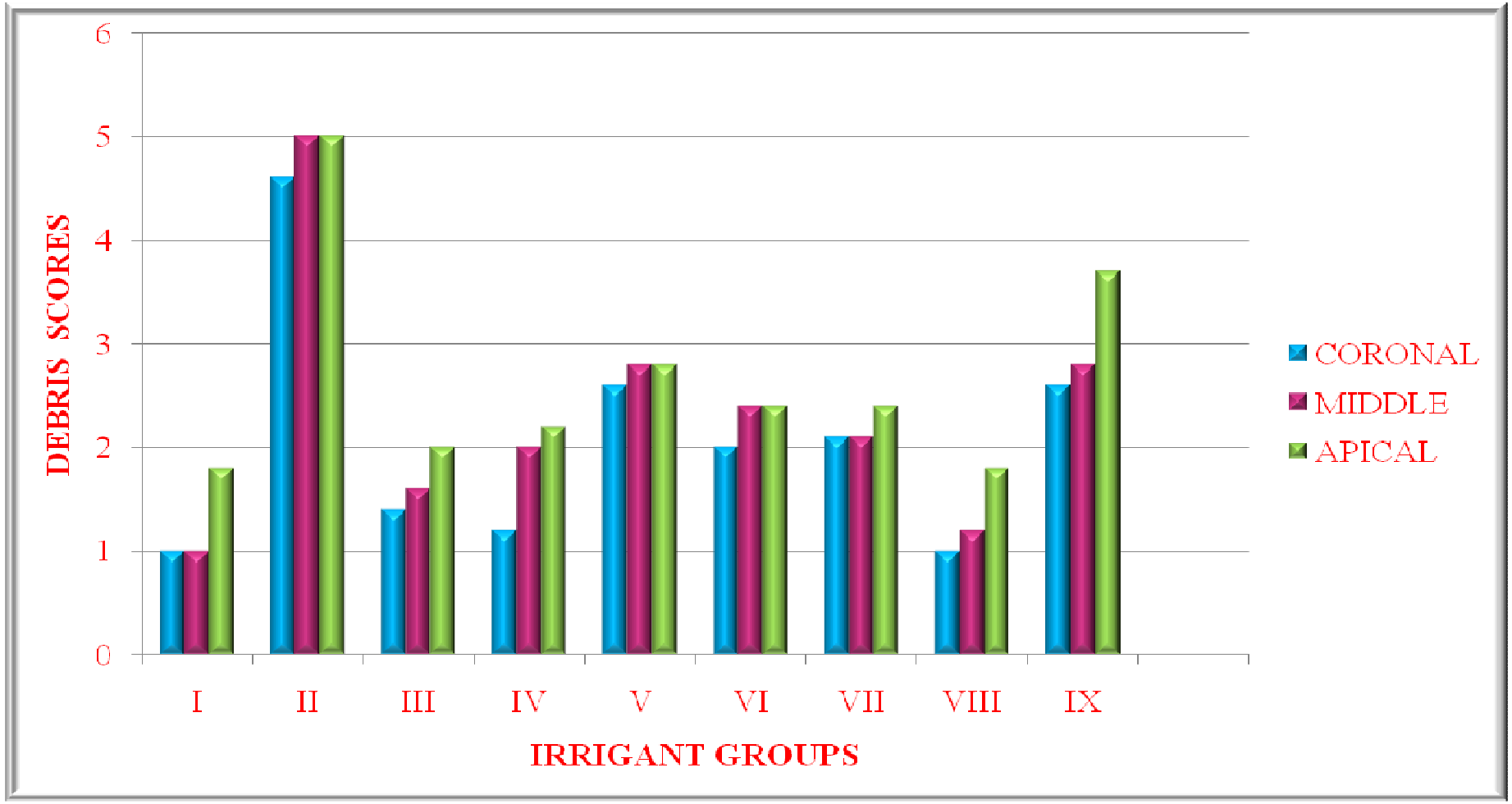


TABLE 4: AVERAGE EROSION SCORES

GROUP	CORONAL	MIDDLE	APICAL
I	2.0	2.0	2.5
II	1	1	1
III	2	1.7	1.1
IV	3	1.8	2.2
V	2.8	1.8	1.3
VI	1.5	1.3	2.4
VII	1.8	2.0	2.0
VIII	1.2	2	2.2
IX	1	1	1
MEAN	**1.81**	**1.62**	**1.74**
SD	**0.73**	**0.41**	**0.63**

CHART III: AVERAGE EROSION SCORES

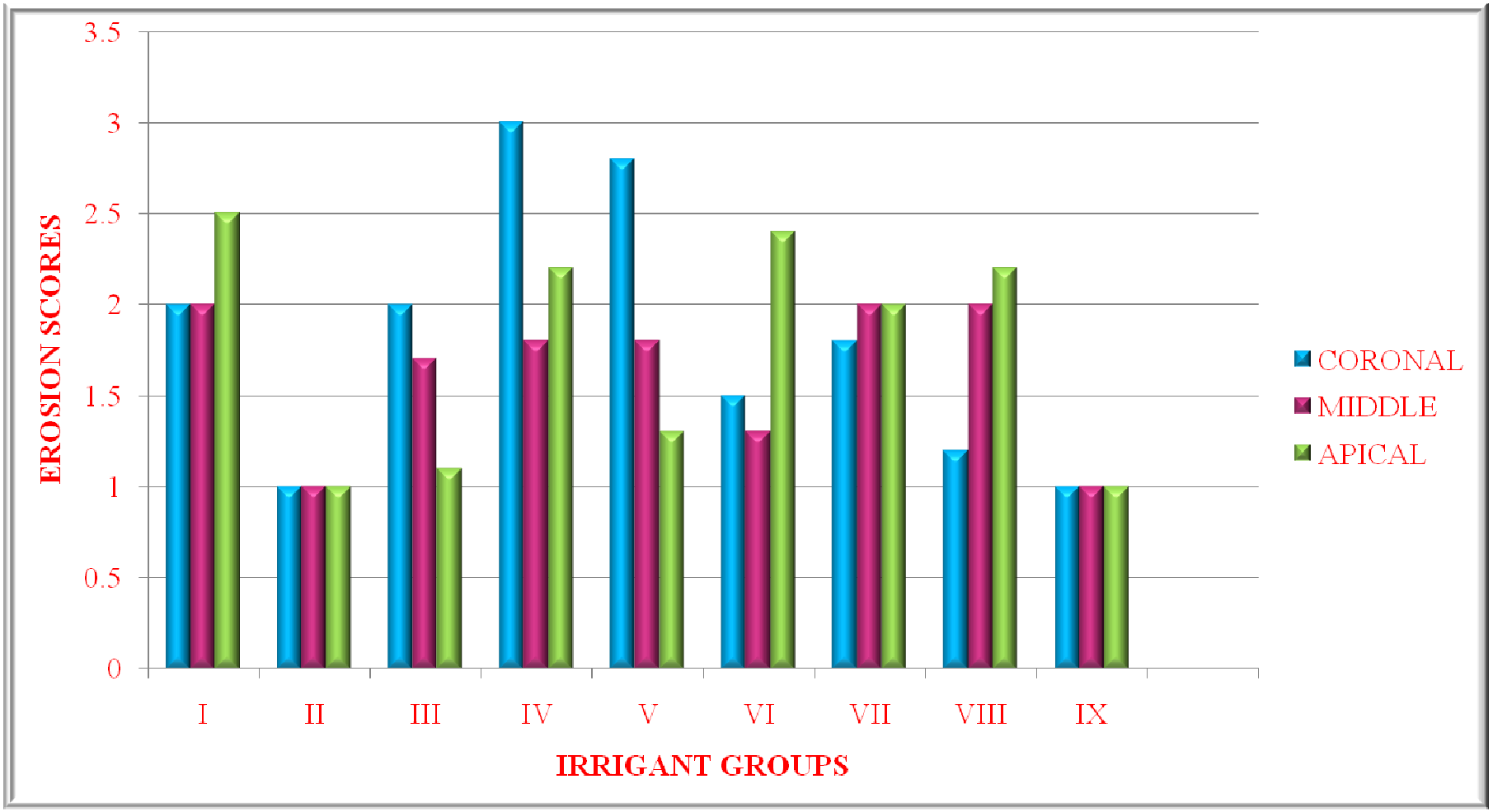


TABLE 5: MEAN SCORES

MEAN VALUES	CORONAL	MIDDLE	APICAL
Smear	2.02	2.34	2.90
Debris	2.06	2.32	2.68
Erosion	1.81	1.62	1.74

CHART IV: MEAN SCORES

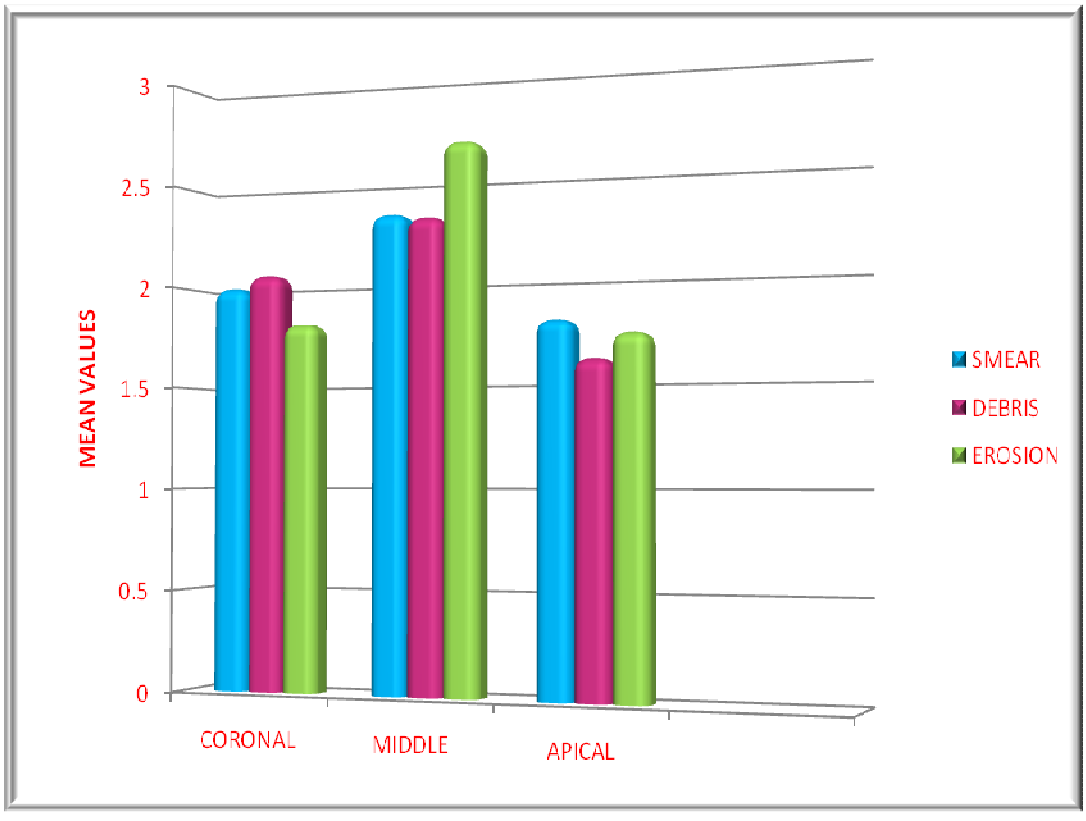


TABLE 6: MEAN SCORES OVERALL

GROUP	SMEAR	DEBRIS	EROSION
I	1.23	1.27	2.17
II	5.00	4.87	1.00
III	2.40	1.67	1.60
IV	1.93	1.80	2.33
V	2.50	2.73	1.97
VI	2.00	2.27	1.73
VII	1.53	2.20	1.93
VIII	2.00	1.33	1.80
IX	3.13	3.03	1.00
MEAN	**2.41**	**2.35**	**1.72**
SD	**1.11**	**1.11**	**0.46**

CHART V: MEAN SCORES OVERALL

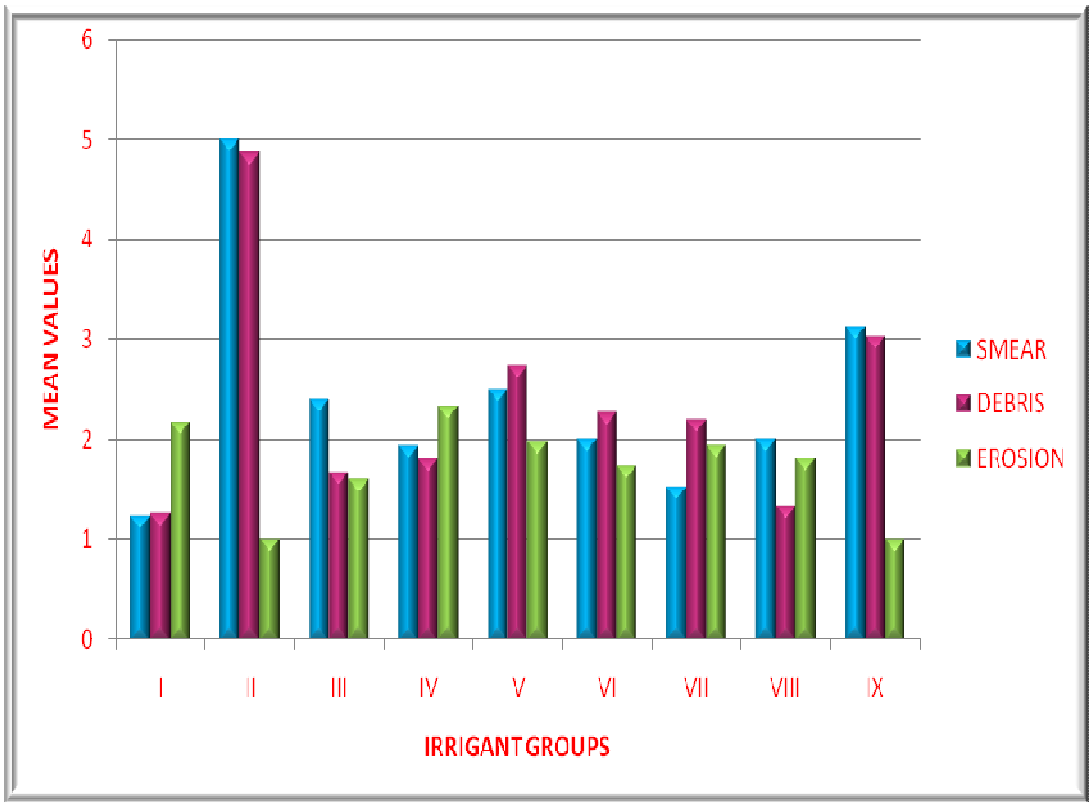


TABLE 7: STATISTICAL COMPARISON BETWEEN GROUPS FOR SMEAR

TWO GROUP COMPARISON Student's t-test

S.NO	GROUPS COMPARED	t-VALUE	p-VALUE	STATISTICAL SIGNIFICANCE
1	**I, II**	16.143	0.00	**SIGNIFICANT**
2	**IV, V**	0.800	0.468	**NOT SIGNIFICANT**
3	**VI, VII**	1.750	0.155	**NOT SIGNIFICANT**
4	**IV, VII**	0.744	0.498	**NOT SIGNIFICANT**
5	**III, VI**	1.225	0.288	**NOT SIGNIFICANT**

ANALYSIS & INTERPRETATION:

Student's "t" test for two independent groups is used to compare the significance of difference between two groups at 5% level of significance.

Note 1: If "p" value is more than 0.05, then we can conclude that there is no significant difference between the two groups considered with regard to mean.

Note 2: If "p" value is less than 0.05, then we can conclude that there is a significant difference between the two groups considered with regard to mean.

TABLE 8: STATISTICAL COMPARISON BETWEEN GROUPS FOR SMEAR

MORE THAN TWO GROUP COMPARISON-Analysis of variance (ANOVA)

S.NO	GROUPS COMPARED	f-VALUE	p-VALUE	STATISTICAL SIGNIFICANCE
1	III, IV, V, VI, VII	1.137	0.393	NOT SIGNIFICANT
2	IV, VI, VII	0.632	0.563	NOT SIGNIFICANT

ANALYSIS & INTERPRETATION:

Analysis of Variance (ANOVA) test is used to compare the significance of difference between more than two groups at 5% level of significance.

Note 1: If “p” value is more than 0.05, then we can conclude that there is no significant difference between the two groups considered with regard to mean.

Note 2: If “p” value is less than 0.05, then we can conclude that there is a significant difference between the two groups considered with regard to mean.

TABLE 9: STATISTICAL COMPARISON BETWEEN GROUPS FOR DEBRIS

TWO GROUP COMPARISON Student's t – test

S.NO	GROUPS COMPARED	t-VALUE	p-VALUE	STATISTICAL SIGNIFICANCE
1	**I, II**	12.075	0.000	**SIGNIFICANT**
2	**IV, V**	2.985	0.041	**SIGNIFICANT**
3	**VI, VII**	0.400	0.710	**NOT SIGNIFICANT**
4	**IV, VII**	1.244	0.281	**NOT SIGNIFICANT**
5	**III, VI**	2.714	0.053	**NOT SIGNIFICANT**

ANALYSIS & INTERPRETATION:

Student's "t" test for two independent groups is used to compare the significance of difference between two groups at 5% level of significance.

Note 1: If "p" value is more than 0.05, then we can conclude that there is no significant difference between the two groups considered with regard to mean.

Note 2: If "p" value is less than 0.05, then we can conclude that there is a significant difference between the two groups considered with regard to mean.

TABLE 10: STATISTICAL COMPARISON BETWEEN GROUPS FOR DEBRIS

MORE THAN TWO GROUP COMPARISON-Analysis of variance (ANOVA)

S.NO	GROUPS COMPARED	f-VALUE	p-VALUE	STATISTICAL SIGNIFICANCE
1	**III, IV, V, VI, VII**	5.674	0.012	**SIGNIFICANT**
2	**IV, VI, VII**	1.578	0.281	**NOT SIGNIFICANT**

ANALYSIS & INTERPRETATION:

Analysis of Variance (ANOVA) test is used to compare the significance of difference between more than two groups at 5% level of significance.

Note 1: If “p” value is more than 0.05, then we can conclude that there is no significant difference between the two groups considered with regard to mean.

Note 2: If “p” value is less than 0.05, then we can conclude that there is a significant difference between the two groups considered with regard to mean.

TABLE 11: STATISTICAL COMPARISON BETWEEN GROUPS FOR EROSION

TWO GROUP COMPARISON Student's t-test

S.NO	GROUPS COMPARED	t-VALUE	p-VALUE	STATISTICAL SIGNIFICANCE
1	I, II	7.000	0.002	SIGNIFICANT
2	IV, V	0.649	0.552	NOT SIGNIFICANT
3	VI, VII	0.580	0.593	NOT SIGNIFICANT
4	IV, VII	1.114	0.328	NOT SIGNIFICANT
5	III, VI	0.310	0.772	NOT SIGNIFICANT

ANALYSIS & INTERPRETATION:

Student's "t" test for two independent groups is used to compare the significance of difference between two groups at 5% level of significance.

Note 1: If "p" value is more than 0.05, then we can conclude that there is no significant difference between the two groups considered with regard to mean.

Note 2: If "p" value is less than 0.05, then we can conclude that there is a significant difference between the two groups considered with regard to mean.

TABLE 12: STATISTICAL COMPARISON BETWEEN GROUPS FOR EROSION

MORE THAN TWO GROUP COMPARISON-Analysis of variance (ANOVA)

S.NO	GROUPS COMPARED	f-VALUE	p-VALUE	STATISTICAL SIGNIFICANCE
1	III, IV, V, VI, VII	0.764	0.572	NOT SIGNIFICANT
2	IV, VI, VII	1.151	0.378	NOT SIGNIFICANT

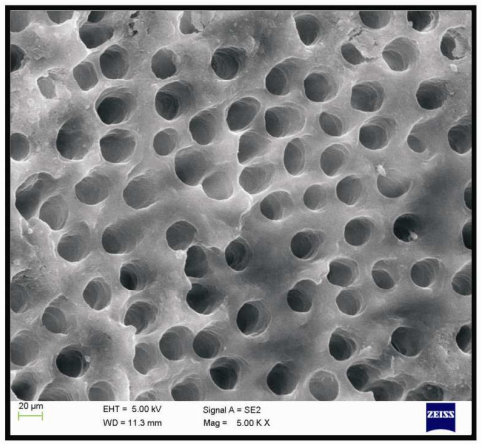
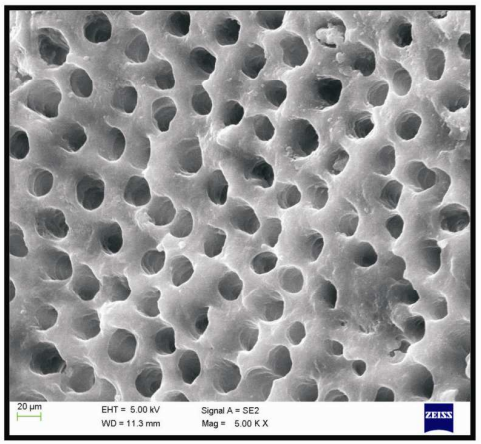
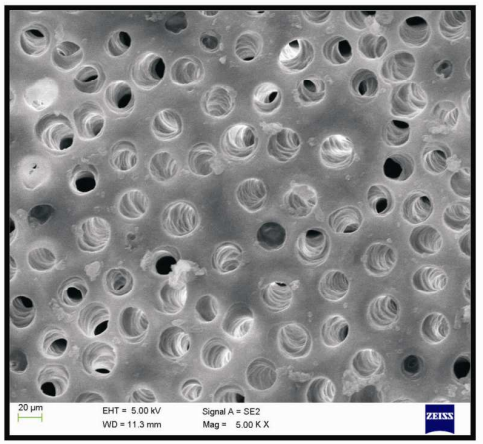
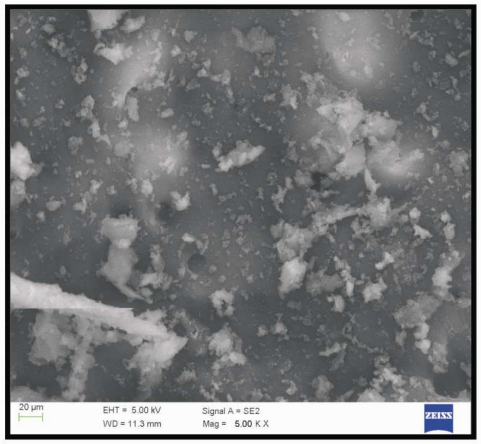
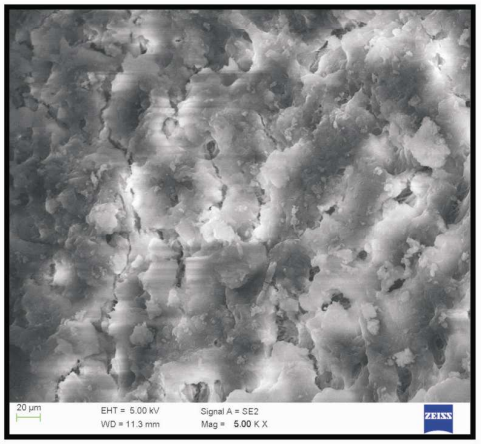
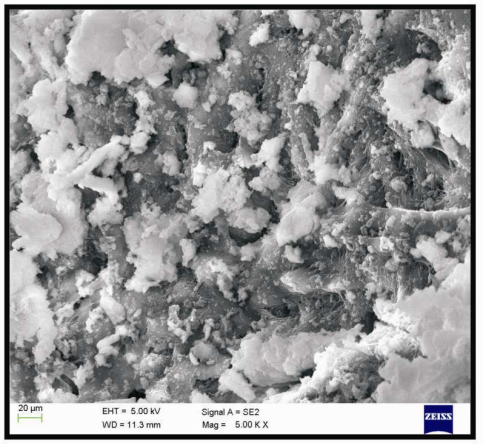
ANALYSIS & INTERPRETATION:

Analysis of Variance (ANOVA) test is used to compare the significance of difference between more than two groups at 5% level of significance.

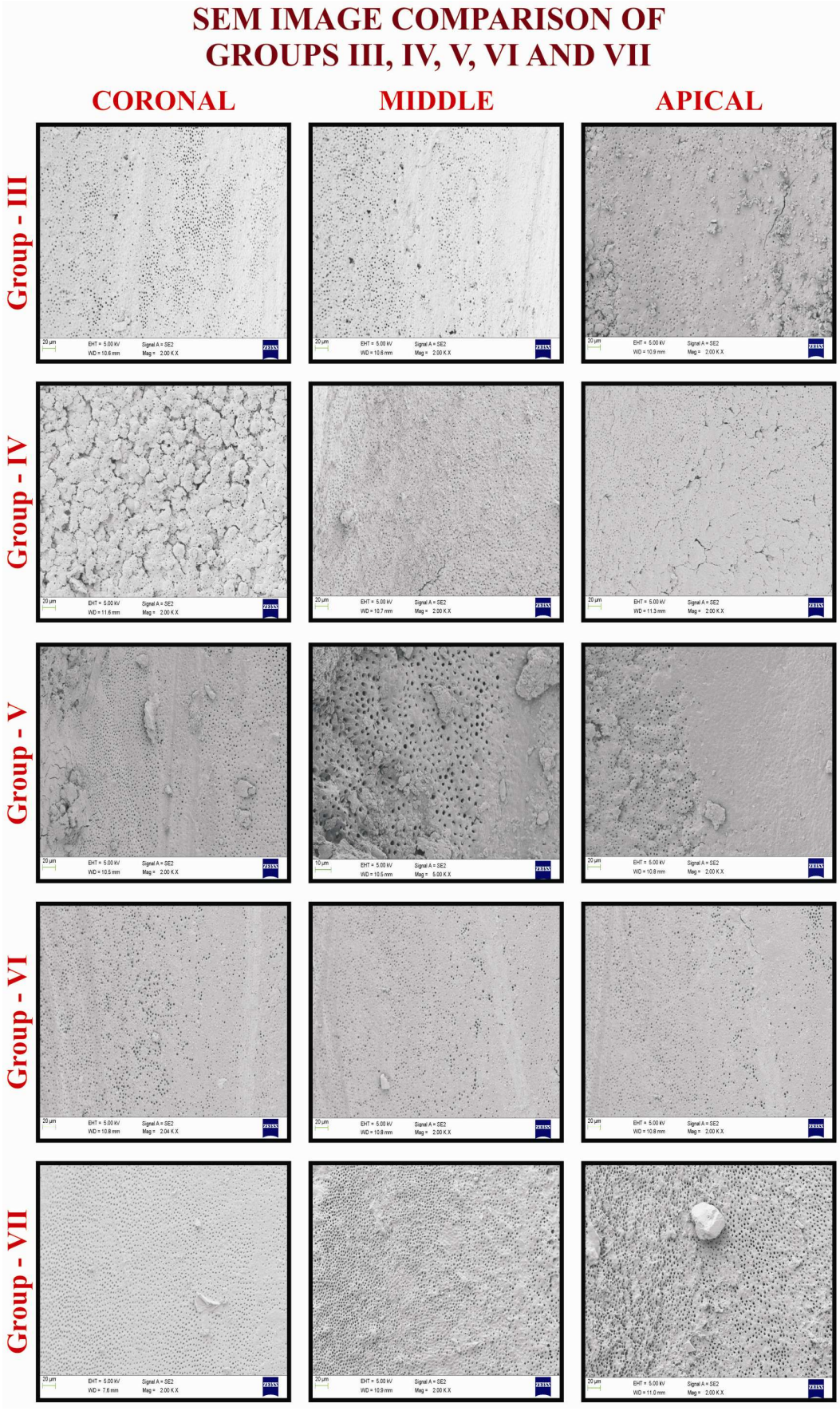
Note 1: If “p” value is more than 0.05, then we can conclude that there is no significant difference between the two groups considered with regard to mean.

Note 2: If “p” value is less than 0.05, then we can conclude that there is a significant difference between the two groups considered with regard to mean.

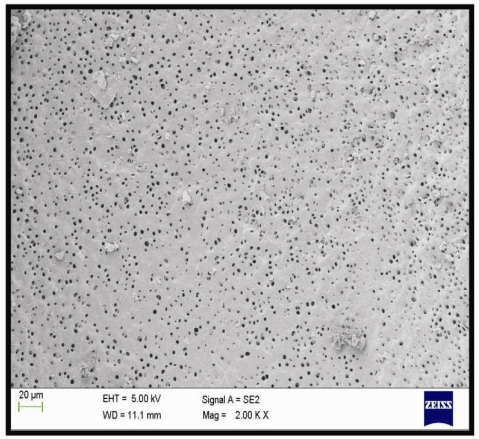
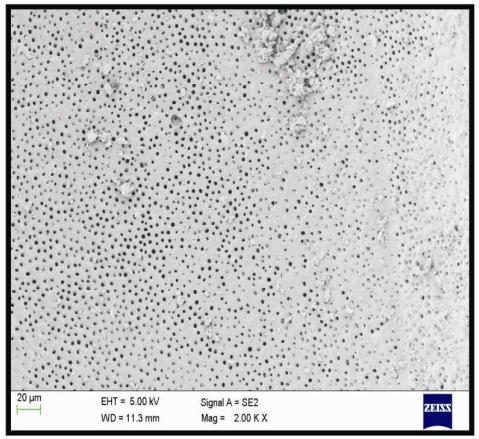
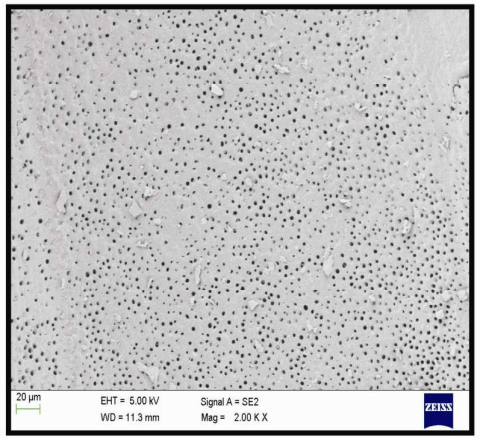
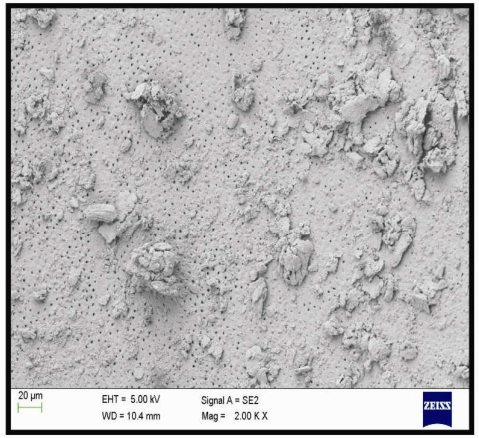
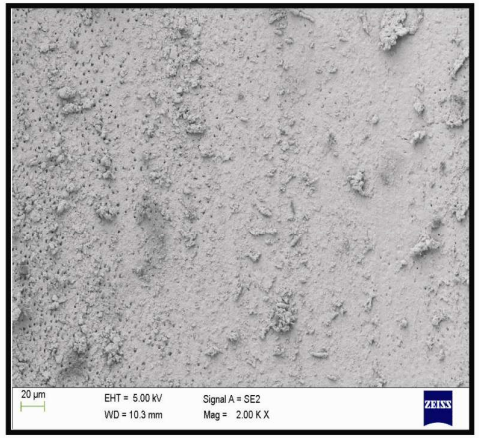
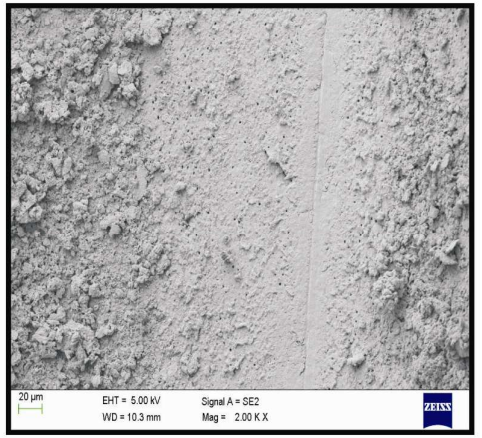
SEM IMAGE COMPARISON OF GROUPS I AND II

	CORONAL	MIDDLE	APICAL
Group - I			
Group - II			

SEM IMAGE COMPARISON OF GROUPS III, IV, V, VI AND VII



SEM IMAGE COMPARISON OF GROUPS VIII AND IX

	CORONAL	MIDDLE	APICAL
Group - VIII			
Group - IX			

The anatomical structure of the root canal space imposes limitations during biomechanical preparation to the root canal space due to the complexity and inherent variations of anatomy. The root canal space in infected teeth contains both vital and necrotic pulp tissue, by-products of the bacterial metabolism, the smear and debris created during the instrumentation procedures. The space is also infected with microbes which have adapted very well to the anaerobic conditions of the root canal. Endodontic therapy aims to clear the canal space of these contaminants. During canal preparation procedures a layer of smear is formed on the surface of the root canal space. Smear layer removal is fairly easy in the occlusal one-third and the middle one third of the root canal. In the apical one third of the root canal it is relatively more difficult to remove smear. The apical third of the root presents challenges with regard to the curvature, size of the canal, the taper and diameter, the ramifications, deltas, isthmuses and permeability of dentin **(Ribero et al in 2012)**[52]. These factors contribute to the apical third being the most difficult to clean.

Eick et al in 1970[21] first reported the presence of a smear layer. This was made possible with the aid of the electron microscope with the scanning electron microscope attachment and was found to be made up of particles of size from 0.5-1.5 micrometers. This was based on their research on cut cavity surfaces of tooth structure. A layer of organic and inorganic material, which contains microorganisms and their byproducts, is formed over the surface of the radicular dentin as a result of the process of biomechanical instrumentation of the root canals. It is made up of small particles of mineralized collagen matrix and is spread over the radicular dentinal surface and is aptly known as the smear layer.

The smear layer produced in a cavity preparation and that during the biomechanical preparation during endodontic therapy is distinctly different and is not directly comparable to smear produced during the cavity preparation procedure, as tooling and procedures are very much different. In addition there is presence of soft tissue remnants. **McCombet al 1975**[44]first reported the presence of smear layer on instrumented radicular dentinal surfaces of the root canal space. They found that this layer contained the remnants of dentinal cutting but also of odontoblastic processes, pulpal remnants and microorganisms. Researchers have reported the thickness of the smear layer to be generally in the range of 1-2μm **(Mader et al 1984)** [40].

The smear produced during a motorized preparation of the canal is much more in volume when compared to hand preparation of the canals (**Czontkowsky et al in 1990**[16]). The use of rotary instruments to prepare root canals results in more volume of smear generation. Smear may vary depending on the nature of dentin, the type and sharpness and geometry of the cutting instruments used to prepare the radicular space. During early stages of biomechanical instrumentation process the smear layer formed on the walls of the root canals can have a relatively high organic content because of necrotic and or viable pulp tissue present in the root canal (**Cameron et al in 1987**[12]. Smear layer has been assessed as having two distinct components, the superficial portion and the deeper layer, which is packed into the dentinal tubules for varying distances ranging from 40 to 110 micrometers. Various mechanisms have been hypothesized for the penetration of the components of smear into the tubular structure. The capillary action between the dentinal tubules and the smear material, the cutting action of the rotary tools (capillary action hypothesis)

possibly explain the tubular packing phenomenon during which strong adhesive forces come into play. Addition of a surface active agent to irrigant solutions increases the depth of penetration of the components of smear layer **(Aktener et al in 1989)** [2].

Though smear layer was first reported almost three decades back, there is a lot of debate on whether to remove or to retain it. The root canal preparation procedure without the removal of a smear has also been proposed by researchers. A hydrodynamic disinfection technique by **Ruddle CJ in 2007**[57] and a non-instrumental hydrodynamic technique by **Lussi et al in 1993**[39] and in which sonically driven polymer instruments with tips of variable diameter have been proposed. The authors who advocated retaining the smear layer proposed that it seals the radicular dentinal tubules restricting penetration of microorganisms and their byproducts into the tubular structure by altering permeability of dentin.

In contrast, others believe that the smear layer must be removed from the radicular dentinal surface as it can harbor microorganisms, debris, prevent effective disinfection of the dentinal tubules and acts as a barrier between the canal wall and the material used for obturation, resulting in an ineffective seal. One of the principal factors affecting the prognosis of endodontic therapy is the failure to obtain a hermetic three-dimensional seal of the root canal system.

A new proposal of smear layer modification in a way that it becomes completely resistant to dissolution or disintegration has been put forward, which results in permanent sealing of the dentinal tubules. Permanent alteration of smear has been observed when treated with Titanium tetra fluoride (TiF4) resulting in a

definitive surface coating which occludes the dentinal tubules regardless of the presence or absence of the smear layer **(Sen and Buyukylimaz in 1998) [60].**

Titanium tetra fluoride used on the radicular surface showed a thicker coating of 1-5μm than the unsmeared surfaces. The commonly used root canal irrigants were not able to remove or reduce the thickness of this surface coating **(Kazemi et al in 1999) [36].** This finding has a tremendous potential in endodontic procedures to minimize microleakage.

Sharavan et al[64] in their meta analysis in **2007** realized that removal of smear from the radicular dentin significantly improves the apical and coronal seal of the obturated root canal and is independent of the type of the sealer type of obturation, the type of dye used for testing, site of leakage, and the duration of the test. A number of reasons have been proposed to support the idea of smear layer removal i.e., the presence of microorganisms, tissue debris, the unpredictable diameter and volume of the canal system, prevents the penetration of irrigants and intracanal medicaments into the dentinal tubules, acts as a bacterial substrate, loosely adherent structure which possibly leads to microleakage, and affects the bond between the sealer material and radicular dentinal structure. Those who recommend that the smear layer should not be removed based their arguments on that it prevents inward or outward movement of microorganisms, other irritants and toxins and effectively blocks the tubules in radicular dentin.

In this study Group VII -presented the least amount of smear among all groups at the apical, middle and coronal third levels with mean value of **1.53 ±0.42 [Table 6 Chart V]**. The Group IV, Group VI and group VIII were found to be close next in removal of smear at a mean value of **1.93 ±0.83, 2.00 ±0.20 and 2.00 ±0.72**

respectively **[Table 6 Chart V]**. On statistical comparison and analysis there was no statistically significant difference between experimental groups III to VIII **(p > 0.05). [Table 7]**

Overall the coronal third presented the least amount of smear with a mean value of **2.02 ±1.26** followed by the middle third with a mean value of **2.34 ±1.15** and the most amount of smear was in the apical third of the canals with a mean value of **2.90 ±0.98. [Table 2 Chart I]**

The chitosan – shrimp shell (groupIV) was comparable to the water soluble chitosan oligosaccharide (groupVI) and chitosan citrate (groupVII) and there was no statistically significant difference. Though the crab shell derived chitosan (groupV) presented with the highest amount of smear amongst the chitosan groups and there was no statistically significant difference **(p > 0.05). [Table8]**

Contrary to the thought that removal of smear layer opens up the dentinal tubules and would thereby increase permeability, smear removal has been shown to alter the diffusion permeability of radicular dentinal surface as reported by **Galvan et al in 1994**[24]. The permeability of dentin increased slowly over a period of two months. They postulated that probably the methodologies created precipitates deep within tubules, which reduced permeability initially, but as they dissolved the permeability increased. There is a possibility that various materials and medicaments that are kept within the root canal space can penetrate and pass through the dentinal tubules to the periodontium and can affect the periodontal status. The diffusion of the medicament into the dentinal tubules also depends on the diffusion properties of the intra canal medicament and not just on the permeability of the dentinal tubules.

Different techniques have been adopted for effective smear removal. Chemical, ultrasonics, LASERS, pressure alternation devices, vaccum assisted devices and more recently sonic cleansing techniques have been used in conjunction with specific irrigant combinations. These irrigants should ideally be able to remove both the organic components of the smear layer and most of the inorganic components. No single irrigant solution complies with all the above mentioned requirements and therefore use of more than one irrigant and specialized techniques have been advocated for effective removal of smear. The concept of a working solution and an irrigant solution was proposed by **Kaufmann in 1986**[35], where the working solution was the one which was first used to cleanse the canal during the preparation procedure and the irrigant solution was the one which was essential to remove the debris and smear layer.

Sodium hypochlorite is anti bacterial, has excellent debriding capacity, which increases with rise in temperature up to 60º Celsius. Its ability to remove smear layer from instrumented canal walls when used alone is insufficient. Chlorine dioxide produces little or no trihalomethanes when compared to sodium hypochlorite. Chlorine dioxide (ClO_2) is similar to sodium hypochlorite and was found to be as efficient in organic tissue dissolution. Trihalomethane is an animal carcinogen and a suspected human carcinogen.

Chelating agents have been tried as root canal irrigant solutions. They have previously been used in endodontics to negotiate calcified canals. EDTA (Ethylene diamine tetra acetic acid) being the most popular in the concentration of 17%. They effectively remove the inorganic component of the smear and radicular dentin surface by a process of chelation. Other chelating agents like citric acid in a

concentration of 10% have been tried. It has also been modified by addition of other substances in an attempt to improve and achieve certain desirable properties. Phytic acid, polyacrylic acid, maleic acid and other organic acids have been tried as chelating agents with varying amounts of success. A 7% Maleic acid solution that is used as a conditioner in adhesive dentistry has been found to be very effective in removal of smear. Poly acrylic acid has also been found to be very effective in concentrations of 10-40%. and a exposure time of not more than 30 seconds was recommended as stated by New Berry et al in 1987 **(M Torabinejad 2003)[71].**

Liquid form of ethylene diamine tetra acetic acid has been most effective at smear removal. Gel types of formulations are also available. Further detergents, peroxides and surfactants have been added to improve the efficacy of removal of debris and smear. Surfactants reduce surface tension and help irrigant solution to effectively penetrate the tubules of radicular dentinal structure. Combinations of ethylene diamine tetra acetic acid and Cetavlon, EGTA (Ethylene Glycol Tetra Acetic acid), REDTA, have also been tried. A 2% ethylene diamine tetra acetic acid and a surface active antibacterial agent Bisdequalinium Acetate was found to be very effective with minimal erosion of peritubular and intertubular dentin. MTAD and Teraclean are formulations where citric acid has been modified suitably with addition of detergents and antimicrobials. In addition it is available as a freeze dried powder, which is freshly constituted before the process of irrigation.

Irrigant solutions with antimicrobial and property of substantivity through adherence to radicular dentin have been tried lately. Tetracycline hydrochloride, Minocycline and doxycycline in addition to their antibacterial properties, at low pH values, have an ability to act as calcium chelators and demineralizes enamel and root

surfaces **(Bjorvatn in 1982)** [8] . Doxycycline in a concentration of 100mg/ml was effective in removing smear layer from the surface of the instrumented canals and also speculated to remain within the tubules for a period of time providing a reservoir of anti-bacterial agent. Peroxides as solutions were more effective in debris removal. But they posed potential risks and hazards when small quantities were inadvertently extruded from the apical foramen.

Researchers tried out new methodologies and found that combinations of irrigants were the most effective at smear removal in the root canal. The sequential use of sodium hypochlorite and 17% ethylene diamine tetra acetic acid was found to be particularly effective at smear layer and debris removal. As there was no single solution that could dissolve the organic tissues and demineralize the inorganic layer, the sequential use of organic and inorganic solvents were advocated **(Baumgartner in 1984)** [5] .

A 5% sodium hypochlorite solution and 17% ethylene diamine tetra acetic acid solution were found to be the most effective in combination. The chemo-mechanical action of sodium hypochlorite removes the loosely attached debris and organic material while chelating action of effectively removes the inorganic part of the smear layer. Various combinations of sodium hypochlorite and other chelating agents have been tried for effective smear removal. Etidronic acid (HEBP:1-Hydroxyethylidene-1,1-bisphosphonate) does not react with sodium hypochlorite in short term and is a potential alternative to ethylene diamine tetra acetic acid or citric acid and is non-toxic.

Cleanliness of the canal is vital for successful outcomes of endodontic therapy. The rotary instruments act primarily in the central body of the canal. The isthumi, cul-de sacs are untouched and under prepared after the completion of biomechanical preparation. These areas serve as a reservoir for microbial ingress, growth and impair the achievement of an effective hermetic seal of the root canal system. The role of the irrigant is vital and necessary to cleanse these areas. Addition of surfactants, irrigant volume, alteration of irrigant temperature, duration of exposure, and activation has been advocated to improve irrigant efficacy. The irrigant solution must be brought into close contact with the entire canal wall for a sufficient period of time, to be effective. An important parameter is mode of irrigant delivery and of date various methods have been attempted.

This study used a side vented needle with the vent at 1mm from the tip in a customized irrigant protocol. Computational dynamic fluid flow has demonstrated the limitations of a side vent needle on irrigant replacement and suitable modifications were made in this study to enhance irrigant replacement. The volume of the irrigant is vital and in this study a volume of 8ml during the initial rinse and 5ml during the final rinse was used. The duration of the exposure of the final rinse is important and a final rinse exposure time of five minutes was used in this study.

To further improve the reach and effectiveness of irrigating solutions within the canal system various agitation techniques have been developed which are either manual or machine assisted. Manual dynamic activation where a well fitting guttapercha point is placed to the working length and moved up and down in 2-3mm strokes, can sufficiently improve the displacement and exchange of the irrigant. This method has been found to be very effective. This method was incorporated into the

custom protocol used in this study in the second minute of the final rinse. Passive irrigation techniques have shortcomings in delivery of irrigant. Machine activated systems are very popular as they are aggressively promoted, which result in a reduced preparation time. Brushes (motorized), plastic files, sonic and ultrasonic systems, reciprocating and pressure alternation devices have been tried. There are no evidence-based studies, which correlate the efficacy of these devices with improved treatment outcomes. LASERS can vaporize tissues in the main canal, remove microorganisms and eliminate residual tissue in the apical portion of the root canals. The main limitations with the laser systems in removal of smear layer are the access to the small canal spaces in the apical one-third of the root and the relatively large laser probes that are available. Research is on to develop thinner probes. LASER activation of the irrigant was found to be effective in smear layer removal **(Peeters and Suardita in 2011)** [49]. Laser activation causes cavitation which is the formation of a vapor or a cavity that contains bubbles inside a fluid which expand 1600 times their volume which allows the irrigants to access the apical portion of the canal more readily and in addition these bubbles become unstable and collapse what is called as an implosion resulting in a shock wave. LASERS generate waves, which enhances the action of the irrigants. LASER activation is done via, a fiber tip and this technique of irrigant activation appears promising in the apical thirds of the canals with closed apices

Certain specially designed file systems, which use vibration and continuous irrigation, have been lately introduced, known as the self-adjusting file system. This system adapts longitudinally to the canal and prepares the canal symmetrically and minimize unnecessary buildup of stresses in the dentin that later

lead to cracks and propagation. The vibrating motion of the file and its delicate meshwork has a synergistic effect with the fluid in the canal that is constantly replaced and this new system has been found to be especially active in the apical one third of the curved root canal.

Elimination of microorganisms from within the canal space, which survive in biofilms and within the radicular dentinal tubules have been a concern and challenge during root canal therapy. Recurrent infections as a result of microbes that survive have necessitated means and mechanisms for their elimination from within the canal space. Antibacterial properties of the irrigant solutions is an important parameter, and as these microbes survive for long periods of time in a dormant state, solutions containing antibacterial components that can bind to the dentinal structure and be released over a period of time (substantivity) have become popular.

Vapor lock effect is as a result of the reaction of the irrigant with smear and debris, releasing bubbles, forming close ended micro-channels, which take a very long time to flood back with the irrigant. A simple method to disrupt the vapor lock effect would be to insert a file or gutta-percha of the size of the prepared canal to working length after instrumentation. Acoustic streaming and cavitation becomes impossible after a vapor lock. Removal of a vapor lock before activation of these systems is necessary in a clinical setting. The present study used a manual dynamic activation technique in the second minute of the final irrigation protocol as it effectively negates vapor lock effect especially in the curved apical thirds of the root. This study adopted a closed ended root canal system. The effectiveness of irrigant protocols is dependent on how effectively it can bring the irrigant solution in contact with the contents of the root canal space.

In this study Group VIII presented the least amounts of debris among all experimental groups at the apical, middle and coronal third levels with mean value of **1.33 ±0.42 [Table 6 Chart V].** Among the chitosan groups Group III had the least debris scores with mean value of **1.67 ±0.31 [Table 6 Chart V] .** The Group IV, Group VII and group VI were found to be close next in removal of debris at a mean value of **1.80 ±0.53, 2.20 ±0.17 and 2.27 ±0.23** respectively. On statistical comparison and analysis there was no statistically significant difference between experimental groups III to VIII **(p > 0.05). [Table 10]**

Overall the coronal third presented the least amount of debris with a mean value of **2.06 ±1.14** followed by the middle third with a mean value of **2.32 ±1.19** and the most amount of debris was in the apical third of the canals with a mean value of **2.68 ±1.05. [Table 3 Chart II]**

The low molecular weight chitosan (groupIII) and (groupIV) presented with least scores for debris among the chitosan groups and the results were not statistically significant when compared to the water soluble chitosan oligosaccharide (groupVI) and chitosan citrate (groupVII). Though the crab shell derived chitosan (groupV) presented with the highest amount of debris amongst the chitosan groups and there was no statistically significant difference **(p > 0.05). [Table 10]**

Attrition, abrasion, occlusal trauma, caries etc lead to formation of sclerotic dentin at the apical third of the root canal. The difficulties of irrigation could be effectively encountered by a four walled access preparation to hold more irrigant, creating sufficient taper, sufficient apical enlargement, pre operative analysis of the apical third, deeper positioning of the irrigant delivery, vapour lock elimination, sufficient irrigant activation, volume and time of exposure.

New machine assisted systems have been designed, with the aim of achieving apical cleanliness. Predictability and to overcome the difficulties commonly encountered during the irrigation procedures. The sonic and the ultrasonic systems employ the principle of activation of the irrigant. The Rins-endo system that is a pressure alternation system delivers the irrigation at a flow rate of 6.2ml/min using pressure–suction technology for irrigant activation. A mechanical action is generated by the device that produces a hydrodynamic change **(Caron et al in 2010)**[13]. Vaccum assisted apical negative pressure systems have been introduced which aim at delivering the irrigant safely to the working length. Here the irrigant solution is pulled into the canal from the pulp chamber and removed by negative pressure at working length.

Though the chemical based irrigant solutions are effective in cleansing the canal they do have attendant clinical complications even when a small amount of the solution is extruded beyond the apical foramen. This has led to the search of new efficient irrigant solutions that are biocompatible, biodegradable and easily available for use as a final rinse solution. Ethylene diamine tetra acetic acid is not originally found in nature and is considered an environmental pollutant. Other acids like citric acid, apple cider vinegar have been tried and have low cytotoxicity. Sodium hypochlorite a recommended irrigant solution has also been found to be hazardous when extruded beyond the apex and has other disadvantages. Electrochemically activated water, Morinda citrifoila have been found to be much more safer for use and cause very less side effects. The use of these naturally available substances should be considered for use as a irrigating solutions. Chitosan has excellent

qualities of biocompatibility, biodegradability, lack of toxicity and bio adhesion and is abundantly available in nature. It has also low production costs.

Chitosan is a polysaccharide with a chemical name of 2-amino2-deoxy-b-D- glucopyranose with a molecular formula of $(C_6H_{11}O_4N)_n$ derived from the shells of crustaceans such as shrimp, crab etc,. It also includes Pandalus borealis and the cell walls of fungi. This is essentially a waste product of the crab and shrimp industries. This substance is derived from chitin, is insoluble in water and alcohol. The poor solubility of chitin is a limitation in the use of the material. In spite of the limitations they have found many applications that include, sutures that are absorbable, wound dressings, raw material for man made fibers, as chelating agents. The different applications of chitosan and chitin require different properties, which are achieved with the degree of deacetylation and the variations in molecular weight. They have found applications in different industries like medical, engineering, food processing, textiles paper, agriculture photography, biomedical and tissue engineering **(Silva P V., et al in 2012)** [65].

Chitosan is derived by the partial deacetylation of chitin. Both chitin and chitosan are in commercial production in countries like India, Japan, Poland, Norway and Australia. They have excellent properties such as biocompatibility, bio-degradability, non-toxicity and adsorption. Chitosan is only soluble in dilute acid solutions. Water soluble chitosan is also available. Oxidation caused by sodium hypochlorite alters the structure and molecular weight of chitosan. The high cost of extraction and purification of chitin chemically limits the use of this polymer to high value applications. Biomedical and pharmaceutical are the most promising fields of application.

Sodium hypochlorite has been used as an antibacterial and for debriding the canals during the irrigation process. It causes damage to the collagen structure of dentinal tubules by denaturation and dissolution and it is not very effective against biofilms. Chitosan has been shown to have an antibacterial effect against gram positive and gram negative organisms and fungi. The covalent immobilization of chitosan on collagen has been proposed to induce the remineralization of the dentin surface. The calcium ions on the dentinal surface bind to functional phosphate groups of the chitosan molecule. This leads to the formation of a favourable surface for crystal nucleation. The antibacterial mechanism of chitosan has been attributed to its poly-cationic nature. It interacts with microorganisms altering their cell permeability and subsequent leakage of intra cellular constituents. It also improves the resistance of collagen of radicular dentinal surface to degradation by collagenase. The use of chitosan has been shown to alter the bacterial adhesion mechanisms thereby preventing biofilm formation **(Carpio-perochina., et al in 2015)**[14] .

Various theories have been put forward for chelation mechanism of chitosan to dentinal structure. The first is the bridge model theory, which states that two or more amino groups of a chain of chitosan bind to the same metal ion. The second theory states that only one amino group of the chitosan is involved in the binding. **(Blair H S., et al in 1981)**[7] Chitosan polymer is composed of several units of dimer of chitin that has got nitrogen atoms with free pairs of electrons, which lead to ionic interaction between the metal and the chelating agent. In an acid medium this forms an ionic form, which results in the amino groups being protonated, which is responsible for attraction to other molecules and results in adsorption. The process

of formation of complexes between chitosan and metal ions occurs as a result of ion exchange, adsorption, and chelation. The prevalent conditions like the pH of the solution, the chemical structure of chitosan and the type of ions determine the type of interaction, which takes place.

Chitosan has been used to repair bone and has been shown to be one of the most promising dental biomaterials. It improves bone regeneration in dental bone loss **(Ezoddikini et al in 2011)** [22]. It has also been shown to increase salivary secretion when incorporated in chewing gums. It also exerts an anti-bacterial effect and suppresses the growth of oral microorganisms. **(Hayashi Y., in 2007)**[30] A reduction in the microhardness of dentin clinically facilitates the negotiation of narrow and curved canals. The reduction in microhardness facilitates easy instrumentation procedures. Chitosan has been shown to reduce the micro hardness of dentin when used as an irrigating solution within the root canal and its effects greater in higher concentrations. **(Pimenta J A., in 2007)**[50]

Citric acid has been tried out as an irrigant in endodontics successfully in varying concentrations. A 10% concentration of citric acid has been found to be very effective in chelation of calcium ions. Higher concentrations have been effective in the removal of smear layer, but have been known to cause dentinal erosion. It has also got an anti-bacterial property, which is directly proportional to its concentration. There arises a situation where citric acid alone is unable to provide effective antimicrobial and chelation properties at the same concentration. To be able to have both these desirable properties researchers have attempted to modify citric acid by addition of other antimicrobials and detergents eg., MTAD and Tetraclean. These formulations have been found effective at smear layer, and very effective against

enterococcus faecalis. These formulations have been reported to cause bacterial resistance. **(Rossi-Fedele G et al in 2007)** [55]

Citric acid has been accepted as an endodontic irrigant and has been modified to improve its properties. Chitosan has excellent anti bacterial properties and is a chelating agent. It is soluble fully in dilute acids. The mixture of chitosan and citric acid would be beneficial in improving the antibacterial and smear clearing efficacy. In the present study the mixture of chitosan and citric acid as chitosan citrate solution has been evaluated as an irrigant solution in the apical, middle and coronal thirds of the root canal.

Overall the middle third presented the least amount of erosion with a mean value of **1.62 ±0.41** followed by the apical third with a mean value of **1.74 ±0.63** and the most amount of erosion was in the coronal third of the canals with a mean value of **1.81 ±0.73. [Table 4 Chart III]**

In this study Group III -presented the least amounts of erosion among all groups at the apical, middle and coronal third levels with mean value of **1.60 ±0.46 [Table 6 Chart V].**The Group VI, Group VII and group V were found to be close next in erosion at a mean value of **1.73 ±0.59, 1.93 ±0.12 and 1.97 ±0.76** respectively. On statistical comparison and analysis there was no statistically significant difference between experimental groups III to VIII **($p > 0.05$). [Table 12]**

The results of this study indicate that Group VII is the most efficient in removal of smear, Group VIII for debris, and Group III minimal in erosion values. Though the result of this study appears promising and Chitosan is available abundantly, the processing and commercial deacetylation process makes the

chitosan very expensive which is a limitation for a material which is available in plenty. Preparation of these chitosan solutions were done freshly in this study and needed a special mixing protocol to incorporate the chitosan into solution and higher concentrations tended to be more viscous. The incorporation of citric acid with chitosan seems a step in the right direction. The biocompatibility of this material, lack of toxicity and excellent biodegradability makes this material and ideal choice for a final rinse solution.

One hundred and fifty two maxillary incisors and canines were collected cleaned and stored in normal saline. They were investigated for the presence of root with normal apex and a patent straight canal devoid of any irregularities,defects or anomalies. Subsequently one hundred and twenty teeth were selected and standardized.

The apices of the selected teeth were sealed with wax and embedded in polyvinyl siloxane material after appropriate coding. They were divided into control (n=5) and experimental groups (n=10). A total of eighty of the selected teeth were used for the purpose of the study. The canals were prepared using Protaper rotary files with X-Smart plus endomotor with 1:16 reduction hand piece as per the manufacturer recommendations. Protocols for initial irrigant rinse during instrumentation and final rinse after instrumentation were implemented. Chitosan was evaluated for it’s efficacy as a root canal irrigant solution in different available forms and concentrations in the apical, middle and coronal thirds of the root canal using a new tool the field emission scanning electron microscope. The images were recorded, results tabulation was done and analysed statistically.

On conclusion of the study, on the effect of different irrigation protocols followed on the removal of smear, debris and erosion in straight canals using field emission Scanning Electron Microscopy, the following conclusions are made:

- Overall the **Group VII** presented the least amounts of smear among the experimental groups at the apical, middle and coronal one-thirds of the root canal with a mean value of **1.53 ±0.42 [Table 6 chart 5].**

- Overall the **Group VIII** presented the least amounts of debris among the experimental groups at the apical, middle and coronal one-thirds of the root canal with mean values of **1.33 ±0.42 [Table 6 chart 5].**

- Overall the **Group III** presented the least amounts of erosion among experimental groups at the apical, middle and coronal one-thirds of the root canal with mean values of **1.60 ±0.46** for **[Table 6 chart 5].**

- On comparison of the groups III, IV, V, VI and VII there was no significant difference statistically **(p > 0.05) [Table 7,9,10].**

- Among the experimental groups, **Group IV** presented with the highest amount of erosion with loss of peri-tubular and intertubular dentin at all levels mean values of **2.33 ±0.61** for **[Table 6 chart 5].**

- Based on the results of this study the use of Chitosan citrate and Chitosan oligosacchride as a final rinse irrigant seems promising. Further evaluation of these irrigants in a clinical setting is recommended.

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