EFFICACY OF COLD ATMOSPHERIC PRESSURE (CAP) PLASMA JET FOR SMEAR LAYER REMOVAL IN INTRA-RADICULAR DENTIN WHEN COMPARED WITH TWO OTHER CHELATING AGENTS USED AS A FINAL RINSE – AN IN-VITRO SCANNING ELECTRON MICROSCOPIC EVALUATION

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CERTIFICATE

This is to certify that thesis entitled "Efficacy of Cold Atmospheric Pressure (CAP) Plasma Jet for smear layer removal in intra-radicular dentin when compared with two other chelating agents used as a final rinse – an In-vitro Scanning Electron Microscopic Evaluation" is an original work of **Dr. Ankita Kapoor** carried out under our direct supervision and guidance at Department of Dentistry, All India Institute of Medical Sciences, Jodhpur.

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DECLARATION

I, hereby declare that the work reported in the thesis entitled **"Efficacy of Cold Atmospheric Pressure (CAP) Plasma Jet for smear layer removal in intra-radicular dentin when compared with two other chelating agents used as a final rinse – an In-vitro Scanning Electron Microscopic Evaluation"** embodies the result of original research work carried out by me in the Department of Dentistry, All India Institute of Medical Sciences, Jodhpur.

I further state that no part of the thesis has been submitted either in part or in full for any other degree of All India Institute of Medical Sciences or any other institution/University.

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LIST OF ABBREVIATIONS

ANOVA : Analysis of variance

APNP : Atmospheric Pressure Nonequilibrium Plasma

APPJ : Atmospheric Pressure Plasma Jet

AP : Apexit

BisGMA : Bisphenol A-Glycidyl Methacrylate

CAP : Cold Atmospheric Pressure

CAPP : Cold Atmospheric Pressure Plasma

CFU : Colony Forming Units

CHX : Chlorhexidine

CMP : Chemo Mechanical Preparation

CP : Cold Plasma

CTR : Cetrimide

DBD : Dielectric Barrier Discharge

EDTA : Ethylene Diamine Tetraacetic Acid

E.Faecalis : Enterococcus Faecalis

Er: YAG : Erbium- doped Yttrium Aluminium Garnet

HEBP : Etidronic acid

HEMA : 2-hydroxyethyl methacrylate

KV : Kilo Voltage

LTAPP : Low Temperature Atmospheric Pressure Plasma

MTAD : Mixture of Tetracycline Acid and Detergent

µm : Micro-meter

µTBS : Micro Tensile Bond Strength

NaOCl : Sodium Hypchlorite

Nd: YAG : Neodymium- doped Yttrium Aluminium Garnet

Ni-Ti : Nickel Titanium

NTAPP	: Non Thermal Atmospheric Pressure Plasma
NTP	: Non Thermal Plasma
PAD	: Photo Activated Disinfection
PDT	: Photo Dynamic Therapy
pH	: Potential of Hydrogen
PTU	: ProTaper Universal
PW	: Pulse Width
RCT	: Root Canal Treatment
ROS	: Reactive Oxygen Species
RNS	: Reactive Nitrogen Species
RS	: Real Seal
SS	: Stainless Steel
SEM	:Scanning Electron Microscope
TEM	:Transmission Electron Microscope

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INTRODUCTION

INTRODUCTION

The goals of endodontic treatment are the removal of diseased tissue, elimination of bacteria present in the canals and dentinal tubules, thereby minimizing the possibility of post treatment recontamination. These can be achieved by thorough cleaning, shaping, and disinfection of the root canal system; by 3-dimensional obturation, thus achieving a fluid-tight seal, and by placing an effective coronal seal (1). Shaping of the root canal system not only involves prime debriding function but also provides radicular access for the irrigant and root canal filling material to complex pulp space anatomy. High-resolution computed tomography shows that nearly 35-53 % of the root canal surfaces remain un-instrumented, which indicates that instrumentation alone is inadequate (2). Thus, when files produce shaping, it is equally essential that irrigants clean and disinfect the root canal system.

Instrumentation, depending upon the design of the instruments, removes most of the residual pulpal tissue and dentinal debris by engaging them, however some gets compacted and burnished against the root canal walls. Whenever dentin is being cut using hand or rotary instruments, the mineralized tissue does not get shredded or cleaved but is shattered to produce a considerable amount of dentinal mud resulting in a smear layer that covers root canal walls and the openings to the dentinal tubules (3). According to American Association of Endodontists (2020), endodontic smear layer is defined as “a surface film of debris retained on dentin or other surfaces after instrumentation with either rotary instruments or endodontic files; it consists of dentin particles, remnants of vital or necrotic pulp tissue, bacterial components, and retained irrigant" (4).

The identification of the smear layer using scanning electron microscope (SEM) was first reported by Eick (1970). McComb and Smith were the first researchers to report the presence of a smear layer on the surface of instrumented root canals in 1975. According to Mader et al. (1984), the smear layer consists of a superficial layer on the surface of the canal wall approximately 1 to 2μm in thickness and a deeper layer packed into the dentinal tubules to a depth of up to 40μm (5). It has been observed that bacteria can persist in the smear layer and in the dentinal tubules despite instrumentation of the root canals. Therefore, the presence of smear layer may block the antimicrobial effects of intracanal disinfectants to reach into the tubules. The systematic review by Shahravan et al. (2007) concluded that it is the eradication of smear layer that enhanced the fluid tight seal of the root canal system, while no significant effect was observed with other factors such as filling technique or the type of sealer (6).

So far the most commonly used method of smear layer removal is the chemical method using chelating agents (7). Other currently employed methods of smear layer removal include the use of ultrasonics and laser techniques. The properties of various chemicals have been studied and it was observed that the pH and time of exposure of the materials determine the quantity of smear layer removed from the dentinal surfaces. Chelating agents remove the inorganic part of the smear layer and demineralize dentin (upto a depth of 20-50μm). The irrigants combining chelating and antimicrobial agents have a wide spectrum of antimicrobial activity and low toxicity along with chelation properties (8).

Nygaard-Ostby first introduced chelators in endodontics in 1957. They recommended the use of 15% EDTA solution (pH 7.3) which comprised of disodium salt of EDTA (17.00g), aqua distilla (100.00ml) and 5M sodium hydroxide (9.25ml) (9). EDTA is a polyaminocarboxylic acid and a colorless, water soluble solid. Its usefulness arises because of its role as a hexadentate ligand and chelating agent, i.e. its ability to sequester metal ions such as Ca^{2+} and Fe^{3+}. After being bound by EDTA, metal ions remain in solution, but exhibit diminished reactivity. EDTA chelates with the divalent calcium ions of the dentinal surfaces which results in formation of soluble calcium chelates. It has been reported that EDTA decalcified dentin up to a depth of 20-30μm in 5 min (10).

EDTA normally used at a concentration of 17%, removes the smear layer in less than 1 minute if the solution reaches the surface of the root canal wall (8). Since EDTA solutions change pH during demineralization, the effect is self-limiting; as pH decreases, both the rate of dentin demineralization and the amount of dentin dissolved decrease (11).

An alternative solution for removal of the smear layer employs the use of MTAD as final rinse. MTAD was introduced by Torabinejad and Johnson at the Loma Linda University in 2003, which is an aqueous solution of 3% doxycycline, a broad-spectrum antibiotic; 4.25% citric acid, a demineralizing agent; and 0.5% polysorbate 80 detergent (Tween 80). In this product, doxycycline hyclate is used instead of its free-base doxycycline monohydrate, to increase the water solubility. It is commercially available as a 2-part mixture (Biopure MTAD; Dentsply). According to some studies use of MTAD has been reported to be more efficient in removing smear layer as compared to the use of EDTA and NaOCl, especially in the apical third (12). Torabinejad et al. showed that MTAD effectively removed smear layer without significantly changing the structure of the dentinal tubules when used after NaOCl (5.25%) in comparison to irrigation with 17% EDTA and 5.25% NaOCl (13).

In recent years, Cold (less than 40 °C at the point of application) Atmospheric Pressure (CAP) Plasma sources have been introduced that provides the possibility to extend plasma treatment to living tissue (14). Many previous studies have realized that non-thermal plasma is a promising technology because of the existence of ultraviolet (UV), reactive oxygen species (ROS), and reactive nitrogen species (RNS). Possible applications of CAP Plasma in Conservative Dentistry and Endodontics include: decontamination of dental caries, sterilization, elimination of biofilms, root canal disinfection, increase in bond strength at the dentin/composite interface and bleaching (15).

Many different types of plasma devices, including nanosecond pulsed plasma pencils, radio-frequency plasma needles, direct-current plasma brushes, and Dielectric Barrier Discharge (DBD) plasma jets have been developed for non-thermal atmospheric-pressure plasma generation.

Dielectric Barrier Discharge (DBD) plasma jet consists of dielectric layers covering the electrodes to restrict the flow of charge carriers generated as soon as the gas is ionized. This causes voltage drop across the plasma when it is ignited. It doesn’t allow discharge current to increase very high which ceases arcing (maintained in glow discharge). Uniform diffused DBD plasma can be produced under certain special conditions by adjusting the frequency range and gas mixture, which is free from filaments. DBDs, typically driven by sine-wave high voltages at several kHz, cover at least one of the two electrodes with dielectric materials to limit the discharge current. The plasma plume generated causes minimal heating of biological materials and is safe to touch with bare hands without causing burning sensation or pain.

Jiang C et. al in 2009 (16) observed surface changes in dentin after exposing the same with a pulsed plasma dental probe. Biological specimen temperatures did not exceed 35°C even after 5 min of plasma exposure under ambient conditions. SEM images showed predominantly clean surfaces revealing open dentinal tubules in the treated area with a visible contrast line distinguishing the zones for plasma treated and non-plasma-treated surfaces. The plasma-treated surface extends 1 mm long in the root canal.

In another study Lehmann et al. in the year 2013, observed changes in surface morphology of dentin after plasma treatment as the inter-tubular areas appear slightly roughened. After exposure to plasma on etched dentin, smear plugs were removed from the dentinal tubules and orifices of the tubules appeared irregularly enlarged (17).

Studies comparing the smear layer removal effectiveness of CAP Plasma Jet with the currently available chelating agents are few and far between. Therefore, this study was envisaged to compare the smear layer removal ability of CAP Plasma Jet, with the known chelating agents like EDTA, MTAD and a control group i.e., Normal Saline.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Ciucchi et al.,(18) **(1989)** compared the effectiveness of different irrigation procedures on the removal of the smear layer. Forty curved canals were manually prepared in vitro under copious irrigation with 3 percent NaOCl. Ten canals served as controls. The remaining 30 were equally distributed in three experimental groups and subsequently irrigated with ultrasound with NaOCl; EDTA; ultrasound with EDTA. In the scanning electron microscope, the appearance of the smear layer was rated over the distribution of dentinal tubule openings for the coronal, middle and apical segments of the canals. Irrigation with NaOCl produced consistently smeared surfaces. Ultrasonic stirring of NaOCl removed the smear layer moderately, while EDTA produced almost smear-free surfaces. Ultrasound in association with EDTA did not enhance the dissolving capability of this chelating agent. A definite decline in the efficiency of the irrigation procedures was also observed along the apical part of the canals.

Garberoglio R et al.,(11) **(1994)** evaluated in an in-vitro study, the effect of six endodontic irrigants on smear layers created by hand instrumentation in the middle and apical sections of 53 root canals. The irrigants evaluated were: 1% and 5% sodium hypochlorite, a combination of 24% phosphoric acid and 10% citric acid, 0.2%, 17%, and 3% ethylenediaminetetraacetic acid. After instrumentation and treatment with the respective irrigants, the root canal specimens were examined by scanning electron microscopy to determine the presence or absence of smear layer. The two sodium hypochlorite solutions did not remove the smear layer, even when 5% sodium hypochlorite was scrubbed on the dentinal walls. The 0.2% ethylenediaminetetraacetic acid solution was more effective than sodium hypochlorite, but it did not completely remove the smear layer, especially at the orifices of the dentinal tubules. The other three solutions effectively removed the smear layer, but no significant difference was found between them ($p > 0.05$). The solution of 3% ethylenediaminetetraacetic acid was as effective as phosphoric-citric acid and 17% ethylenediaminetetraacetic acid. Ethylenediaminetetraacetic acid, however, did not show the marked demineralizing effect on the dentinal walls and tubules as the acidic solution.

Sen B.H. et al.,(19) **(1995)** reviewed Smear layer: a phenomena in root canal therapy. It stated that when the root canals are instrumented during endodontic therapy, a layer of material composed of dentine, remnants of pulp tissue and odontoblastic processes, and sometimes bacteria, is always formed on the canal walls, this layer has been called the smear layer. It has an amorphous, irregular and granular appearance under the scanning electron microscope. The advantages and disadvantages of the presence of smear layer, and whether it should be removed or not from the instrumented root canals, are still controversial. It has been shown that this layer is not a complete barrier to bacteria and it delays but does not abolish the action of endodontic disinfectants. Endodontic smear layer also acts as a physical barrier interfering with adhesion and penetration of sealers into dentinal tubules. In turn, it may affect the sealing efficiency of root canal obturation. When it is not removed, the durability of the apical and coronal seal should be evaluated over a long period. If smear layer is to be removed, EDTA and NaOCl solutions have been shown to be effective, among various irrigation solutions and techniques, including ultrasonics, that have been tested. Once this layer is removed, it should be borne in mind that there is a risk of reinfecting dentinal tubules if the seal fails. Further studies are needed to establish the clinical importance of the absence or presence of smear layer.

S Calt et al.,(20) **(2002)** in his study evaluated the effects of EDTA on smear layer removal and on the structure of dentin, after 1 and 10 min of application. Six extracted single-rooted teeth were instrumented to #60. Apical and coronal thirds of each root were removed, leaving a 5 mm middle third which was then cut longitudinally into two equal segments. Using 10 ml of 17% EDTA solution, halves belonging to the same root were irrigated for 1 and 10 min, respectively. All specimens were subjected to irrigation with 10 ml of 5% NaOCl. All the specimens were prepared for SEM evaluation which showed that 1 min EDTA irrigation is effective in removing the smear layer. However, a 10-min application of EDTA caused excessive peritubular and intertubular dentinal erosion.

Torabinejad M et al.,(13) **(2003)** investigated the effect of a mixture of a tetracycline isomer, an acid, and a detergent (MTAD) as a final rinse on the surface of instrumented root canals. Forty-eight extracted single-rooted human teeth were irrigated using Sterile-distilled water or 5.25% sodium hypochlorite as intracanal irrigant and with 5ml of sterile distilled water, 5.25% sodium hypochlorite, 17% EDTA, or a new solution, MTAD as a final rinse. The SEM evaluation results concluded that MTAD is an effective solution for the removal of the smear layer and does not significantly change the structure of the dentinal tubules when used as a final rinse of MTAD.

Torabinejad M et al.,(21) **(2003)** did a study to investigate the effect of various concentrations of sodium hypochlorite (NaOCl) as an intracanal irrigant before the use of MTAD (a mixture of a tetracycline isomer, an acid, and a detergent) as a final rise to remove the smear layer. Ten operators prepared 80 single- and multirooted human teeth, using a combination of passive step-back and rotary 0.04 taper, nickel-titanium files. The canals were then treated for 2min with 5ml each solution as a final rinse: 5.25% NaOCl, sterile distilled water, 17% EDTA, or MTAD. The presence or absence of smear layer and the amount of erosion on the surface of the root canal walls at the coronal, middle, and apical portion of each canal were examined under a scanning electron microscope. The results showed that although MTAD removed most of the smear layer when used as an intracanal irrigant, some remnants of the organic component of the smear layer remained scattered on the surface of the root canal walls. The effectiveness of MTAD to completely remove the smear layer was enhanced when low concentrations of NaOCl were used as an intracanal irrigant before the use of MTAD as a final rinse.

Hulsmann M et al.,(9) **(2003)** reviewed chelating agents in endodontics. They mentioned that chelating agents were introduced into endodontics as an aid for the preparation of narrow and calcified root canals in 1957 by Nygaard-Østby. A liquid solution of ethylenediaminetetraacetic acid (EDTA) was thought to chemically soften the root canal dentine and dissolve the smear layer, as well as to increase dentine permeability. Although the efficacy of EDTA preparations in softening root dentine has been debated, chelator preparations have regained popularity recently. Almost all manufacturers of nickel-titanium instruments recommend their use as a lubricant during rotary root canal preparation. Additionally, a final irrigation of the root canal with 15-17% EDTA solutions to dissolve the smear layer is recommended in many textbooks. They reviewed the relevant literature on chelating agents, and presented an overview of the chemical and pharmacological properties of EDTA preparations and the recommendations for their clinical use.

Tay FR et al.,(22) **(2006)** examined the structure of mechanically instrumented intraradicular dentin after initial irrigation with NaOCl and BioPure MTAD as the final rinse from the coronal, middle, and apical parts of root canal walls using TEM. The respective positive and negative controls had distilled water and EDTA as final rinse. Both irrigants created a zone of demineralized collagen matrices in eroded dentin and around the dentinal tubules, with the mildly acidic BioPure MTAD being more aggressive exposing collagen matrices 1.5-2 times thicker than produced with EDTA.

Shahravan A et al.,(6) **(2007)** conducted a systematic review to determine whether smear layer removal reduces leakage of obturated human teeth in-vitro. Among 26 eligible papers with 65 comparisons, 53.8% of the comparisons reported no significant difference, 41.5% reported a difference in favor of removing the smear layer, and 4.7% reported a difference in favor of keeping it; differences were significant ($p<0.001$). Of the 65 comparisons, 44 used the dye leakage test for evaluation which showed smear layer removal decreases dye leakage. It was 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 significant effects.

Saleh IM et al.,(23) **(2007)** studied effect of the smear layer on the penetration of bacteria along different root canal filling materials and to examine the dentine/sealer and sealer/core material interfaces for the presence of bacteria. Out of 110 human root segments half of the roots were irrigated with a 5-mL rinse of 17% EDTA. Roots with and without smear layer were filled with gutta-percha (GP) and AH Plus sealer (AH), GP and Apexit sealer (AP), or RealSeal cones and sealer (RS). It was concluded that removal of the smear layer did not impair bacterial penetration along root canal fillings, but it slowed down the leakage. A comparison of the sealers revealed no difference except that AH performed better than RS in the absence of the smear layer.

Ballal et al.,(24) **(2009)** assessed the ability of 17% EDTA and 7% maleic acid in the removal of the smear layer from the human root canal system by scanning electron microscopic (SEM) analysis. Eighty single-rooted human anterior teeth were subjected to standardized root canal instrumentation (step-back technique) and were irrigated with 2.5% NaOCl after each instrument. Based on the final irrigating solution used, samples were divided randomly into three groups: the EDTA group: 17% EDTA+ 2.5% NaOCl (n = 30), the maleic acid group: 7% maleic acid + 2.5% NaOCl (n = 30), and the control group: 0.9% saline (n = 20). After final irrigation, teeth were prepared for SEM analysis to evaluate the cleaning of the coronal, middle, and apical thirds of radicular dentin by determining the presence or absence of smear layer. They found that at the coronal and middle thirds, there was no significant difference between EDTA and maleic acid. In the apical third, maleic acid showed significantly better smear layer removing ability than EDTA.

Mancini M et al.,(25) **(2009)** compared the efficacy of Bio-Pure MTAD, 17% EDTA, and 42% citric acid in endodontic smear layer removal and degree of erosion in the apical third of endodontic canals. They randomised ninety six extracted single rooted teeth into four groups (n = 24), and final irrigation was done by: BioPure MTAD, 17% EDTA, 42% citric acid, or 5.25% NaOCl (control) followed by 5.25% NaOCl. SEM evaluation showed no significant differences among test irrigants in removing the smear layer. It was concluded that efficacy of BioPure MTAD and 17% EDTA in smear layer removal was significantly greater than 5.25% Na0C1 (control).

Mozayeni MA et al., (26) **(2009)** compared the effectiveness of MTAD with that of 17% EDTA in smear layer removal as the final irrigant following irrigation with 5.25% sodium NaOCl. Fifty-five single-rooted human teeth were subjected final rinse of 5ml of 5.25% sodium hypochlorite or 17% EDTA or MTAD. SEM evaluation showed MTAD to be an effective final rinse solution for removing smear layer in canals irrigated with NaOCl. While 17% EDTA removed smear layer from the middle and coronal thirds of canal preparations, but it was less effective in the apical third of the canals.

Jiang C et al.,(16) **(2009)** gave a novel coaxial tubular device capable of generating a 2.5 cm long pencil-like plasma plume in ambient atmosphere recently developed to disinfect root canal systems during endodontic treatment. Powered with short (100 ns), intense (6 kV) electric pulses at 1 kHz, the plasma dental probe was safe for operation, electromagnetic noise free, with low power consumption (an average power of 1 W) and minimal heating of materials under treatment. It thus had the essential features required for oral and dental disinfection. Scanning electron microscopy showed complete destruction of endodontic biofilms for a depth of 1 mm inside a root canal after plasma treatment for 5 min. Plasma emission spectroscopy identified atomic oxygen as one of the likely active agents for the bactericidal effect.

Ritts AC et al.,(27) **(2010)** conducted a study to investigate the effects of non-thermal atmospheric gas plasmas on dentin surfaces used for composite restoration. The dentin surfaces of extracted unerupted human third molars were treated using a non-thermal atmospheric argon plasma brush for various periods of time. The molecular changes of the dentin surfaces were analyzed using Fourier transform infrared spectrophotometry/attenuated total reflectance (FTIR/ATR) and the test samples were restored using Filtek Z250 dental composite. To evaluate the dentin/composite interfacial bonding, the teeth thus prepared were sectioned into micro-bars and analyzed using tensile testing. It was found that the bonding strength of the composite restoration to peripheral dentin was significantly increased (by 64%) after 30 s of plasma treatment. Results concluded that plasma treatment of the peripheral dentin surface for up to 100 s resulted in an increase in the interfacial bonding strength.

Violich D.R. et al.,(28) **(2010)** reviewed smear layer in endodontics. The review said that root canal instrumentation produces a layer of organic and inorganic material called the smear layer that may also contain bacteria and their by-products. It can prevent the penetration of intracanal medicaments into dentinal tubules and influence the adaptation of filling materials to canal walls. The PubMed database was used initially; the reference list for smear layer featured 1277 articles, and for both smear layer dentine and smear layer root canal revealed 1455 publications. Smear layer endodontics disclosed 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 include chemical, ultrasonic and laser techniques – 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 ethylenediaminetetraacetic acid and sodium hypochlorite solutions. Conflict remains regarding the removal of the smear layer before filling root canals, with investigations required to determine the role of the smear layer in the outcomes of root canal treatment.

Dai L et al.,(29) **(2011)** compared canal wall smear layer removing the ability of two versions of QMix with Biopure MTAD and 17% EDTA. Single-rooted human teeth were irrigated with one of the following as the final irrigant: QMix I (pH = 8), QMix II (pH = 7.5), distilled water, 17% EDTA, and BioPure MTAD after cleaning and shaping and the samples were evaluated under SEM. It was concluded that the two experimental QMix versions were as effective as 17% EDTA in removing canal wall smear layer from entire root canal space and effectiveness of MTAD was comparable to QMix II and better than the other final irrigants used.

Singla MG et al.,(12) **(2011)** reviewed MTAD in endodontics. The major objective in endodontic therapy is to achieve complete chemomechanical debridement of the entire root canal system. This can be accomplished with biomechanical instrumentation and chemical irrigation. Various endodontic irrigants, such as sodium hypochlorite, chlorhexidene, and iodine potassium iodide, are available, each having its own advantages with some limitations. MTAD, a new endodontic irrigant, has been introduced to fulfill these limitations. MTAD is a mixture of doxycycline, citric acid, and a detergent (Tween 80). Since its introduction, it is a material that has been researched extensively for its properties. They reviewed the numerous properties of MTAD, such as antimicrobial activity, smear layer- and pulp-dissolving capability, effect on dentin and adhesion, and biocompatibility.

Prado M et al.,(30) **(2011)** compared the effectiveness of 37% phosphoric acid with that of 17% EDTA and 10% citric acid in the removal of smear layer. Fifty-two maxillary single-rooted human canines were accessed and instrumented. Between each instrument used, the canals were irrigated with sodium hypochlorite. After instrumentation, the teeth were irrigated with distilled water and then divided into groups according to the time and substances employed. The substances used were 17% EDTA, 10% citric acid, and 37% phosphoric acid solution and gel. The experimental time periods were of 30 seconds, 1 minute, and 3 minutes. The samples were prepared and observed by means of scanning electron microscopy. Three photomicrographs (2,000x) were recorded for each sample regarding the apical, middle, and cervical thirds. A score system was used to evaluate the images. It was observed that none of the substances analyzed was effective for removing the smear layer at 30 seconds. In the 1-minute period, the phosphoric acid solution showed better results than the other substances evaluated. In the 3- minute period, all the substances worked well in the middle and cervical thirds although phosphoric acid solution showed excellent results even in the apical third.

Lotfi M et al.,(31) **(2012)** evaluated the effect of MTAD as final rinse on removal of the smear layer. Division of forty teeth into MTAD group (1.3% NaOCI solution was used for flushing canals during instrumentation keeping MTAD for the final rinse); EDTA group (5.25% NaOCI used as irrigant during instrumentation and 17% EDTA as the final rinse); positive control group (sterile distilled water was used for irrigation). They concluded that use of 5.25% NaOCI during instrumentation and 17% EDTA as the final rinse is more effective than using 1.3% NaOCI as primary irrigation and MTAD as final rinse on removal of the smear layer.

Wu L et al.,(32) **(2012)** evaluated the efficacy of smear layer removal using 4 decalcifying agents (17% EDTA, 20% citric acid, BioPure MTAD, and SmearClear). Forty-five single-rooted human teeth were randomized into 5 groups according to the final irrigants: 17% EDTA, 20% citric acid, BioPure MTAD, SmearClear, and 3% NaOCI (control). The smear layer removal of all groups at the apical, middle, and coronal thirds was observed under the SEM. The decalcifying agents could effectively, but not completely, remove the smear layer, especially in the apical third. The efficacy of 17% EDTA was observed to be better than that of MTAD and SmearClear.

Lehmann et al., (17) **(2013)** conducted a study to see the modifications by CAP jet on surface properties of tooth substances. Surfaces of polished and etched enamel and dentin slices from the vestibular face of bovine incisors were modified by plasma jet at a distance of 2mm and with a scan velocity of 5.5mms^{-1}. Plasma treatment caused changes of surface roughness (evaluated using laser scanning) and morphological alterations (evaluated using SEM) on dentin, while no such changes were measureable on etched dentin and polished and etched enamel surfaces. Plasma caused contact angle reductions for both, water and ethylene glycol.

Paul ML et al.,(33) **(2013)** conducted a study to compare the efficacy of different irrigants including EDTA, EDTA with ultrasonic activation, citric acid, and MTAD as final irrigants. Forty-five human upper anterior teeth were divided into 5 groups, one control group and four experimental groups (n=9); based on final irrigating solution i.e. 5m1 of 17% EDTA, 17% EDTA along with ultrasonic activation, 25% citric acid, and MTAD, respectively. SEM analysis revealed that none of the combined irrigants was completely effective. Smear layer removal by all tested irrigants is comparable in cervical and middle third whereas, MTAD showed excellent results in the apical third as compared to the other groups.

Bansode AS et al.,(34) **(2013)** developed the non-thermal atmospheric plasma torch to study the effect of plasma treatment on the growth rate of E. faecalis culture and biofilms. E. faecalis treated with plasma was then compared with helium gas exposed and chlorohexidine treated cultures and biofilms. All the results were analysed for significance ($P < 0.001$) using ANOVA and TUCKEY'S test. Optical emission spectroscopy technique was employed in situ to identify the species interacting with the samples. It was found that atmospheric non-thermal plasma proved to be a promising alternative to traditional disinfectants for disinfection during endodontic treatment.

Dong X et al.,(35) **(2013)** in his study evaluated and verified the effectiveness of plasma treatment for improving adhesive/dentin interfacial bonding by performing micro-tensile bond strength (μTBS) test using the same-tooth controls and varying cross-sectional surface areas. Extracted unerupted human third molars were used by removing the crowns to expose the dentin surface. For each dentin surface, one half of it was treated with a non-thermal argon plasma brush, while another half was shielded with glass slide and used as untreated control. Adper Single Bond Plus adhesive and Filtek Z250 dental composite were then applied as directed. The teeth thus prepared were further cut into micro-bar specimens with cross-sectional size of 1×1 mm^2, 1×2 mm^2 and 1×3 mm^2 for μTBS test. The test results showed that plasma treated specimens gave substantially stronger adhesive/dentin bonding than their corresponding same tooth controls. As compared with their untreated controls, plasma treatment gave statistically significant higher bonding strength for specimens having cross-sectional area of 1×1 mm^2 and 1×2 mm^2, with mean increases of 30.8% and 45.1%, respectively.

Arora V et al.,(14) **(2013)** reviewed plasma therapy in dentistry. The review includes a summary of the current status of this emerging field, its scope, and its broad interdisciplinary approach. Matter usually includes liquids, solids, and gases. But a fourth category of matter has been discovered called plasma that's actually the most unusual and the most abundant. It could become a new and painless way to prepare cavities for restoration with improved longevity. Also, it is capable of bacterial inactivation and non-inflammatory tissue modification, which makes it an attractive tool for the treatment of dental caries and for composite restorations. Plasma can also be used for tooth whitening. Various dental applications of plasma were discussed in the mentioned review.

Mohammadi Z et al.,(10) **(2013)** reviewed EDTA in endodontics. Ethylene-diaminetetraacetic acid (EDTA) is a chelating agent can bind to metals via four carboxylate and two amine groups. It is a polyamino carboxylic acid and a colorless, water-soluble solid, which is widely used to dissolve lime scale. It is produced as several salts, notably disodium EDTA and calcium disodium EDTA. EDTA reacts with the calcium ions in dentine and forms soluble calcium chelates.

Zhang et al.,(36) **(2014)** investigated the influence of non-thermal plasma treatment on the penetration of a model dental adhesive into the demineralized dentin. Prepared dentin surfaces were conditioned with Scotchbond Universal etchant for 15 s and sectioned equally perpendicular to the etched surfaces. The separated halves were randomly selected for treatment with an argon plasma brush (input current 6 mA, treatment time 30 s) or gentle argon air blowing (treatment time 30 s, as control). The plasma-treated specimens and control specimens were applied with a model adhesive containing 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy) phenyl]-propane (BisGMA) and 2-hydroxyethyl methacrylate (HEMA) (mass ratio of 30/70), gently air-dried for 5 s, and light-cured for 20 s. Cross-sectional specimens were characterized using micro-Raman spectral mapping across the dentin, adhesive/dentin interface, and adhesive layer at 1∼micron spatial resolution. SEM was also employed to examine the adhesive/dentin interfacial morphology. The micro-Raman result disclosed that plasma treatment significantly improved the penetration of the adhesive, evidenced by the apparently higher content of the adhesive at the adhesive/dentin interface as compared to the control. The results further suggested that plasma treatment could benefit polymerization of the adhesive, especially in the interface region.

Han GJ et al.,(37) **(2014)** investigated the effect of low-power, non-thermal atmospheric pressure plasma (NT-APP) treatments, in pulsed and conventional modes, on the adhesion of resin composite to dentin and on the durability of the bond between resin composite and dentin. A pencil-type NT-APP jet was applied in pulsed and conventional modes to acid-etched dentin. The microtensile bond strength (MTBS) of resin composite to dentin was evaluated in one control group (no plasma) and in two experimental groups (pulsed plasma and conventional plasma groups) using the

Scotchbond Multi-Purpose Plus Adhesive System. Fractured surfaces and the bonded interfaces were evaluated using a FE-SEM. The plasma treatment improved the MTBS compared with the control group. It was concluded that plasma treatment using NT-APP improved the adhesion of resin composite to dentin and after using a pulsed energy source.

Dong et al.,(38) **(2014)** did a study to achieve mechanistic understanding of the plasma treatment effects on dentin surface through investigating the plasma treated dentin surfaces and their interaction with adhesive monomer, 2-Hydroxyethyl methacrylate (HEMA). The plasma treated dentin surfaces from human third molars were evaluated by water contact angle measurements and scanning electron microscopy (SEM). It was found that plasma-treated dentin surface with subsequent HEMA immersion (Plasma/HEMA Treated) had much lower water contact angle compared with only plasma-treated (Plasma Treated) or only HEMA immersed (HEMA Treated) dentin surfaces. With prolong water droplet deposition time, water droplets spread out completely on the Plasma/HEMA Treated dentin surfaces. SEM images of Plasma/ HEMA Treated dentin surfaces verified that dentin tubules were opened-up and filled with HEMA monomers. The results demonstrated that non-thermal argon plasma treatment was very effective in loosing collagen structure and enhancing adhesive monomer penetration, which were beneficial to thicker hybrid layer and longer resin tag formation, and consequently enhance adhesive/dentin interface bonding.

Dong et al.,(39) **(2015)** did a study to evaluate plasma treatment effects on dentin surfaces for improving self-etching adhesive and dentin interface bonding. Extracted unerupted human third molars were used after crown removal to expose dentin. One half of each dentin surface was treated with atmospheric non-thermal argon plasmas, while another half was untreated and used as the same tooth control. Self-etching adhesive and universal resin composite was applied to the dentin surfaces as directed. After restoration, the adhesive-dentin bonding strength was evaluated by micro-tensile bonding strength (μTBS) test. Bonding strength data was analyzed using histograms and Welch's t-test based on unequal variances. μTBS test results showed that, with plasma treatment, the average μTBS value increased to 69.7±11.5 MPa as compared with the 57.1±17.5 MPa obtained from the untreated controls. After 2 months immersion of the restored teeth in 37^{o}C phosphate buffered saline (PBS), the adhesive-dentin bonding strengths of the plasma-treated specimens slightly decreased from

69.7±11.5 MPa to 63.9±14.4 MPa, while the strengths of the untreated specimens reduced from 57.1±17.5 MPa to 48.9±14.6 MPa. Water contact angle measurement and scanning electron microscopy (SEM) examination verified that plasma treatment followed by water rewetting could partially open dentin tubules, which could enhance adhesive penetration to form thicker hybrid layer and longer resin tags and consequently improve the adhesive/dentin interface quality.

Kuruvilla A et al.,(40) **(2015)** conducted a study is to evaluate and compare the efficacy of 17% EDTA, 18% etidronic acid, and 7% maleic acid in smear layer removal using scanning electron microscopic image analysis. Thirty, freshly extracted mandibular premolars were used. The teeth were decoronated to obtain working length of 17mm and instrumentation up to 40 size (K file) with 2.5% NaOCl irrigation between each file. The samples were divided into Groups I (17% ethylenediaminetetraacetic acid (EDTA)), II (18% etidronic acid), and III (7% maleic acid) containing 10 samples each. Longitudinal sectioning of the samples was done. Then the samples were observed under scanning electron microscope (SEM) at apical, middle, and coronal levels. Data was analyzed statistically using Kruskal–Wallis analysis of variance (ANOVA) followed by Mann-Whitney U test for individual comparisons. The level for significance was set at 0.05. It was observed that all the three experimental irrigants removed the smear layer from different tooth levels (coronal, middle, and apical). Final irrigation with 7% maleic acid is more efficient than 17% EDTA and 18% etidronic acid in the removal of smear layer from the apical third of root canal.

Vemuri S et al.,(7) **(2016)** compared the smear layer removal efficacy of different irrigating solutions at the apical third of the root canal in their invitro study. Forty human single-rooted mandibular premolar teeth were taken and decoronated to standardize the canal length to 14 mm. They were prepared by ProTaper rotary system to an apical preparation of file size F3. Prepared teeth were randomly divided into four groups (n = 10); saline (Group 1; negative control), ethylenediaminetetraacetic acid (Group 2), BioPure MTAD (Group 3), and QMix 2 in 1 (Group 4). After final irrigation with tested irrigants, the teeth were split into two halves longitudinally and observed under a scanning electron microscope (SEM) for the removal of smear layer. The SEM images were then analyzed for the amount of smear layer present using a three-score system. Intergroup comparison of groups showed statistically significant difference in the smear layer removal efficacy of irrigants tested. QMix 2 in 1 was most effective in removal of smear layer when compared to other tested irrigants.

Likhitkar MS et al.,(41) **(2016)** conducted an in-vitro study to assess the effect of the presence/absence of a smear layer on the microleakage of root canal filled teeth. 100 extracted mandibular premolars were divided into positive control group (n=5) (smear layer removed with 17% EDTA + no obturation); Negative control group (n=5) (smear layer not removed); three experimental groups A, C, E (n=15 each) received a final rinse with 3 ml of 17% EDTA, followed by irrigation with 10 ml 5.25% NaOCl to clear the smear layer; three experimental groups B, D, F received a final rinse of 5.25% NaOCl after complete instrumentation. Group E showed the highest microleakage value and Group A showed the lowest value showing that elimination of smear layer enhanced the resistance to microleakage.

Abreu et al.,(42) **(2016)** evaluated the effect of non-thermal argon plasma on the bond strength of a self-etch adhesive system to dentin exposed to NaOCl, in their study. Thirty-two flat dentin surfaces of bovine incisors were immersed in 2.5% NaOCl for 30 min to simulate the irrigation step during endodontic treatment. The specimens were divided into four groups (n=8), according to the surface treatment: Control (without plasma treatment), AR15 (argon plasma for 15 s), AR30 (argon plasma for 30 s) and AR45 (argon plasma for 45 s). For microtensile bond strength test, the specimens were hybridized with a self-etch adhesive system (Clearfil SE Bond) and resin composite buildups were constructed. After 48 h of water storage, specimens were sectioned into sticks (5 per tooth, 25 per group) and subjected to microtensile bond strength test (μTBS) until failure, evaluating failure mode. Three specimens per group were analyzed under FTIR spectroscopy to verify the chemical modifications produced in dentin. AR30 showed the highest μTBS (20.86±9.0). AR15 (13.81±6.4) and AR45 (11.51±6.8) were statistically similar to control (13.67±8.1). FTIR spectroscopy showed that argon plasma treatment produced chemical modifications in dentin.

Vemuri S et al.,(7) **(2016)** conducted an in vitro study to compare the smear layer removal efficacy of different irrigating solutions at the apical third of the root canal. Forty human single-rooted mandibular premolar teeth were prepared by ProTaper rotary system to an apical preparation of file size F3 and were randomized into four groups (n=10); saline (negative control), ethylenediaminetetraacetic acid, BioPure

MTAD, and QMix 2 in 1. SEM evaluation for the amount of smear layer was done and it was concluded that QMix 2 in 1 is the most effective final irrigating solution for smear layer removal followed by MTAD.

Sonu KR et al.,(43) **(2016)** conducted a study to compare the dentinal tubule penetration of MTA Fillapex, GuttaFlow 2 sealer with standard sealer AH Plus in instrumented root canals obturated by using cold lateral compaction techniques in either the presence or absence of the smear layer. Sixty extracted human mandibular premolars were randomised into three groups of 20 teeth each; GuttaFlow 2(Group I), MTA Fillapex(Group II) and AH Plus(Group III). Groups were divided into two subgroups in which either the smear layer was removed (using 2ml of 17% EDTA) or retained. It was concluded, with the removal of smear layer AH plus sealer showed deeper penetration into the dentinal tubules at cervical and middle third of root compared with apical third.

Liu Y et al.,(44) **(2016)** in his article aimed to collect and summarize recent advances in utilizing nonthermal atmospheric plasmas (NTAPs)—a novel technology that delivers highly reactive species in a gaseous medium at or below physiologic temperature—to improve the durability of dentin bonding by addressing 3 issues simultaneously. Overall, NTAP has demonstrated efficacies in improving a number of critical properties for dentin bonding, including deactivation of oral pathogens, modification of surface chemistry/properties, resin polymerization, improvement in adhesive-dentin interactions, and establishment of auxiliary bonding mechanism.

Zhou H et al.,(45) **(2018)** aimed to compare the efficacy of chitosan and MTAD for the smear layer removal from the root canal through a scanning electron microscope (SEM). Thirty teeth were randomly divided into three groups according to the final irrigants: 0.2% chitosan, MTAD, saline (control group). After the mechanical preparation, the samples were irrigated with saline (control group), 0.2% chitosan and MTDA respectively. Then, the samples were split and the smear layer at the apical, middle, and coronal thirds of each root canal was imaged using SEM. The statistical analysis was performed using the Kruskal-Wallis test and the Mann-Whitney U test ($\alpha = 5\%$). The difference between chitosan and MTDA was statistically significant in the apical region ($p < 0.05$), no significant difference was obtained in the coronal and middle regions in these two experiment groups ($p > 0.05$). The control group exhibited the lowest efficacy in smear layer removal in all regions. It was concluded that chitosan was more effective in smear layer removal than MTAD especially in the apical third.

Stancampiano A et al.,(46) **(2019)** investigated the use of a CAP source (handheld DBD-jet prototype) in an endodontic procedure to enhance the bond strength of a dental adhesive in root canal restoration. In extracted monoradicular teeth, the dentin surface was conditioned according to different protocols including; chelating agents 17% EDTA, 1%IP6 and CAP treatment (180s). Light curing was done after application of a self-etch adhesive and luting material to seal the root canal. Tooth sections were obtained from coronal and middle portions of the root canal, and the push-out test was used to evaluate the bond strength between the adhesive and dentin. Results demonstrated that plasma treatment greatly improved (>two fold) the mechanical properties of the adhesive-dentin interface along the whole length of the root canal. Contact angle measurements and SEM analyses showed that plasma treatment facilitated adhesive permeation into dentinal tubules, hence enhancing the effects of the bonding procedure.

AIMS AND OBJECTIVES

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AIM:

The study aims to assess the efficacy of Cold Atmospheric Pressure (CAP) Plasma Jet, MTAD, and EDTA in removing smear layer from intra-radicular dentin using a scanning electron microscope (SEM).

OBJECTIVES:

Primary Objective

To have a comparative evaluation of the effectiveness of Cold Atmospheric Pressure (CAP) Plasma Jet, MTAD, and EDTA for smear layer removal.

Secondary Objective

To compare the effectiveness of smear layer removal by Cold Atmospheric Pressure (CAP) Plasma Jet, MTAD, and EDTA in coronal, middle and apical thirds of root canal dentin surfaces.

The **Null hypothesis** states that there will be no significant difference in the efficacy of smear layer removal on intra-radicular dentinal surfaces using Cold Atmospheric Pressure (CAP) Plasma Jet, when compared with MTAD and EDTA.

MATERIALS AND METHODS

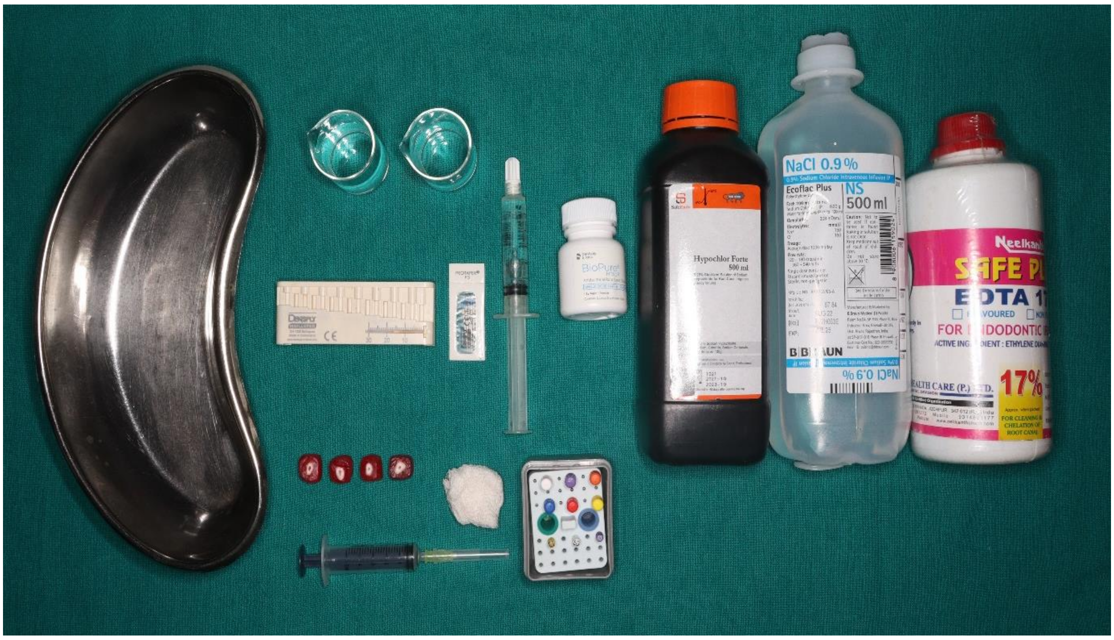

Fig. 1 Armamentarium for preparation of samples

MATERIALS AND METHODS

The present in-vitro study was conducted at the Department of Dentistry, All India Institute of Medical Sciences, Jodhpur in collaboration with Department of Physics, Indian Institute of Technology, Jodhpur. Approval to conduct this study was taken from the Institutional Ethics Committee (IEC), AIIMS, Jodhpur.

MATERIALS

1. 84 Human extracted single rooted mandibular premolar teeth
2. Micromotor handpiece (Confident, India)
3. Diamond disc (safe sided) (DFS, Germany)
4. Barbed broach (Dentsply, Maillefer, India)
5. Hand K files # 10, 15, 20 (Dentsply M access K files)
6. Protaper Universal Hand Files (Dentsply, Maillefer, India)
7. 30G side vented needles (ENDO-TOP Endo irrigation needles)
8. Disposable syringe (5ml) (Omnivan)
9. 5.25% Sodium hypochlorite solution (NaOCl) (SafeEndo Dental India Pvt. LTD)
10. Normal saline (0.9%) (B|Braun group company, India)
11. 17% EDTA (Neelkanth Healthcare (P.) LTD, India)
12. MTAD solution (Biopure)
13. Paper points (F_3) (Dentsply, Maillefer, India)
14. Sticky wax (MDM Corp)
15. Osteotome (ORACRAFT)
16. Modelling Wax (Pyrax)
17. Distilled water
18. SEM sample preparation armamentarium
    - Centrifuge tubes
    - 2% glutaraldehyde
    - Phosphate buffer (pH 7.3)
    - Ethanol- 70%, 80%, 90%, 100%
    - Platinum particles

Fig. 2 Eighty Four single rooted mandibular premolars

EQUIPMENTS

1. Autoclave (Sternweber)
2. Ultrasonic scaler with tips
3. Radiovisiography (RVG)
4. Cold Atmospheric Plasma jet unit
5. Zero-point desiccator
6. Scanning Electron Microscope

SAMPLE COLLECTION

A total of 84 single-rooted mandibular premolars (Fig. 2), recently extracted for orthodontic/periodontal reasons, were collected.

INCLUSION CRITERIA

- Teeth with intact and mature root apices.
- Degree of root curvature $\leq 15^{o}$.
- Teeth having single root canal with single canal orifice and apical foramen.

EXCLUSION CRITERIA

- Teeth with caries, cracks, endodontic treatments, or restorations.
- Degree of root curvature $> 15^{o}$.
- Teeth with any calcification, extra canals, internal and external resorptions, root caries and open apices.

The tooth samples were stored in saline till they were used. A digital x ray using RVG was taken for each tooth in buccolingual and mesiodistal views so as to either include or exclude it according to the criteria specified earlier.

Fig. 3 Decoronating the sample

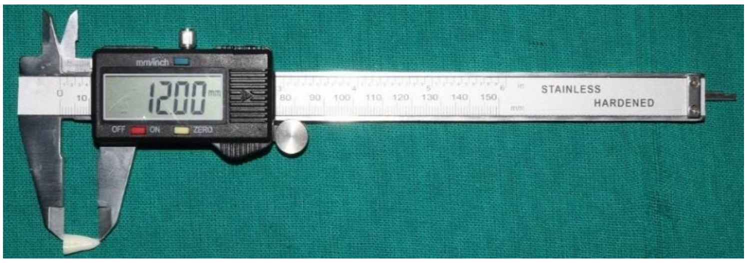

Fig. 4 Standardisation of root length to 12mm measured using vernier callipers

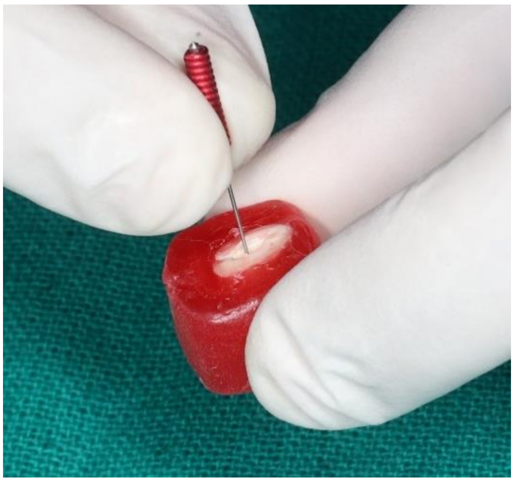

Fig. 5 Pulp extirpation using barbed broach

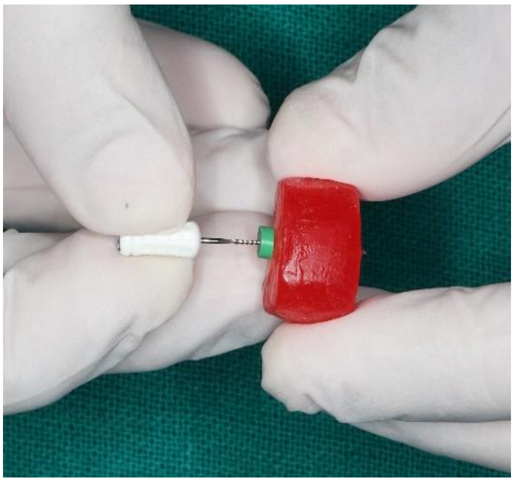

Fig. 6 Establishing apical patency using 15k file

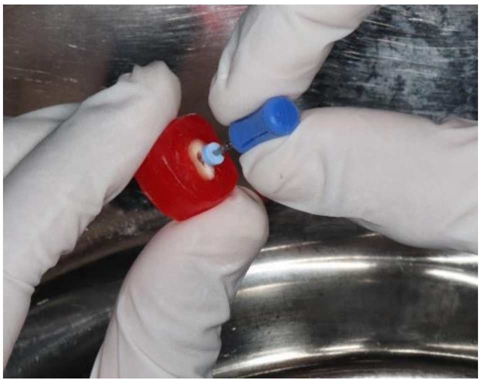

Fig. 7 Canal preparation using F3 ProTaper Universal Hand file

Fig. 8 Canal irrigation

SAMPLE PREPARATION

After collection, teeth were kept in NaOCl for 24 hours and subsequently cleaned with an ultrasonic scaler to remove any remaining debris or tissue tags. Teeth were autoclaved at 121°C at 15psi for 15minutes following which they were stored in 0.9% normal saline solution until used. After removal from normal saline, the anatomical crowns of these teeth were decoronated with a diamond disc under cooling with distilled water (Fig. 3) to prevent crack generation and root length was standardized to 12mm (Fig. 4). The samples were mounted on wax blocks, ensuring apical 2mm of root remained visible. Pulp extirpation was done by a suitable barbed broach (Fig. 5). The patency of the apical foramina was established by using a size 15 K-file and working length established by subtracting 0.5 millimeters from the length recorded when tip of #15 K-file was just visible at the apical foramen (Fig. 6). Chemo-mechanical preparation of teeth was done by crown-down technique using Hand ProTaper files upto F3 size file (Fig. 7). During preparation, the canals were rinsed with 5millilitre of 5.25% NaOCl after each file sequence, using 30G side-vented irrigation needle, placing the needle 2mm short of apex (Fig. 8).

COLD ATMOSPHERIC PRESSURE (CAP) PLASMA JET

A dielectric barrier discharge CAP plasma jet consists of a SS/copper tube as the central electrode and an axially aligned SS/copper ring as the grounded electrode. A dielectric barrier (Teflon/Quartz) was used between two electrodes to reduce the flowing current and prevent electrical arching and control plasma discharge in flow region. The produced plasma was cold at atmospheric pressure. The central electrode was connected through a low power bi-polar high voltage source with a 5 KV peak-to-peak voltage using 0-6 KV, 1 amp, 2μm PW power source. Helium was used as a working gas at atmospheric pressure, at a flow rate of 2.5 standard litre per min. The generated CAP plasma plume was exposed via a 23 gauge needle and the geometry developed was unique to extend the plasma jet length in a controlled way (Fig. 9).

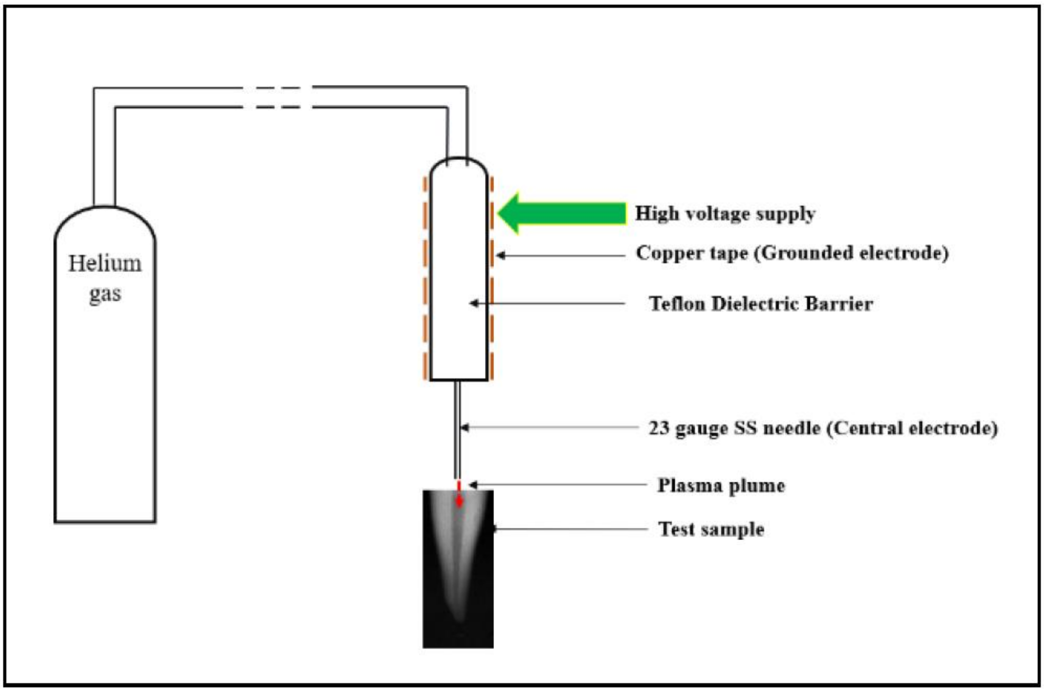

Fig. 9 Generation of CAP plasma jet using Helium gas

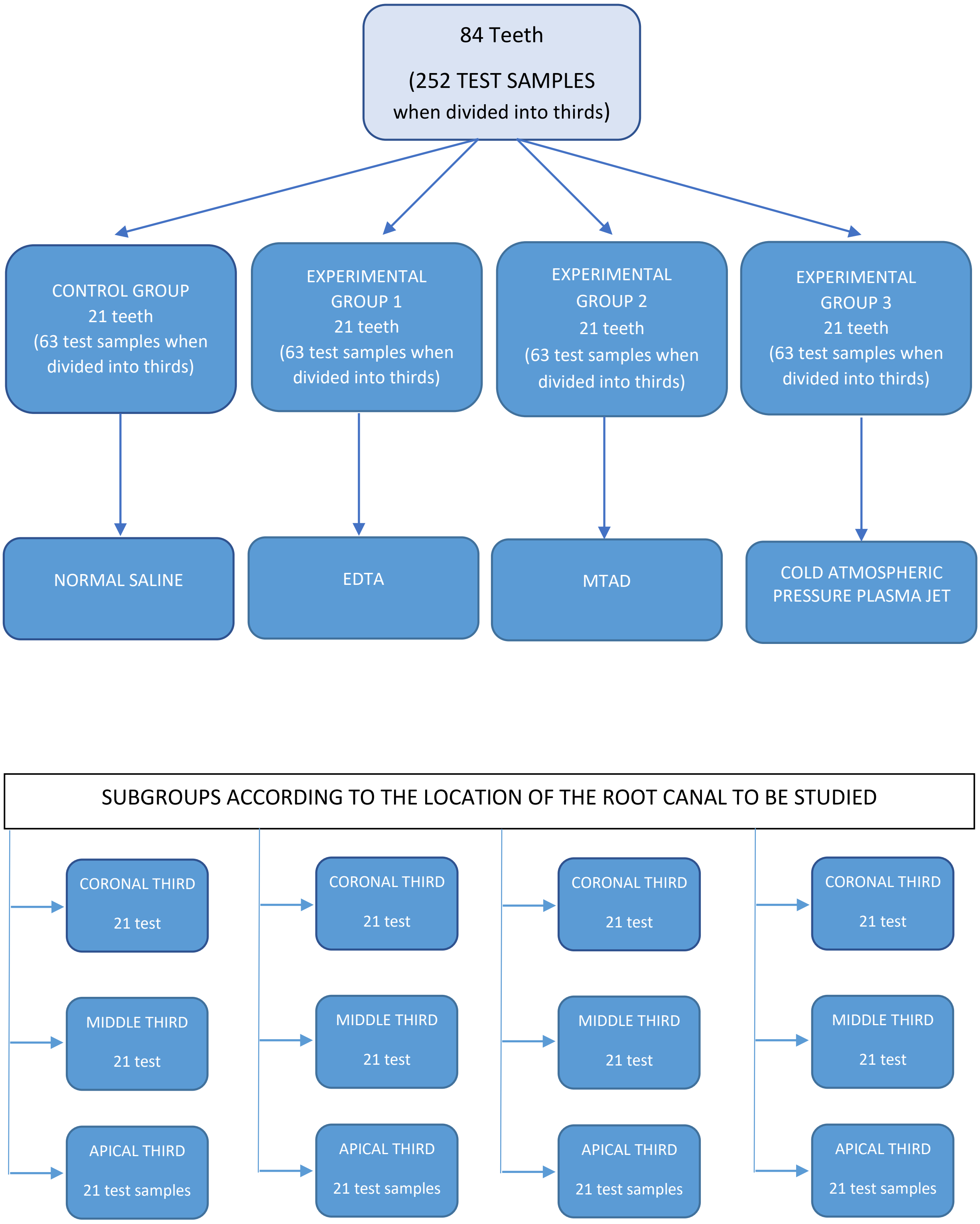

Fig. 10. Flow chart depicting the division of test samples into control and experimental groups

FINAL IRRIGATION

After instrumentation, 84 teeth were divided equally into 4 groups (1 control and 3 experimental) depending upon the final irrigation regimen (Fig. 10). The apical foramen was sealed with sticky wax to enable the final irrigant to retain in the canal.

Control group

Teeth in the control group were rinsed with 5millilitre of normal saline using 30G side vented needle placing it 2mm short of the apex and saline was retained in the canal at least for 2 minutes. The canal was then dried with F3 paper points.

Experimental group 1

Teeth in the experimental group 1 were rinsed with 5millilitre of 17% EDTA using 30 G side vented needle placing it 2mm short of the apex and solution was kept in the canal for 2 minutes. The canal was dried with F3 paper points.

Experimental group 2

Teeth in the experimental group 2 were rinsed with 5millilitre of MTAD using 30 G side vented needle placing it 2mm short of the apex and solution was retained in the canal for 2 minutes. The canal was dried with F3 paper points.

Experimental group 3

The samples were subjected to exposure of Cold Atmospheric Pressure (CAP) Plasma Jet for a duration of 2 minutes.

After the final irrigation regimen, the samples were removed from the wax blocks. The apical foramen and canal orifice was sealed with a sticky wax to prevent contamination of the root canals. The samples were then prepared to be evaluated under the SEM.

Fig. 11 Longitudinal grooves cut using diamond disc

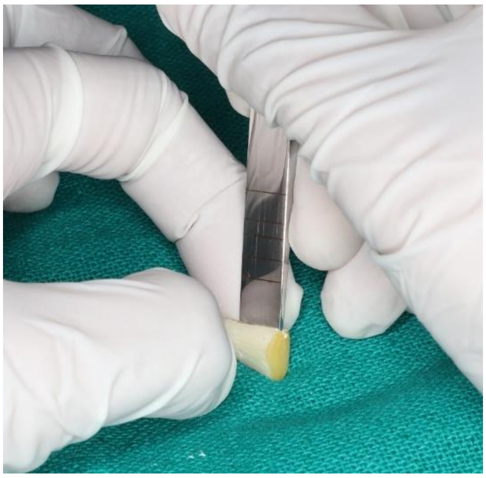

Fig. 12 Splitting samples using an osteotome

Fig. 13 Samples stored in zero-point desiccator

Fig. 14 Mounting of samples for sputtering and sputtering of the samples

Fig. 15 Scanning Electron Microscope

SAMPLE PREPARATION FOR SEM ANALYSIS

A Scanning Electron Microscope was used to evaluate endodontic smear layer removal from intra-radicular dentinal surface. To prepare samples for imaging the roots were split longitudinally in the buccolingual plane, so as to facilitate fracture into two halves. Two grooves were made on the buccal and lingual aspects of each tooth using safe-sided diamond disc under cooling with distilled water avoiding penetration of the root canals (Fig. 11). An osteotome was used to split the grooved roots into two halves, the mesial and the distal (Fig. 12). **The half containing major part of the root canal was prepared for SEM evaluation.** These specimens were immersed, for tissue fixation, in 2% glutaraldehyde with phosphate buffer (pH = 7.3) for 12 hours. The specimens were then washed with 20 mL of phosphate buffer for 15 min followed by dehydration in a graded ethanol series: 70%, 80%, 90% for 15 min each, and in 100% ethanol for 30 min. The specimens were then dried in Zero-point desiccator (Fig. 13). The specimens were sputter coated with a Platinum film (Fig. 14). The specimens were ready for observation under a scanning electron microscope (SEM) (Fig. 15). The total test samples in each group (21 teeth) were further sub-divided into three different sub-groups based upon the location (coronal, middle and apical third) for SEM evaluation at 3000x magnification. Thus, in each group with 21 teeth, a total of 63 samples were evaluated (Fig. 12).

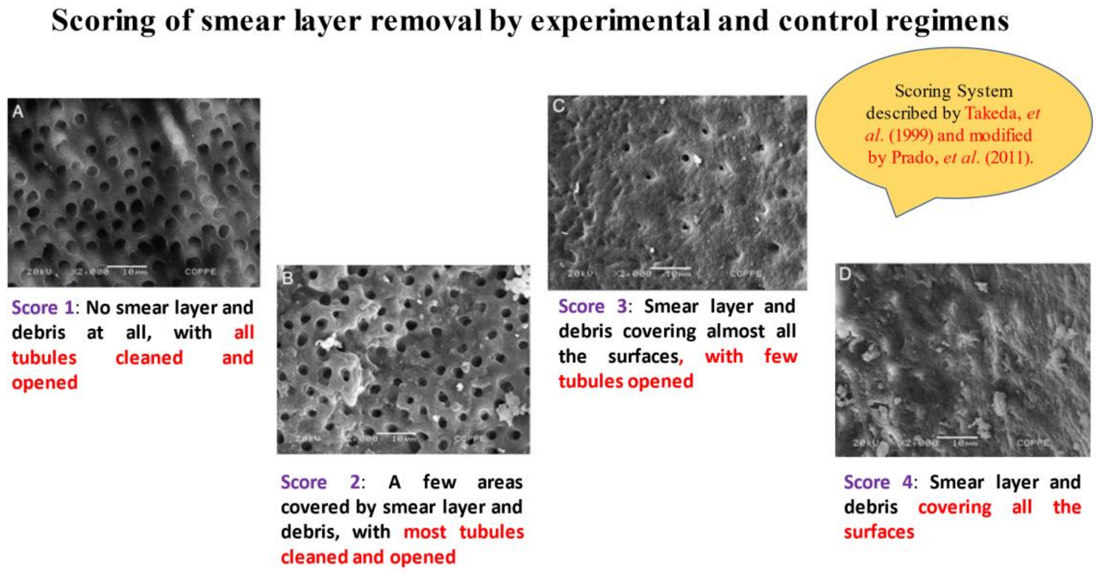

Fig. 16 Scoring criteria for evaluation of SEM images

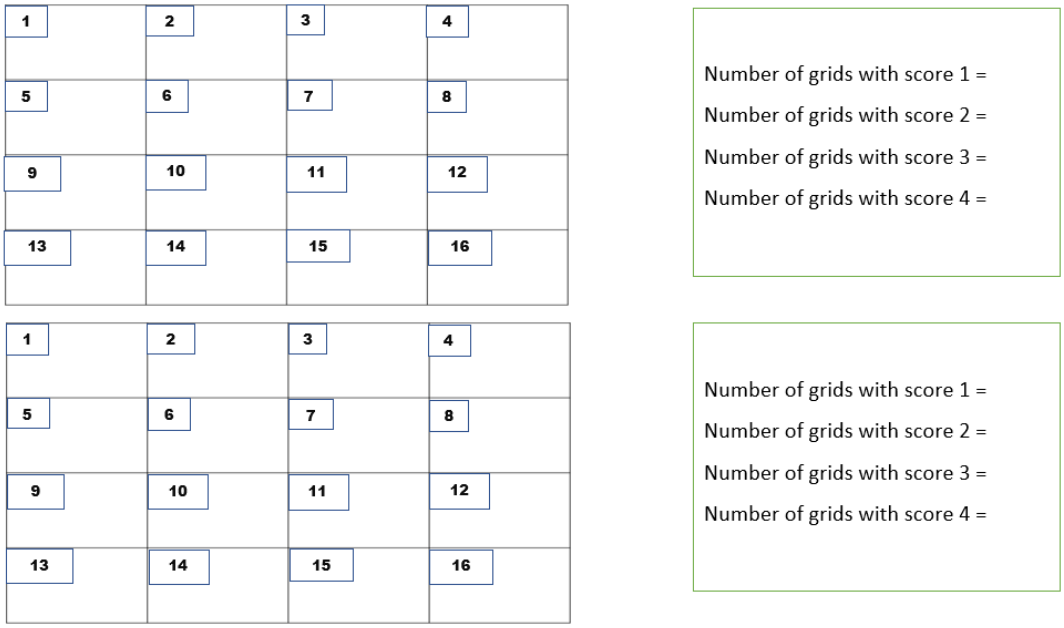

Fig. 17 Data extraction form for scoring of SEM images

EVALUATION OF PHOTOGRAPHS

Photographs were captured such that the area to be analyzed represented the center of coronal, middle or apical thirds of the canal lumen of each test sample at a magnification of 3000x. The photographs were stored in TIF Image format and analyzed in a Microsoft PowerPoint format. Each one of them was then divided into 16 equal subareas by an overlaying a grid. Each subarea of an image was evaluated for the presence of smear layer using the scoring system suggested by Prado, et al. (2011) (Fig. 16) (30).

The scoring criteria given by Prado, et al. included scores 1 to 4 which are as follows:-

Score 1: No smear layer and debris at all, with all tubules clean and open.

Score 2: A few areas covered by smear layer and debris, with most tubules clean and open.

Score 3: Smear layer and debris covering almost all the surfaces, with few tubules open.

Score 4: Smear layer and debris covering all the surfaces.

The scoring of each subarea was done by two trained and experienced examiners and any disagreement between the two examiners was solved after discussion with the third examiner (Principal Investigator). The decision of the third examiner for reporting the final score of the test sample was considered final. Number of subareas with different scores (1, 2, 3 and 4) of smear layer removal for each test sample was then counted and recorded in a separate data extraction form (Fig. 17). The number of subareas with score 1 and 2 were further combined to get a final score which represent the area with either no smear layer/debris or few areas covered by smear layer/debris. The final score of each test sample in different groups and subgroups was used for statistical analysis.

STATISTICAL ANALYSIS

STATISTICAL ANALYSIS

All the pre-processing and the downstream analysis was carried out in R studio (version = 4.2.1) data analysis software. The Cohen's Kappa statistics was employed to evaluate the correlation between the three observers during SEM analysis. Inter-examiner reliability was found to be 0.85. For the Intergroup and Intra group comparison, data pre-processing performed using *tidyr (V1.2.0) and* visualisation was performed using *ggplot2* (V3.3.5) package. Kruskal–Wallis test was performed to test the significant differences across multiple groups, because the count data of scores followed a non-parametric distribution. The p-value less than 0.05 was considered as statistically significant. Dunn's test was performed to check the pair wise comparison between different subgroups. The Bonferroni correction was used to reduce the type-1 error while multiple pair comparisons were performed. The p-value of less than 0.05 was considered statistically significant.

RESULTS

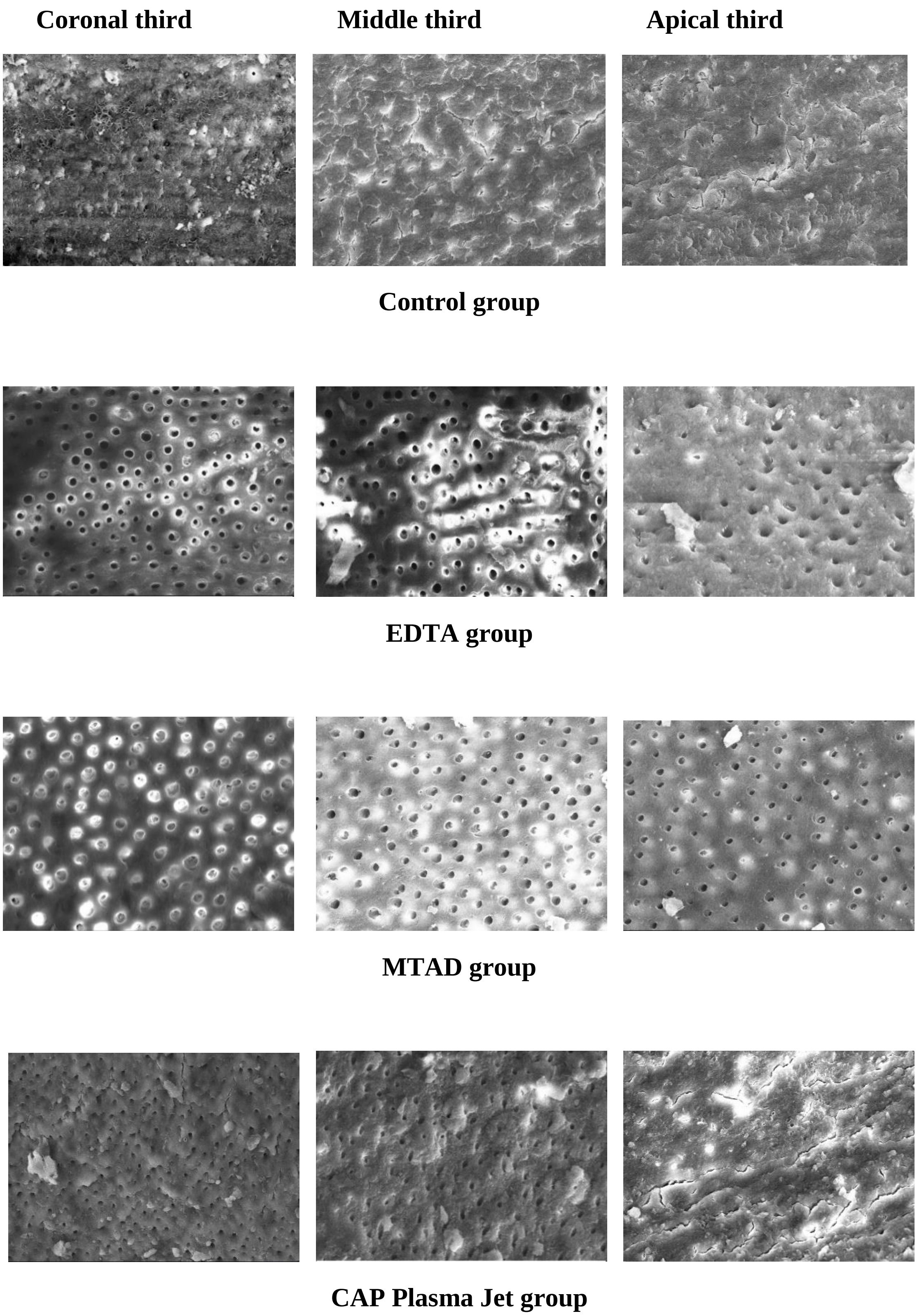

Fig. 18 Representative SEM images of the samples analysed

RESULTS

The final smear layer scores in each group and subgroup with respective median and interquartile range (IQR) values are summarized and tabulated as (Table 1, Table 2 and Table 3) (Fig. 18).

Table 1: Inter-group comparison of median values of the final scores in Control, EDTA, MTAD and CAP Plasma Jet

Group	Median (IQR)	P value (Pairwise groups)		
		EDTA	MTAD	CAP
Control	0.0 (0.0, 0.0)	**2.32×10^{-10}**	**3.64×10^{-15}**	0.06
EDTA	12.0 (0.0, 16.0)		0.83	**3.27×10^{-4}**
MTAD	16.0 (0.5, 16.0)			**2.1×10^{-7}**
CAP	0.0 (0.0, 8.0)			

Table 1 shows intergroup comparison of the final scores in the control and experimental groups. The two experimental groups, EDTA and MTAD, were significantly better than the control group in smear layer removal. Both EDTA and MTAD performed significantly better than CAP Plasma Jet while, there was no significant difference amongst EDTA and MTAD. Also there was no significant difference between CAP Plasma Jet and control group.

On the basis of intergroup comparison of all the irrigating solutions, it can be concluded that:

MTAD ≥ EDTA > CAP Plasma Jet = Normal Saline

Table 2: Intra group comparisons of the median values of the final scores in coronal, middle and apical third of the samples analyzed.

<table>
<tr><th rowspan="2">Group</th><th rowspan="2">Region</th><th rowspan="2">Median (IQR)</th><th rowspan="2">P-value (All sub-groups)</th><th colspan="2">P value</th></tr>
<tr><th>Middle</th><th>Apical</th></tr>
<tr><td rowspan="3">Control</td><td>Coronal third</td><td>0.0 (0.0, 0.0)</td><td rowspan="3">0.05</td><td>0.0933</td><td>0.0933</td></tr>
<tr><td>Middle third</td><td>0.0 (0.0, 0.0)</td><td></td><td>1</td></tr>
<tr><td>Apical third</td><td>0.0 (0.0, 0.0)</td><td colspan="2"></td></tr>
<tr><td rowspan="3">EDTA</td><td>Coronal third</td><td>15.0 (7.0, 16.0)</td><td rowspan="3">0.0001</td><td>1</td><td>0.0005</td></tr>
<tr><td>Middle third</td><td>16.0 (6.0, 16.0)</td><td></td><td>0.0007</td></tr>
<tr><td>Apical third</td><td>0.0 (0.0, 5.0)</td><td></td><td></td></tr>
<tr><td rowspan="3">MTAD</td><td>Coronal third</td><td>16.0 (16.0, 16.0)</td><td rowspan="3">0.0009</td><td>0.4125</td><td>0.0006</td></tr>
<tr><td>Middle third</td><td>16.0 (5.0, 16.0)</td><td></td><td>0.0805</td></tr>
<tr><td>Apical third</td><td>0.0 (0.0, 16.0)</td><td></td><td></td></tr>
<tr><td rowspan="3">CAP</td><td>Coronal third</td><td>8.0 (0.0, 14.0)</td><td rowspan="3">8.12×10^{-6}</td><td>5.59×10^{-3}</td><td>5.57×10^{-3}</td></tr>
<tr><td>Middle third</td><td>0.0 (0.0, 0.0)</td><td></td><td>0.292</td></tr>
<tr><td>Apical third</td><td>0.0 (0.0, 0.0)</td><td></td><td></td></tr>
</table>

Median and interquartile range (IQR) of the count with scores 1 and 2 across different samples

P-value < 0.05 is significant

Table 2 shows intra group comparisons of the median (IQR) of the final scores (sum of the number of grids with score 1 & score 2) in coronal, middle and apical third regions of the samples analysed. The median (IQR) values for the **control group** in the coronal, middle and apical third were 0.0 (0.0, 0.0), 0.0 (0.0, 0.0) and 0.0 (0.0, 0.0) respectively. Intra-group comparison revealed that a few samples in control group showed complete or almost complete smear layer removal and this is limited to the coronal third only. In the **EDTA group** median (IQR) values for the coronal, middle and apical third were 15.0 (7.0, 16.0), 16.0 (6.0, 16.0) and 0.0 (0.0, 5.0) respectively. The maximum smear layer removal in the EDTA group was seen in the coronal third followed by middle and apical third. Difference in smear layer removal ability of EDTA was statistically significant when comparison was made between the coronal v/s the apical third and the middle v/s the apical third ($P<0.05$). The median (IQR) values for the **MTAD group** in the coronal, middle and apical third were 16.0 (16.0, 16.0), 16.0 (5.0, 16.0) and 0.0 (0.0, 16.0) respectively. In the MTAD group maximum smear layer removal was seen in the coronal third, followed by middle and apical third, with statistically significant difference observed only between coronal v/s apical third ($P<0.05$). In the **CAP Plasma Jet group** median (IQR) values for the coronal, middle and apical third values were found to be 8.0 (0.0, 14.0), 0.0 (0.0, 0.0) and 0.0 (0.0, 0.0) respectively. In the CAP Plasma Jet group maximum smear layer removal was seen in the coronal third, followed by the middle third. Difference in smear layer removal ability of CAP Plasma Jet was statistically significant when comparison was made between the coronal v/s the middle third and the coronal v/s the apical third ($P<0.05$).

Table 3: Inter-group comparison of median values of the final scores in coronal, middle and apical third regions.

Region	Group	Median (IQR)	P-value (All groups)	P value (Pairwise groups)		
				EDTA	MTAD	CAP
Coronal third	Control	0.0 (0.0, 0.0)	**5.02 x 10^{-8}**	**1.09 x 10^{-4}**	**3.80 x 10^{-8}**	**3.46 x 10^{-2}**
	EDTA	15.0 (7.0, 16.0)			0.7683	0.7633
	MTAD	16.0 (16.0, 16.0)				**0.0138**
	CAP	8.0 (0.0, 14.0)				
Middle third	Control	0.0 (0.0, 0.0)	**4.19 x10^{-9}**	**4.00 x 10^{-6}**	**1.04 x 10^{-6}**	1.00
	EDTA	16.0 (6.0, 16.0)			1.00	**1.00 x 10^{-3}**
	MTAD	16.0 (5.0, 16.0)				**3.54 x 10^{-4}**
	CAP	0.0 (0.0, 0.0)				
Apical third	Control	0.0 (0.0, 0.0)	**9.27 x 10^{-5}**	**0.0254**	**0.0020**	1.0000
	EDTA	0.0 (0.0, 5.0)			1.0000	**0.0254**
	MTAD	0.0 (0.0, 16.0)				**0.0020**
	CAP	0.0 (0.0, 0.0)				

Median and interquartile range (IQR) of the count with scores 1 and 2 across different samples.

P-value < 0.05 is significant

Table 3 shows inter-group comparison of median (IQR) values in coronal, middle and apical third regions of the test samples. **In the coronal third** the median (IQR) values of control, EDTA, MTAD and CAP Plasma Jet were 0.0 (0.0, 0.0), 15.0 (7.0, 16.0), 16.0 (16.0, 16.0) and 8.0 (0.0, 14.0) respectively. MTAD showed the highest smear layer removal ability followed by EDTA, CAP Plasma jet, and normal saline. Statistically significant difference was found between control, EDTA, MTAD and CAP Plasma Jet (P value <0.05). Smear layer removal by MTAD was statistically significant when compared to CAP Plasma jet and normal saline (P value<0.05). However, the difference in the smear layer removal ability of MTAD was not statistically significant when compared with EDTA (P value=0.76). EDTA showed statistically significant results (P<0.05), in smear layer removal ability, when compared with normal saline group while the results weren't statistically significant when the comparison was with CAP plasma jet (P value=0.76). The difference in the smear layer removal ability of CAP plasma jet was statistically significant in contrast to normal saline group (P value<0.05).

To summarize the effect of treatment regimens in the coronal third - MTAD ≥ EDTA ≥ CAP Plasma Jet > Normal Saline.

In the middle third median (IQR) values of control, EDTA, MTAD and CAP Plasma Jet were 0.0 (0.0, 0.0), 16.0 (6.0, 16.0), 16.0 (5.0, 16.0) and 0.0 (0.0, 0.0) respectively. In the middle third too, MTAD showed the highest smear layer removal ability followed by EDTA, CAP Plasma jet, and normal saline. Statistically significant difference was found between control, EDTA, MTAD and CAP Plasma Jet (P value <0.05). MTAD showed statistically significant smear layer removal when compared to CAP Plasma jet and normal saline (P<0.05). However, the difference in the smear layer removal ability of MTAD was not statistically significant in comparison to EDTA (P value=1.0), though it was marginally better than the latter. The difference in the smear layer removal ability of EDTA was statistically significant in contrast to CAP plasma jet and normal saline group (P<0.05). CAP Plasma jet showed similar results when compared with the normal saline group (P>0.05).

The effect of treatment regimens in the middle third were - MTAD ≥ EDTA > CAP Plasma Jet = Normal Saline.

In the apical third median (IQR) values of control, EDTA, MTAD and CAP Plasma Jet were 0.0 (0.0, 0.0), 0.0 (0.0, 5.0), 0.0 (0.0, 16.0) and 0.0 (0.0, 0.0) respectively. MTAD showed the highest smear layer removal ability followed by EDTA, while CAP Plasma jet and normal saline showed similar results. Here the difference in the smear layer removal ability of MTAD was statistically significant in contrast to the other 2 treatment regimens employed (i.e., CAP Plasma jet and normal saline) ($P<0.05$). However, the difference in the smear layer removal ability of MTAD was not statistically significant in comparison to EDTA (P value=1.0), though it was marginally better than the latter. A statistically significant difference was observed when the smear layer removal ability of EDTA was compared to CAP plasma jet and normal saline groups ($P<0.05$). Whereas, CAP plasma jet did not show statistically significant results in contrast to the normal saline group (P value =1).

To summarize the effect of treatment regimens in the apical third - MTAD ≥ EDTA > CAP Plasma Jet = Normal Saline.

DISCUSSION

DISCUSSION

Endodontic treatment involves the removal of the vital and necrotic contents of the root canal through chemo-mechanical means followed by obturation of the prepared root canal to prevent the ingress of fluids thereby avoiding bacterial infection or regrowth (47).

Mechanical instrumentation of the root canal produces a smear layer that covers the dentinal tubules (48). The smear layer is an irregular amorphous layer containing inorganic dentin debris and organic materials like pulp tissue, odontoblastic process, necrotic debris, microorganisms, and their metabolic products (1). The smear layer structure can be divided into two zones: first, which is 1–2 µm thick, is attached to the surface of the root canal wall; and second, which is forced into the dentinal tubules to a depth of upto 40 µm, forming smear plugs (49). Some authors favor the preservation of the smear layer as they suggest that maintaining the smear layer may block the dentinal tubules and limit bacterial or toxin penetration by altering dentinal permeability (Michelich et al. 1980, Pashley et al. 1981, Safavi et al. 1990) (50)(51)(52). Others believe that the smear layer, being a loosely adherent structure, should be removed entirely from the surface of the root canal wall because it can harbour bacteria and provide an avenue for leakage (Mader et al. 1984, Cameron 1987a, Meryon & Brook 1990) (49)(53)(54). Since the smear layer is nonhomogeneous and may potentially be dislodged from the underlying tubules (Mader et al. 1984), it may slowly disintegrate, dissolving around a leaking filling material to leave a void between the canal wall and sealer (49). It may also limit the effective disinfection of dentinal tubules by preventing disinfectants like sodium hypochlorite, intracanal-medicaments like calcium hydroxide and even sealers from penetrating the dentinal tubules (5). A recent systematic review and meta-analysis of leakage studies concluded that the removal of the smear layer improves the fluid tight seal of the root canal system (6). Various methods employed for smear layer removal include chemical, ultrasonic and laser, however none has proven to be fully effective.

In the present study, effectiveness of smear layer removal by CAP Plasma Jet as a final treatment regimen was evaluated, when compared to 17% EDTA, MTAD and a control group (normal saline) when used as a final rinse, following irrigation with 5.25% NaOCl during instrumentation.

According to literature incidences of a single canal in mandibular 1st and 2nd premolar is 74% and 97.5% respectively (55). Their root canal shape tends to become round in the middle of the root, and as a consequence of which there are greater chances of the file contacting all the surfaces of root canals at the same time during instrumentation. Further, buccolingual and mesiodistal width of mandibular premolars is nearly equal so the dentin thickness all around the root canal is also nearly the same. Therefore, single rooted mandibular premolar teeth with single canal, extracted for orthodontic or periodontal reasons were used for the present study. There is less possibility of cracks and fractures developing in the sample since teeth removed for orthodontic or periodontal reasons experience minimal trauma during extraction.

Preoperative radiographs were taken for these teeth in the mesiodistal and the buccolingual view to rule out the possibility of additional canals, root resorption, calcification or carious involvement and to maintain a standard sample distribution amongst the groups. Samples selected had a root curvature less than 15 degrees when measured using schneider method on the preoperative radiographs. The Schneider method involves first drawing a line parallel to the long axis of the canal, in coronal third (AC), a second line was then drawn from the apical foramen to intersect the point where the first line left the long axis of the canal (BC). The Schneider angle was the outer angle created by the line AC and BC (angle BCE) (56).

The mandibular premolars were decoronated to standardise the sample length to 12mm. The samples were prepared using ProTaper Universal (PTU) files. PTU is a nickel titanium (NiTi) rotary system of instruments manufactured with variable taper over the length of the cutting blades, has convex triangular cross-sections and noncutting tips. The PTU contains three files (SX, S1, and S2) for the preparation of the coronal, middle and apical thirds of root canal and three finishing files (F1, F2, and F3) (57). PTU files are characterised by variable taper and modified triangular cross section that results in a higher cutting efficiency and less space for collection of dentinal chips. These accumulated dentinal chips and pulpal remnants form the smear layer on the dentinal walls. The mandibular premolars in the present study were prepared till F3 size apical preparation i.e. the apical portion was enlarged to size 30 file to allow adequate cleaning and penetration of the solution to the apical third of each root canal. The canal enlargement done was based on Weine's rule of enlarging the canal to three sizes larger than the first file that binds.

Chemo mechanical preparation of the canals was done using Sodium hypochlorite. Sodium hypochlorite solution remains the most widely recommended irrigant in endodontics on the basis of its unique capacity to dissolve necrotic tissue remnants and excellent antimicrobial potency. However, sodium hypochlorite even at concentrations of 5.25% was only able to remove the organic component of the smear layer. So, there was a need for chelating agents to remove the inorganic component.

Ethylenediaminetetraacetic acid (EDTA) refers to a chelating agent with the formula $[CH_2N(CH_2CO_2H)_2]_2$. Its usefulness arises because of its role as a hexadentate ligand and chelating agent, i.e. its ability to sequester metal ions such as Ca^{2+} and Fe^{3+}. After being bound by EDTA, metal ions remain in solution, but exhibit diminished reactivity (10). It has been reported that EDTA decalcified dentin to a depth of 20 to 30 μm in 5 min (58). EDTA acts as a chelator to form a stable complex with calcium in dentine and forms soluble calcium chelates. When all available ions have been bound, equilibrium is formed and no further dissolution takes place; therefore, chelating action of EDTA is self-limiting (59). The chelating effect of EDTA was almost neglected in the apical part of root canals. For root canal preparation, EDTA has limited value alone as an irrigation fluid. Combined application of ethylenediaminetetraacetic acid (EDTA) and sodium hypochlorite (NaOCl) is frequently recommended for effective removal of the smear layer from the root canal system. EDTA removes the mineralized portion of the smear layer by chelation, but has to be used with a proteolytic agent (NaOCl) to remove the organic component (60). EDTA itself does not possess disinfecting ability and also has been shown to inactivate chlorine, the active agent in NaOCl. However, there is concern that this combined irrigation regimen causes inadvertent erosion of the intra-radicular dentin (61)(62).

MTAD, introduced by Torabinejad and Johnson at Loma Linda University in 2003, is an aqueous solution of 3 % doxycycline, a broad-spectrum antibiotic; 4.25 % citric acid, a demineralizing agent; and 0.5 % polysorbate 80 detergent (Tween 80)(13). It is commercially available as a 2-part mixture (Biopure MTAD; Dentsply). MTAD has been recommended in clinical practice as a final rinse after completion of conventional chemo-mechanical preparation. The antimicrobial efficacy of MTAD is because of anti-collagenase activity of doxycycline, its low pH, ability to be released gradually over time, and its action is facilitated by citric acid which removes the organic and inorganic substances (13). Tween-80 reduces surface tension on the dentinal tubules and allows deeper penetration of Doxycycline into the tubules (63). Doxycycline, citric acid, and Tween 80 together may have a synergistic effect on the disruption of the bacterial cell wall and on the cytoplasmic membrane. MTAD has been reported to be effective in eliminating microbes that are resistant to conventional endodontic irrigants and medications and providing sustained antimicrobial activity through the binding affinity of doxycycline for dental hard tissues.

Torabinejad et al. showed that MTAD was an effective solution for the removal of the smear layer and doesn't not significantly change the structure of the dentinal tubules when canals were irrigated with sodium hypochlorite and then underwent a final rinse of MTAD (13). In an another in vitro study Torabinejad et al (2003) showed that MTAD has the ability to remove most of the smear layer and it possesses superior bactericidal activity compared with NaOCl or EDTA when tested against E. *faecalis* (64). The effectiveness of MTAD to completely remove the smear layer is enhanced when a low concentration of NaOCl (1.3 %) is used as an intracanal irrigant before placing 1 ml of MTAD in a canal for 5 min and rinsing it with an additional 4 ml of MTAD as the final rinse (according to manufacturer's instructions). Unlike the use of EDTA, minimal erosion of intra-radicular dentin has been reported when NaOCl and MTAD were used as the final rinse (21). The shortcomings of MTAD may be summarised as, it has less than optimal antimicrobial activity, lesser compatibility to dental pulp cells for revascularization procedure, its high cost and reduced shelf life (12).

In the present study final rinse was done by 5ml of each of the test irrigants and the solutions were held in the canal for 2 minutes similar to the irrigation regime followed by Torabinejad M. et. al. (2003) (64).

Plasma, the fourth state of matter, is a collection of stripped particles and it makes up for more than 99 percent of the visible universe. Plasmas are naturally energetic because stripping electrons uses constant energy. Based on the relative temperatures of the electrons, ions and neutrals, plasmas are classified as "thermal" or "non-thermal". Thermal plasmas have electrons and the heavy particles at the same temperature (higher than the room temperature), i.e., they are in thermal equilibrium with each other. Non-thermal plasmas on the other hand have the ions and neutrals at a much lower temperature (sometimes room temperature), whereas electrons are much "hotter" (14). In recent years, cold (less than 40 °C at the point of application) atmospheric pressure plasma (CAPP) sources have been introduced that provide the possibility to extend plasma treatment to living tissue. Cold Atmospheric Pressure Plasma (CAPP) is known as non-thermal because it has electrons at a hotter temperature than the heavy particles that are at room temperature and its temperature is less than 40°C at the point of application (15). CAPP Plasma may be produced by various methods, the one commonly employed is the use of Dielectric Barrier Discharge (DBD). Atmospheric Pressure Plasma Jet (APPJ), plasma needle, and plasma pencil are the various methods of delivery of the Plasma discharge. Gases that can be used to produce CAPP are Helium, Argon, Nitrogen, Heliox (a mix of helium and oxygen), and air (65).

In the present study we used a cross-field configured CAP Plasma-Jet which is a double dielectric barrier discharge (DBD) system generating plasma jet of a length of up to 35 mm through a dielectric material tube having dielectric constant 4.6 in which a copper wire mounted on a similar tube acts as the central power electrode and a thin copper material as the outer ring electrode. The central electrode was connected through a pulsed high-voltage power source, and the outer electrode was grounded. Helium was used as the working gas at atmospheric pressure. The plasma discharge was optimized at different combinations of input parameters like its applied voltage, frequency, average power consumption and gas flow rate. The optimized voltage applied was 4 KV at a frequency 20 KHz. The working distance (distance from nozzle to the canal lumen) was kept 1 cm.

Due to its ability to deactivate microorganisms, cause cell detachment, and cause death in cancer cells, researchers have been interested in finding uses for CAP Plasma in dentistry and oncology (65). Non-thermal plasmas are partially ionized gases that contain highly reactive particles including electronically excited atoms, molecules, ionic and free radical species. Depending on the plasma chemistry or gas composition, these highly reactive plasma species react with the surface, clean the surface, and etch surface materials, bond to various substrates, or combine to form a thin layer of plasma coating, and consequently alter the surface characteristics. Non-thermal plasmas combine exceptional chemical reactivity with relatively mild, non-destructive character due to cold gas phase (27). Ritts et al. (2010) and Zhang Y et al. (2014), showed that non-thermal plasma treatment provides a unique opportunity in modifying dentin surfaces in order to improve the interfacial bonding of the dental composite restoration (27)(36). Somewhat similar results were shown by Dong X et al. (2013) using argon plasma treatment (35). The hydrophilicity of the dentin surface was shown to increase and an increased number of carbonyl groups were found on the surface which improved the penetration of hydrophilic monomer components into collagen fibrils and dentin tubules.

Plasma treatment can generally change surfaces in two ways: modification and etching. Each interaction competes with one another on the applied surface. Modification of the surface could change the surface chemistry by introducing new functionalities, such as new chemical structures (27). Plasma consists of many energetic and chemically reactive species including high energy electrons, ionic species, electronically excited neutrals, and free radicals, etc. These active species in argon plasma can react with the treated surface and thus modify the surface chemistry and properties without affecting the bulk material properties (35). By lowering the temperature of the plasma and reducing the quantity of high energy ions, the plasma intensity decreases and limits the etching effect that can be seen in certain conditions. However, if a surface undergoes prolonged exposure to plasma under even low temperature, the surface structure such as the collagen fibrils on dentin surfaces can be etched away by the plasma (27).

Lehmann A. et al. (2013) studied changes in surface morphology of dentin after plasma treatment and observed inter-tubular areas appear slightly roughened. It was supposed that plasma treatment might chemically translate organic substance on dentin surface into volatile compounds and lead to the enlargement of dentin tubules (17). Dong X et al. in the year 2015 observed partially opened dentinal tubules and less smear layer under SEM examination after plasma treatment (39). Jiang C et al. showed surface alterations of the tooth surface by plasma such that in the plasma-treated root canal, a visible contrast line distinguishes the zones for plasma treated and non-plasma-treated surfaces, SEM images at higher magnification show predominantly clean surfaces revealing open dentinal tubules for this area (16). Similar findings were found in our study, where smear layer removal was noted in the coronal and middle thirds of root canal specimens.

Normal Saline, which was used as the control in the present study, had no effect on the smear layer removal, in accordance with previous work by Baumgartner J.C. et al. (1987) and also as shown by numerous other researchers (66).

Smear layer on the surface of instrumented root canals was studied using Scanning Electron Microscope (SEM). Other microscopy techniques such as Light microscopy has consistently failed to identify the smear layer, principally due to the fact that light microscopy depends upon histologic sections or shadowing techniques. The improvement in resolution of microscopic detail with SEM compared to that revealed by the light microscope, coupled with a large depth of field, makes this instrument ideally suited to detailing surface morphology. Transmission Electron Microscopy (TEM), another form of electron microscope, uses transmitted electrons (electrons that are passing through the sample) to create an image. As a result, TEM offers valuable information on the inner structure of the sample, such as crystal structure, morphology and stress state information. Whereas, the SEM on the other hand provides information on the sample's surface characteristics, which was the objective of the present study.

In the inter group comparison it was observed that MTAD and EDTA were the most efficient in smear layer removal and both showed comparable results. While smear layer removal ability of CAP Plasma Jet was significantly less efficient than EDTA. Both EDTA and MTAD performed significantly better than the control group i.e. normal saline. While CAP Plasma Jet and normal saline showed comparable results. **The effect of treatment regimens in the root canal can be summarised as - MTAD ≥ EDTA > CAP Plasma Jet = Normal Saline.** This result is in contrast to studies by Lotfi M et al. (2012) and Wu L et al. (2012) where EDTA performed better than MTAD. This could be attributed to the different methodology employed by them. In their study they used 1ml of the chelating agent for a duration of 1 minute which could be the factor producing different results.

The results of the present study show that the efficacy of smear layer removal in each of the experimental groups was in the order - Coronal third > Middle third > Apical third. This is in agreement with findings in endodontic literature that the apical third is the most challenging to clean. In the inter group comparison, the efficacy of smear layer removal in the **coronal third** was shown to be highest with the use of

MTAD followed by EDTA, CAP Plasma Jet and then Control group. Interestingly, the EDTA and CAP Plasma Jet groups showed comparable results in the **coronal third**, i.e. similar cleaning ability with no statistically significant difference in their values. All the test regimes, MTAD, EDTA, CAP Plasma Jet showed statistically significant superior smear layer removal ability when compared with the control group in the coronal third. These results may be attributed to greater surface wetting in the coronal third owing to a larger reservoir in the region allowing for extended irrigant contact with the canal walls. **To summarize the effect of treatment regimens in the coronal third - MTAD ≥ EDTA ≥ CAP Plasma Jet > Normal Saline**

In the **middle third**, MTAD was marginally better in its efficacy for smear layer removal than EDTA, though numerically not statistically significant. Both MTAD and EDTA showed statistically significant better results for smear layer removal when compared to CAP Plasma Jet group and control group ($P<0.05$). While CAP Plasma Jet showed comparable results when compared to Control group. **The effect of treatment regimens in the middle third were - MTAD ≥ EDTA > CAP Plasma Jet = Normal Saline.**

In the **apical third** the MTAD alone showed statistically significant better results than CAP Plasma Jet and control i.e. Normal Saline ($P<0.05$). MTAD and EDTA showed comparable results. EDTA showed statistically significant better smear layer removal ability than CAP Plasma Jet and Normal Saline. Complete or near complete smear layer removal i.e. score 1 and 2 was not seen in the test samples when exposed to CAP Plasma Jet as well as Normal Saline. **To summarize the effect of treatment regimens in the apical third - MTAD ≥ EDTA > CAP Plasma Jet = Normal Saline.**

These findings are in conjunction with the study by Mancini M. et al. (2009) where efficacy of smear layer removal by MTAD and EDTA showed no significant difference in the coronal, middle and apical third of the root canal (25). Both MTAD and EDTA showed statistically significant better smear layer removal when compared to the CAP Plasma Jet and Normal saline as well.

Contrary to our results, study by Mozayeni MA et al. (2009) and Torabinejad M. et al. (2003) showed that efficacy of smear layer removal by MTAD and EDTA showed no significant difference in the coronal and middle third (26)(21), while MTAD showed statistically significant better results than EDTA in the apical third. This difference in result from our study could be attributed to the different methodology in the above-mentioned studies. In the study by Mozayeni MA et al. the total exposure time to the final solution was approximately 5 min, while in our study it was 2 minutes. Dai L. et al. (2011) also concluded that MTAD showed better smear layer removal ability vis a vis EDTA, when the contribution from different thirds of the root canal was taken into consideration (29). They used 1.3% sodium hypochlorite before final rinse with MTAD, according to manufacturer's instructions whereas in our study 5.25% sodium hypochlorite was used to maintain standardization amongst the groups. Similar results were seen later in studies by Paul M.L. et al. (2013) and Vemuri S. et al. (2016) (33)(7).

On the other hand, research by Lotfi M et al. (2012) and Wu L et al. (2012) demonstrated that EDTA had superior smear layer removal ability when compared to MTAD (31)(32). This could be attributed to a different methodology. Lotfi M et al. used 1.3% Sodium hypochlorite during instrumentation of the canals. While in the study by Wu L et al. they used 1ml of the chelating agent for a duration of 1 minute which could be the factor producing different results.

The limitation of CAP Plasma Jet in its smear layer removal efficiency can be attributed to its design geometry which left much to be desired. There needs to be an optimisation of delivery system of plasma plume which affects the distance of plasma plume where it acts on a surface. The gas used in the present CAP Plasma Jet was Helium, whereas other optimised devices which are used for plasma delivery systems were Argon based and had oxygen gas used with in them. This may be a factor which makes the used device less efficient in smear layer removal. Lastly most optimised devices showed efficient results in 15-20 seconds whereas, the present device wasn't efficient even after exposure for 2 minutes. Further research after optimisation of the plasma device is warranted for efficient smear layer removal.

CONCLUSION

CONCLUSION

Hence it may be safely be concluded from the results of the present study that:

1. Both MTAD and EDTA had the best smear layer removal ability in all the thirds of the root canal when the samples were irrigated with 5ml of the solution for a period of 2 minutes.
2. CAP Plasma Jet showed comparable smear layer removal ability when compared to EDTA in the coronal third, while in the middle and apical third, EDTA was significantly better than CAP Plasma Jet.
3. Control group i.e. Normal Saline showed smear layer removal only in the coronal third of the root canal possibly owing to the mechanical effect of irrigation.
4. A 2 minute holding time for 5 ml of solution was found to be optimal for smear layer removal.

SUMMARY

SUMMARY

Plasma treatment had been shown to induce surface changes on dentin. Partially opened dentinal tubules and less smear layer under SEM examination were observed after plasma treatment.

Aim: The present in-vitro study aimed to assess the effectiveness of three final irrigation regimens (Cold Atmospheric Pressure Plasma Jet, MTAD, and EDTA) in removing smear layer from intra-radicular dentin using a scanning electron microscope (SEM).

Methodology: Eighty-four Human mandibular premolars extracted for orthodontic or periodontic purposes were decoronated and root length was standardized to 12 millimeters. Chemo-mechanical preparation was done with ProTaper Universal hand files up to size F3. The prepared samples were divided into 3 experimental groups (EDTA, MTAD, CAP Plasma Jet) and one Control group (normal saline) with 21 samples in each. Test samples in the experimental (EDTA and MTAD) and control groups were rinsed with 5 milliliters of the irrigant using 30G side-vented irrigation needle, placed 3mm short of apex and the solution was retained in the canal for 2 minutes. In the CAP Plasma Jet group, the samples were exposed to the plasma plume directed towards the canal lumen for a duration of 2 minutes. Test samples were further split longitudinally and the dentinal surfaces of the coronal, middle and apical thirds of the root were examined under SEM to determine the effectiveness of smear layer removal. The SEM images of the test samples were graded according to the scoring criteria given by Prado et al. Statistical analysis was performed using Chi-square test to determine the significant difference between the treatment groups**.**

Results: The samples irrigated with MTAD and EDTA had shown the highest smear layer removal in coronal, middle and apical third regions when compared to CAP Plasma Jet. The smear layer removal ability of MTAD was significantly better CAP Plasma Jet and Normal Saline in the coronal, middle and apical third of the root canal ($P < 0.05$). While, MTAD and EDTA showed comparable results in their smear layer removal ability. Smear layer removal ability of EDTA when compared to CAP Plasma Jet was statistically insignificant in the coronal third, while EDTA performed significantly better in the middle and apical third. CAP Plasma had better efficiency in removing smear layer when compared to control (normal saline) in the coronal third (P

<0.05). However, there was no difference in the smear removal ability amongst the two in the middle and apical third.

Conclusion: MTAD and EDTA aided in the better smear layer removal when compared to CAP Plasma Jet in the coronal, middle and apical third of the test samples. CAP Plasma jet had better smear layer removal ability than the control group in the coronal third only.

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ANNEXURES

ANNEXURES

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर

All India Institute of Medical Sciences, Jodhpur

संस्थागत नैतिकता समिति

Institutional Ethics Committee

No. AIIMS/IEC/2021/3484 **Date: 12/03/2021**

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2021/3319**

Project title: **"Efficacy of cold atmospheric pressure (CAP) plasma jet for smear layer removal in intra-radicular dentin when compared with two other chelating agents used as a final rinse-an In-vitro scanning electron microscopic evaluation"**

Nature of Project:	**Research Project Submitted for Expedited Review**
Submitted as:	**M.D.S. Dissertation**
Student Name:	**Dr. Ankita Kapoor**
Guide:	**Dr. Pravin Kumar**
Co-Guide:	**Dr. Ram Prakash & Dr. Kirti Chaudhary**

Institutional Ethics Committee after thorough consideration accorded its approval on above project.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

Please Note that this approval will be rectified whenever it is possible to hold a meeting in person of the Institutional Ethics Committee. It is possible that the PI may be asked to give more clarifications or the Institutional Ethics Committee may withhold the project. The Institutional Ethics Committee is adopting this procedure due to COVID-19 (Corona Virus) situation.

If the Institutional Ethics Committee does not get back to you, this means your project has been cleared by the IEC.

On behalf of Ethics Committee, I wish you success in your research.

Dr. Praveen Sharma
Member Secretary

Member secretary
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