"EVALUATION OF EFFECT OF CALCIUM HYDROXIDE INTRACANAL MEDICAMENT ON THE DENTINAL TUBULE PENETRATION OF AN EPOXY RESIN BASED SEALER AND A BIOCERAMIC BASED SEALER USING CONFOCAL LASER SCANNING MICROSCOPY - AN IN-VITRO STUDY"

By

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REG.NO. IE0219004

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Under the guidance of

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DEPARTMENT OF CONSERVATIVE DENTISTRY AND ENDODONTICS

KAHER VK INSTITUTE OF DENTAL SCIENCES,

BELAGAVI, KARNATAKA

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This Dissertation is dedicated to

The Almighty God,

My Parents,

&

My Family Members

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Dr Shefali G Pednecar

LIST OF ABBREVIATIONS

SR.NO	ABBREVIATIONS	FULL FORM
1	EDTA	Ethylene diamine tetra acetic acid
2	CLSM	Confocal laser scanning microscope
3	ANOVA	Analysis of variance
4	μm	Micrometres
5	SD	Standard Deviation
6	et al	Additional persons involved in the same study
7	NaOCl	Sodium Hypochlorite
8	SEM	Scanning Electron Microscope
9	mL	Milliliter
10	° C	Degrees Celsius
11	mm	Millimeter
12	hrs	Hours
13	min	Minutes
14	n	Number of specimens
15	p-value	Probability of obtaining a test statistic at least as extreme as the one that was actually observed
16	i.e.	That is
17	nm	nanometers
18	<	Less than

19	>	Greater than
20	$Ca(OH)_2$	Calcium Hydroxide
21	SE	Standard error
22	CI	Central incisor
23	PM	Premolar
24	PUI	Passive ultrasonic irrigation
25	WL	Working Length
26	SC	Single Cone
27	WVC	Warm Vertical Condensation

ABSTRACT

Aim- Comparative evaluation of the effect of Calcium Hydroxide intracanal medicament on the dentinal tubule penetration of an Epoxy resin based sealer, AH Plus and a Bioceramic based root canal sealer, Bio-C using Confocal Laser Scanning Microscopy.

Methodology- Seventy extracted human mandibular premolars were selected and decoronated to obtain a standardized root length of 14±1 mm. Working length was established using a 10 K-file and the specimens were divided into 3 groups: Group 1- Positive control group, Group 2- Negative control group, Group 3- Experimental group. Chemo-mechanical preparation was done using ProTaper Gold file system upto F4 for negative control group and upto F3 followed by calcium hydroxide placement for Experimental group. Calcium hydroxide was then removed and canal was prepared upto F4. All the specimens were then dried with paper points, divided into 2 subgroups based on sealer used and obturated with rhodamine labelled sealer. After incubation for a week, the specimens were sectioned to obtain apical, middle, coronal thirds and viewed under the CLSM for maximum depth of tubule penetration. Statistical analysis was done using One-way ANOVA and Tukey's post-hoc tests.

Results – Highly statistically significant difference was seen between the groups with Bio-C after $Ca(OH)_2$ demonstrating the highest penetration. Among the three thirds, the highest penetration was seen in the coronal third for AH Plus, whereas Bio-C showed highest penetration in the middle third. Least penetration was seen in the apical section for all the groups.

Conclusion- Bio-C sealer showed promising results both with and without calcium hydroxide with highest in the middle third. AH Plus portrayed highest penetration in the coronal third. Future studies assessing long term behaviour of Bio-C sealer need to be taken into consideration.

Keywords: Bio-C sealer, Calcium hydroxide, AH plus, Dentinal tubule penetration, Rhodamine dye, Confocal Laser Scanning Microscope.

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INTRODUCTION:

Root canal treatment aims at meticulous debridement and cleaning of any pulp tissue which is necrosed and infected from the 'root canal system' so that the canal can be adequately prepared and shaped for filling with an inert material thereby averting reinfection. Despite favorable success rate of endodontic treatment, a certain number of cases fail due to various factors like persistence of bacteria, incomplete filling, untreated canals, and improper 'coronal' seal. Persistent microbial infections being one of the prime factors for such treatment failure has led to a paradigm shift during root canal therapy which focuses on elimination of infection, prevention of fluid penetration and proliferation of bacteria[1] within the canal and synchronously protecting the decontaminated tooth from future microbial invasion.[2]

Residual bacteria remaining within the 'root canal' after preparation can result in continued infection when it comes in contact with the 'periapical tissues', the result of which is microbial recolonization of the obturated root canal.[3]

Hence during endodontic therapy, along with various biomechanical preparation techniques and irrigation procedures, 'intracanal medicaments' such as '$Ca(OH)_2$', Ledermix, Triple Antibiotic Paste, Iodine-potassium-iodine, etc. have been widely employed for disinfection of infected root canals during endodontic therapy.[2]

Very often bacteria remaining within the root canal complexities in necrotic teeth damper the accomplishment of total disinfection without the use of an intracanal dressing. Single visit root canal treatment is usually not preferred in root canals which cannot be dried due to exudates in the presence of acute 'apical' periodontitis as it

could result in intensified postoperative pain and flare-up. In such situations, the placement of '$Ca(OH)_2$' which is the most widely used intracanal medicament has shown to increase the healing rates, whereas, when not placed, the bacterial counts are found to swiftly escalate.[4]

'Hermann' in 1920 introduced '$Ca(OH)_2$' which demonstrated good antimicrobial and tissue dissolving ability, hard tissue formation and impediment of tooth resorption and is associated with peri-radicular healing.[2,4] Its biological properties are achieved because of its high pH and ionic activity, diffusion through dentinal tubules and its influence on 'apical' tissues.[5]

The different types of vehicles used along with '$Ca(OH)_2$', that is, 'aqueous, viscous or oily' has a pivotal role to play on account of its direct relationship with the velocity and concentration of ionic liberation to boot with the antibacterial action when the paste is carried into the contaminated area. Aqueous vehicles offer a benefit of rapid and high ionic dissolution. The high molecular weight of these vehicles maintains the paste in the desired area for longer time periods and also minimizes its dispersion into the tissues.[6] The type of vehicle plays an important role and affects the removal of $Ca(OH)_2$.

The complete removal of '$Ca(OH)_2$' from the root canal is a quandary. There are no methods available till date, that can completely remove all the remnants from the canal though it is critical as these remnants affect the bonding capacity between dentin and sealer, penetrability of the sealer into the tubules and also sealing ability of 'root canal sealers'. Thus, the absolute elimination of '$Ca(OH)_2$' medicament prior to obturation is paramount, thereby directly affecting the treatment outcome.[2, 7, 8] These

findings necessitate the need of future studies to assess impact of retention of 'intracanal medicaments' on 'tubular penetration' of sealers.

The most preferred obturation material is gutta percha along with the various 'root canal sealers'. Use of endodontic 'root canal sealers' enhances bonding to dentin by extending into the accessory and lateral canals. Its main role is to acquire a fluid tight hermetic seal through the entire root canal that includes the 'apical' foramen and canal irregularities. Sealers are also essential to obliterate the minor variance between the dentinal wall of the canal and the gutta percha filling material.[9]

A key determinant associated with the clinical outcome and longevity of root canal therapy is the 'tubular penetration' depth of the sealer and also micro-gap between the sealer and root canal wall. The performance of a root canal sealer is measured by its 'dentinal tubule penetration' depth. Deeper 'tubular penetration' augments the contact area between root fillings and dentin, entombs the bacteria within the dentinal tubules and also has the advantage of increasing the fracture resistance of the root canal by improving the retention of root canal fillings. [10,11]

'Epoxy resin-based sealers' were introduced in endodontics by Schroeder and they are found to show improved bonding to the core obturation material and root canal dentin.[12]

Epoxy based sealer, 'AH Plus' has been universally accepted as a gold standard amongst 'root canal sealers'. This is a sealer against which other sealers can be compared because of its propitious handling characteristics and superior physical properties. Although, it has quite a few advantages such as low shrinkage, high radio opacity, low solubility and good biocompatibility; however, it is found to be toxic

when freshly mixed. Besides, it also lacks bioactive potential desired for periapical healing which lead to the pioneering of bioceramic sealers.[3, 13, 14]

'Bioceramic root canal sealers' were first introduced by Krell and Wefel, its major advantages being their biocompatibility. They result in a chemical composition and crystalline structure analogous to tooth and bone apatite materials, thereby improving sealer to root dentin bonding.[15]

Bio-C sealer is a new 'bioceramic root canal sealer' and is composed of calcium silicates, calcium oxide, calcium aluminate, iron oxide, zirconium oxide, silicon dioxide and dispersing agent. It has numerous advantages such as short setting time, high flow rate, alkalinization ability, radio-opacity and a low volumetric shrinkage.[16]

Variations in properties (physical and chemical) of these 'root canal sealers' influences the depth of sealer penetration. Ability of different 'root canal sealers' to penetrate into the 'dentinal tubules' unswervingly and efficiently influences the choice of material for filling the root canals. Current literature has portrayed a similarity in composition between bioceramic sealers and '$Ca(OH)_2$' residues which could enhance its penetration property after '$Ca(OH)_2$' medicament. Hence, it is imperative to collate the penetration of various sealers employed in root canal treatment both with and without the use of intracanal medicament.[17]

Till date there are no studies comparing the dentinal 'tubular penetration' of 'Bio-C' sealer with other sealers after '$Ca(OH)_2$' medicament. Hence, the present study aims at comparing and evaluating the effect of '$Ca(OH)_2$' intracanal medicament on the 'dentinal tubule penetration' of 'epoxy resin-based sealer', 'AH Plus' and 'Bio-C', 'bioceramic root canal sealer.'

AIM AND OBJECTIVES

AIM OF STUDY:

- Comparative evaluation of the effect of '$Ca(OH)_2$' intracanal medicament on the 'dentinal tubule penetration' of Epoxy resin-based root canal sealer, AH Plus and Bioceramic based root canal sealer, Bio-C using Confocal Laser Scanning Microscopy.

OBJECTIVES OF THE STUDY:

- To evaluate effect of '$Ca(OH)_2$' intracanal medicament on 'dentinal tubule penetration' of an Epoxy resin based root canal sealer, AH Plus using confocal laser scanning microscopy.

- To evaluate effect of '$Ca(OH)_2$' intracanal medicament on 'dentinal tubule penetration' of a Bioceramic based root canal sealer, Bio-C using confocal laser scanning microscopy.

- To compare the 'dentinal tubule penetration' of an Epoxy resin-based root canal sealer, AH Plus and a Bioceramic based root canal sealer, Bio-C using confocal laser scanning microscopy.

HYPOTHESIS:

NULL HYPOTHESIS:

'There is no difference in the dentinal tubule penetration of the Epoxy resin based root canal sealer, AH Plus and the Bioceramic based root canal sealer, Bio-C after use of $Ca(OH)_2$ intracanal medicament.'

ALTERNATIVE HYPOTHESIS:

'There is a difference in the dentinal tubule penetration of the Epoxy resin-based root canal sealer, AH Plus and the Bioceramic based root canal sealer, Bio-C after use of $Ca(OH)_2$ intracanal medicament.'

REVIEW OF LITERATURE:

1. An in vitro study was conducted by Eid et al (2019) for evaluating the "tubular penetration" of 3 "root canal sealers", 'AH Plus, Apexit Plus and Smart paste bio' in the 'apical', 'middle' and 'coronal thirds' using single cone obturation technique for which 30 teeth were prepared and then cross sectioned. "Tubular penetration" and adaptation of sealer to the root canals were evaluated under scanning electron microscope. Among the three tested groups, 'apical' third showed the least penetration whereas no statistical difference was noted between the 'coronal' and 'middle' thirds. "AH Plus" reported optimal "tubular penetration" in the 'apical' section of all the sealers tested.[18]

2. Uzunoglu-Özyürek E et al (2018) evaluated the effect of '$Ca(OH)_2$' dressing on the "dentinal tubule penetration" of two "root canal sealers" using CLSM. 52 human mandibular premolars were selected of which 4 representing the positive control tested the penetrability of '$Ca(OH)_2$'. Twenty-four samples received '$Ca(OH)_2$', eliminated after 2 weeks with PUI. Two groups were made of these samples based on sealer type used, i.e., AH 26 and Bioroot-RCS and obturation was completed by single cone technique (F4 ProTaper cone used). Evaluation using image analysis showed least penetration in the 'apical' third and 'Bioroot-RCS' showed higher penetration than 'AH Plus' even in the presence of '$Ca(OH)_2$'.[2]

3. Wang et al (2018) conducted an In-vitro study to evaluate the "dentinal tubule penetration" and filling quality of 'bioceramic sealer (iRoot SP)' for which he instrumented 42 extracted mandibular incisors using ProTaper universal system till F3. Samples were divided into 4 groups: 'iRoot SP single cone group, iRoot SP warm

vertical group, “AH Plus” single cone group and “AH Plus” warm vertical group’. Obturation was done using sealers mixed with “Rhodamine B dye” for better perception using confocal microscopy. Penetration of sealer into dentinal tubules in all the sections and percentage of voids was evaluated after sectioning of samples at 2, 4 and 6 mm. iRoot SP demonstrated statistically higher penetration than “AH Plus” in both the single cone and warm vertical technique at 2 mm from the apex, thereby indicating better filling quality.[14]

4. Cruz et al (2017) in his In-vitro study evaluated the influence of ‘$Ca(OH)_2$’ medicament on the “tubular penetration” of “AH Plus” and ‘MTA Fillapex’. Preparation of mandibular premolars (n=70) with single root canal was done with Profile instruments and were divided into 4 groups. ‘$Ca(OH)_2$’ was placed in 2 of these groups for ‘15 days’ after which lateral condensation was done. Sealer penetration was then assessed using CLSM after transverse sectioning in the ‘middle’ and ‘apical third’. Decreased penetration was observed for “AH Plus” in the ‘middle third’. Overall, ‘$Ca(OH)_2$’ didn’t influence ‘apical’ third sealer ‘penetration’ for the two sealers and “tubular penetration” was higher with MTA Fillapex than with “AH Plus”.[3]

5. A study was done by de CAMARGO EJ et al (2015) evaluating the adaptation and root canal penetration in ‘coronal’, ‘middle’ and ‘apical’ third of methacrylate based resin sealer (Epiphany) after ‘$Ca(OH)_2$’. Profile instruments were used for BMP in 30 mandibular incisors which were randomly distributed into 3 groups, “group 1:no ‘$Ca(OH)_2$’ dressing, group 2: ‘$Ca(OH)_2$ for 14 days’ + removal with ‘saline and 40 K-file’ and group 3: 17% EDTA used in addition for removal of ‘$Ca(OH)_2$’’. Canals were obturated using Rhodamine labelled Epiphany sealer and viewed under

magnification after sectioning. Significantly lower sealer penetration was seen in 'apical third' compared to 'middle' and 'coronal thirds'. Group 1 showed least adaptation than Group 2 and Group 3 and '$Ca(OH)_2$' was shown to favor methacrylate-based sealers penetration.[19]

6. Reynolds et al (2020) compared the depth and percentage of "dentinal tubule penetration" of two bioceramic sealers, BC sealer, BC Sealer HiFlow and an"epoxy resin-based sealer", 2Seal easymiX after single-cone and warm vertical obturation in fifty teeth. Control group were filled using the warn vertical technique only whereas the BCS and BCSHF groups were filled with SC or WV techniques. Samples were sectioned at 3 and 6 mm to visualize under CLSM and the results were observed. No difference which was significant in depth and percent of sealer penetration among the sealer types and obturation techniques was noted. Significantly deeper penetration was observed as 6 mm as compared to 3 mm by Mann Whitney test. The study concluded that the "dentinal tubule penetration" was similar using SC and WV techniques amongst the three sealers tested in the study. [20]

7. Roula El Hachem et al (2018) conducted a study assessing the "dentinal tubule penetration" of 'AH Plus, BC Sealer and a novel tricalcium silicate sealer' for which 96 maxillary CIs were selected. After dividing them into three groups, single cone obturation was completed using 'gutta-percha' with either of the 3 sealers: 'AH Plus, BC Sealer or NTS'. CLSM was used for examination after sectioning at 1 and 5 mm from the apex. The sealer penetration depths were analyzed at their maximum depths and at four circumferential depths that is 9, 12, 3 and 6 o'clock using ImageJ software. 'Penetration' was significantly higher as seen at 5 mm as against 1 mm for all three groups according to the Two-way ANOVA test. Intergroup comparison

showed lower penetration for "AH Plus" at 5 mm than the other two sealers (p = 0.012). In conclusion, the BC Sealer and NTS exhibited better tubule penetration than the "AH Plus" sealer.[21]

8. The "penetration" ability of 'calcium silicate root canal sealers' and 'resin-based sealer' was compared in a study done by Yemi Kim et al (2019) using the CLSM. Root canals of 60 single rooted premolars were instrumented using ProFile rotary instruments to a size 40/0.06 taper and were irrigated with 'NaOCl and EDTA', following which root canals were dried. Random division of the specimens into 3 groups was done based on the sealers: 'Group 1, gutta-percha (GP)/ "AH Plus" with continuous wave compaction; group 2, GP/BioRoot RCS with a single-cone technique; and group 3, GP/Endoseal MTA with a single-cone technique'. Sectioning was done to evaluate penetrability under the CLSM obtaining 'apical', 'middle' and 'coronal' thirds. Statistical analysis done using Kruskal–Wallis and Mann–Whitney U post hoc tests showed greater fluorescence in the "AH Plus" group in 'apical' and 'coronal thirds' as compared to 'BioRoot RCS' and 'Endoseal MTA' groups, whilst 'BioRoot RCS' group displayed a higher intensity in 'middle third', comparable to the "AH Plus" group. In conclusion, type of sealer and root third significantly affected the pattern of penetration and distance.[22]

9. An In-vitro study was done by Diana Eid et al (2021) for evaluating the "dentinal tubule penetration" of two 'calcium silicate-based sealers' using 'warm vertical compaction obturation technique' in comparison with the 'single cone technique' by CLSM. 44 mandibular single-rooted premolars were randomly divided into four experimental and two control groups based on sealer type ("Bio-C" or "HiFlow") with either SC or WVC. After using Rhodamine labelled sealers, the specimens were

sectioned at 1 and 5 mm horizontally from the apex. Images were analyzed in the Image J software followed by statistical analysis using 'Mann–Whitney U and Kruskal–Wallis tests'. Results showed significant difference betwixt the groups at '1 mm ($p = 0.0116$)' in contrast to results noted at '5 mm ($p = 0.20$)' which were similar. 'The study concluded that Warm vertical compaction technique enhanced the penetration of calcium silicate-based sealers into the dentinal tubules in comparison with the Single cone technique at both level'.[23]

10. Tavares et al (2020) evaluated premixed 'calcium silicate-based sealer' for its filling ability and compared it to 'epoxy resin-based sealer' using technique of 'single cone obturation' in root canals which were flattened. 32 second maxillary premolars with such anatomy were prepared by "Hyflex EDM 25/0.08" in adjunct with "ultrasonic tip Flatsonic and ProDesign Logic 25/0.03" and then filled with 2 "root canal sealers", i.e., "Bio-C" Sealer or "AH Plus". Micro-computed tomography (micro-CT) was used to analyze the percentage of voids in the cervical/ 'middle' thirds and in the 'apical' third. Mann–Whitney test revealed no statistical difference between "Bio-C" Sealer and "AH Plus" ($p > .05$). In the 'apical third', the respective percentages for "Bio-C" Sealer and "AH Plus" were 11.84% (4.85–27.00) and 9.21% (1.34–28.78). In conclusion, when single-cone technique was used, filling ability of both was found to be similar.[24]

11.Turker et al (2018) studied the effect of "smear layer" on the depth of penetration and 'push-out bond strength' of "root canal sealers". Herein, 2 groups were made of 90 mandibular premolars: 'smear layer preserved and smear layer removed' and subdivided into 3 subgroups based on the sealer which was tested, i.e., 'BioRoot RCS, AH 26, and MTA Plus'. Obturation was done following which 3 slices of 1 mm

thickness were taken from mid-root area of every root. Of these, 2 slices were for testing of 'push-out bond strength' and 1 was for calculating the "dentinal tubule penetration" depth using CLSM. Statistical analysis was done by Two-way ANOVA and post hoc Tukey test. The results showed that the retention of "MTA Plus" and "BioRoot RCS" was greater than "AH 26" in presence of 'smear layer'. When it was eliminated, the depth of penetration was the lowest with BioRoot RCS ($P<0.05$). In conclusion, depth of penetration and percentage of "root canal sealers" remained unaffected by the presence or absence of smear. [11]

12. Paula Muedra et al (2021) in his In-vitro study compared the 'dentinal penetration of two silicate-based sealers using CLSM'. Human single root teeth (n=32) were instrumented up to 35/0.04 Mtwo system, irrigated with 5.25% NaOCl and 17% EDTA and then randomly assigned to three study groups namely, ES, group 1(EndoSequence BC sealer), BR, group 2 (BioRoot RCS); and a control group with "AH Plus" (AHP). Samples were obtained from 'coronal', 'middle' and 'apical' thirds after single cone obturation and then penetration depth and percentage were measured. "Mann Whitney U test" and Wilcoxon t-test was used for statistical analysis with ES exhibiting greater penetration than "AH Plus" in the 'apical' and 'middle thirds ($p < 0.05$)', and in 'middle' and 'coronal thirds' relative to 'BR ($p < 0.05$)'. The penetration percentage around perimeter of the canal was greater for 'ES in all thirds', except for 'apical' where "AH Plus" was higher ($p < 0.05$) and thereby concluding that the "pre-mixed silicate-based sealer" exhibited superior penetrability.[25]

13. An In-vitro study was done by Chadha et al (2012) evaluating the penetration depth of three resin-based "root canal sealers" into the dentinal tubules at the

'cervical', 'middle' and 'apical' third of the root canal. 32 mandibular PMs were prepared and obturated using 'EndoREZ + resin-coated gutta-percha points (group A), Epiphany + Resilon points (group B), or "AH Plus" + gutta-percha (group C)'. SEM analysis was done after longitudinally sectioning the teeth in a buccolingual orientation. EndoREZ sealer showed maximum penetration into dentinal tubules at 'cervical', 'middle' and 'apical' third (525.2 μ, 327.802 μ and 198.36 μ). Epiphany sealer was the second and least penetration by "AH Plus". The study concluded that the 'penetration depth of EndoREZ and Epiphany is significantly greater than that of "AH Plus"'.[26]

14. A study was done by Greer E. McMichael et al (2016) for measuring the "tubule penetration" with "tricalcium silicate sealers"; EndoSequence BC Sealer, QuickSet2, NeoMTA Plus, and MTA Fillapex using the 'continuous wave (CW) and single-cone (SC) obturation techniques'. Eight groups were made of 80 single rooted teeth and were obturated with the sealers labelled with "Rhodamine dye" using either the "CW or SC technique". Samples were sectioned at 1 mm and 5 mm from the apex and were seen under CLSM. When the maximum sealer penetration was measured, "MTA Fillapex" had significantly greater "tubule penetration" at the 1-mm level. In conclusion, "CW and SC techniques" had similar tubule penetration at 1-mm and the 5-mm level with 'BC Sealer, QuickSet2, and NeoMTA Plus'.[27]

15. Jeong et al (2017) investigated the depths of penetration into dentinal tubules of a calcium silicate–based sealer by using 3 different obturation techniques. One hundred extracted human permanent anterior teeth were endodontically prepared and divided equally into 3 experimental groups and 1 control group as follows: 'C Point single cone (CPSC), gutta-percha single cone (GPSC), gutta-percha vertical condensation

(GPVC), all with a calcium silicate–based sealer and calcium indicator Fluo-3, and C Point single cone with a calcium indicator Fluo-3 (CPF3) without sealer as the control'. Roots were sectioned, visualized under the confocal laser scanning microscope and the "sealer penetration depths" were measured at their maximum and at 4 circumferential depths. There was no statistically significant difference among the mean maximum depth (P = .7553) and among the average depths across all points for the 3 experimental groups as assessed by the One-way ANOVA. The study concluded that the obturation technique did not affect the sealer penetration into dentinal tubules.[28]

16. Coronas et al (2020) evaluated the penetrability of a new "bioceramic root canal sealer" under the CLSM using Fluo-3. Forty distobuccal roots of the maxillary molars were prepared using Wave One Gold files upto size 35.06 and divided into 4 groups based on the filling techniques: 'Sealer Plus BC /Lentulo; "Bioceramic"/EasyClean group, 3x20 s activation (Easy Clean instrument); "Bioceramic"/30 s Irrisonic; and "AH Plus"/Lentulo'. Analysis was done after sectioning of the specimens at 2 and 7 mm, analysis was done using CLSM and sealer penetration assessed using Adobe Photoshop. Statistical tests were "Kruskal Wallis and Wilcoxon T tests". The results were found to be kindred for both the sealers irrespective of the technique used to perform activation. The study concluded that the instrument type used for activation of bioceramic sealers did not affect its penetrability.[29]

17. Caceres et al (2021) in her study compared the "dentinal tubule penetration" and adaptation of "premixed bioceramic sealer" and "epoxy resin-based sealer" using 30 single straight roots. After instrumentation using ProTaper Next, the specimens were assigned to 2 groups according to the sealer used, that is "AH Plus" and "Bio-C"

sealer. The samples were viewed by SEM after sectioning at '2, 5 and 8 mm from the apex' and analyzed for "tubular penetration" of sealers. The results analyzed by 'Shapiro Wilk, Levene and Mann-Whitney tests' showed "Bio-C" sealer to have a significantly more penetration than "AH Plus" sealer in all sections.[30]

18. A study done by Yang et al (2021) evaluated the '"dentinal tubule penetration" and retreatability of Endosequence BC Sealer HiFlow (HiFlow), iRoot SP, and "AH Plus" after using the single-cone (SC) or continuous wave condensation (CWC) technique'. 65 single rooted teeth were prepared using ProTaper Next system and then randomly assigned to 5 groups: 'group 1, "AH Plus"/CWC; group 2, iRoot SP/CWC; group 3, iRoot SP/SC; group 4, HiFlow/CWC; and group 5, HiFlow/SC'. CLSM and SEM was used to assess the tubule penetration and remaining debris after retreatment and statistical analysis done using "Kruskal-Wallis test" and "Dunn's multiple comparisons test". Results showed significantly higher penetration area for HiFlow/CWC than for 'iRoot SP/SC at 4 mm from the apex' and greater depth of penetration at both 8- and 12-mm level. Less remaining sealer was noted with HiFlow/CWC and HiFlow/SC groups than 'AH Plus/CWC group at 4-mm level'. In conclusion, recommendation for root canal therapy is the combined use of 'EndoSequence BC Sealer HiFlow with the continuous wave condensation technique'. [31]

19. Dani Song et al (2021) compared the degree of dentinal penetration between epoxy based sealer applied using two different filling methods and an ultrasonically activated calcium silicate-based sealer using CLSM. 45 maxillary premolars with Vertucci type 2 canals were prepared using ProTaper system, out of which three groups were made as follows: '"AH Plus" + continuous wave technique (AHC

group); “AH Plus” + single cone technique (AHS group); and Endoseal MTA + single cone technique with ultrasonic activation (EMS) group’. Sealer penetration was assessed at ‘2 and 5 mm from the apex’ under confocal microscopy and results obtained assessed with ANOVA or “Kruskal–Wallis test”. Results showed higher values of all the parameters at “5 mm level” with “EMS group” showing the least values. At the 2 mm level, there was no significant difference between the groups. Higher values were observed in the AHC group as against other groups; however, the filling efficacy was questionable in the ‘apical’ third. [32]

20. A confocal microscopic study was conducted by Akcay et al (2016) to study the penetration of “root canal sealers” by using various final irrigation techniques for which 156 mandibular premolars with single root were prepared using “ProTaper system”. Four groups were made at random depending on the type of: “AH Plus”, ‘iRoot SP, MTA Fillapex, and GF Bioseal’ and further subdivided into 3 groups based on irrigation protocol: ‘conventional needle irrigation (CI), photon-induced-photoacoustic streaming activation (PIPS), and passive ultrasonic irrigation (PUI)’. Obturation was completed using rhodamine dye labeled sealer and specimens were visualized under the CLSM after sectioning at 2, 5 and 8 mm to measure tubule penetration area. It was seen that iRoot SP had the greatest penetration area than others, however there was no significant difference between the other three groups, i.e., “AH Plus”, MTA Fillapex, and GF Bioseal. CI demonstrated significantly lower penetration as compared to ‘Er:YAG laser activation with PIPS and PUI’. The ‘coronal’ third demonstrated highest levels of penetration after which were ‘middle’ and ‘apical’ thirds. Concluding, the “root canal sealer” type, “final irrigation” protocol and “root canal third” affected “tubular penetration” and ‘use of iRoot with PIPS tip or PUI” was found to be advantageous’. [33]

MATERIALS AND METHODOLOGY

STUDY DESIGN:

In-vitro study

SOURCE OF DATA:

The study was conducted in the "Department of Conservative Dentistry and Endodontics, KLE Academy of Higher Education & Research, KLE VK Institute of Dental Sciences, Belagavi" and the laboratory procedures were undertaken at Dr. Prabhakar Kore's Basic Science Research Laboratory, KLE University, Belagavi.

Specimens were evaluated under the confocal laser scanning microscope at "Birla Institute of Technology and Science- Pilani, K. K Birla Goa campus".

Extracted human mandibular premolar teeth were collected from "Department of Oral and Maxillofacial Surgery, KLE Academy of Higher Education & Research, KLE VK Institute of Dental Sciences, Belagavi".

INCLUSION CRITERIA:

Human mandibular premolars with single root and single straight canal with closed apex with initial 'apical' binding file size 25 K or less.

EXCLUSION CRITERIA:

- Teeth with radicular resorption, cracks or fracture line.
- Root canal treated teeth.
- Teeth with calcified canals.
- Teeth with root caries.
- Teeth with multiple canals/Anatomic variation.
- Teeth with initial 'apical' binding file size more than 25 K.

SAMPLE SIZE ESTIMATION:

S1=269.13

S2=248.85

d=255.19

Z_α=1.96 at 5% α error

Z_β = 0.842 at 80% power

$$n = \frac{2S^2 (Z_\alpha + Z_\beta)^2}{d^2}$$ = 16 in each sub-group

SAMPLING PROCEDURE:

Samples were randomly allocated to groups and sampling procedure employed for the same was Multistage sampling technique.

MATERIALS AND ARMAMENTARIUM:

MATERIALS:

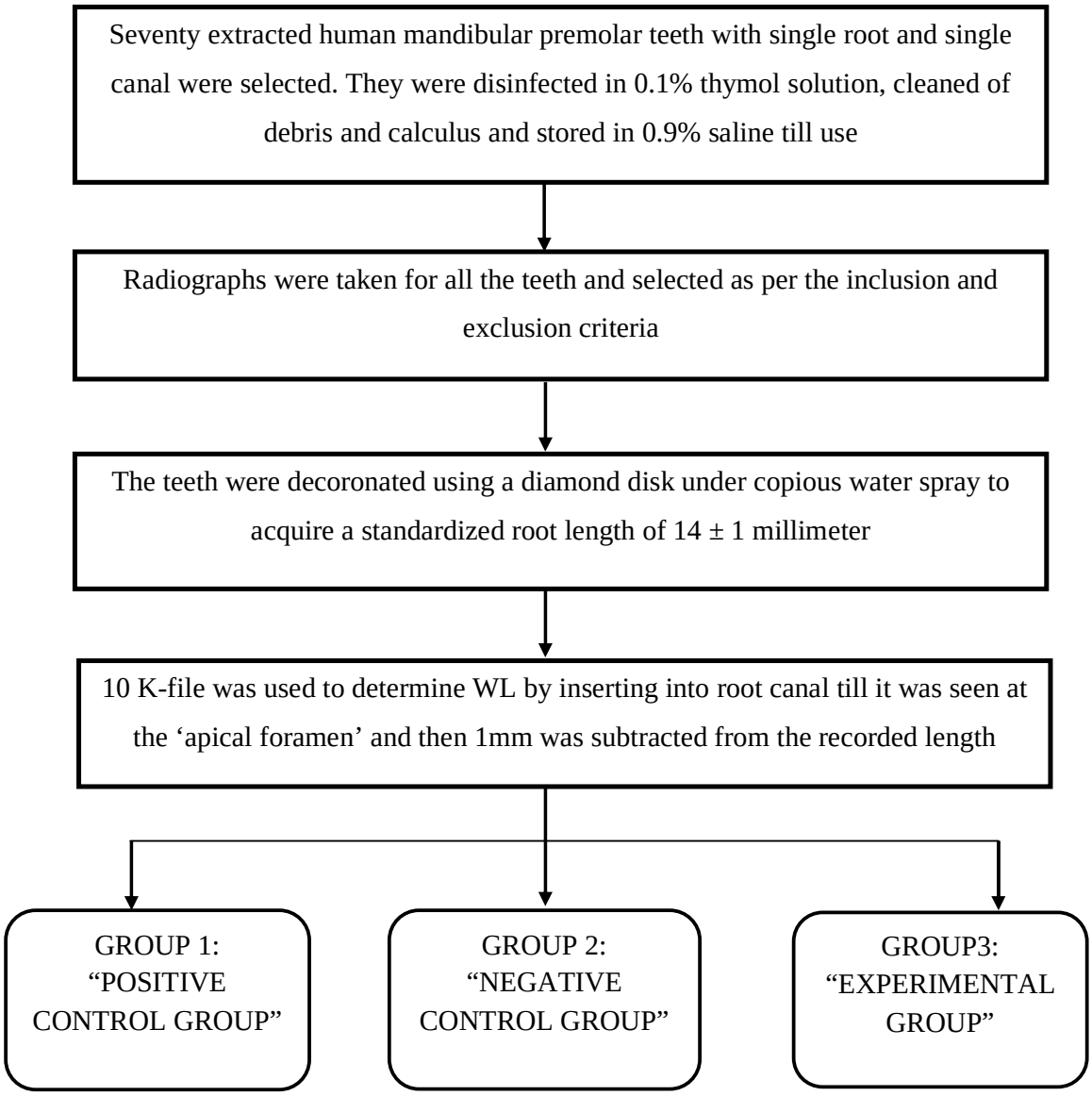
- Human mandibular premolars
- 0.1% thymol (s. d. fine chem limited), 0.9% saline (Amanta Healthcare)
- 3% Sodium Hypochlorite (Vishal Dentocare, Ahmedabad)
- 17% Ethylene diamine tetra acetic Acid (EDTA) (Canalarge, Ammdent, Punjab)
- Paper points (Diadent Group International)
- EDTA gel (Avue Prep)
- Rhodamine B dye (Sigma Aldrich)
- Distilled water
- '$Ca(OH)_2$' intracanal medicament (UltraCal^{TM} XS, Ultradent)

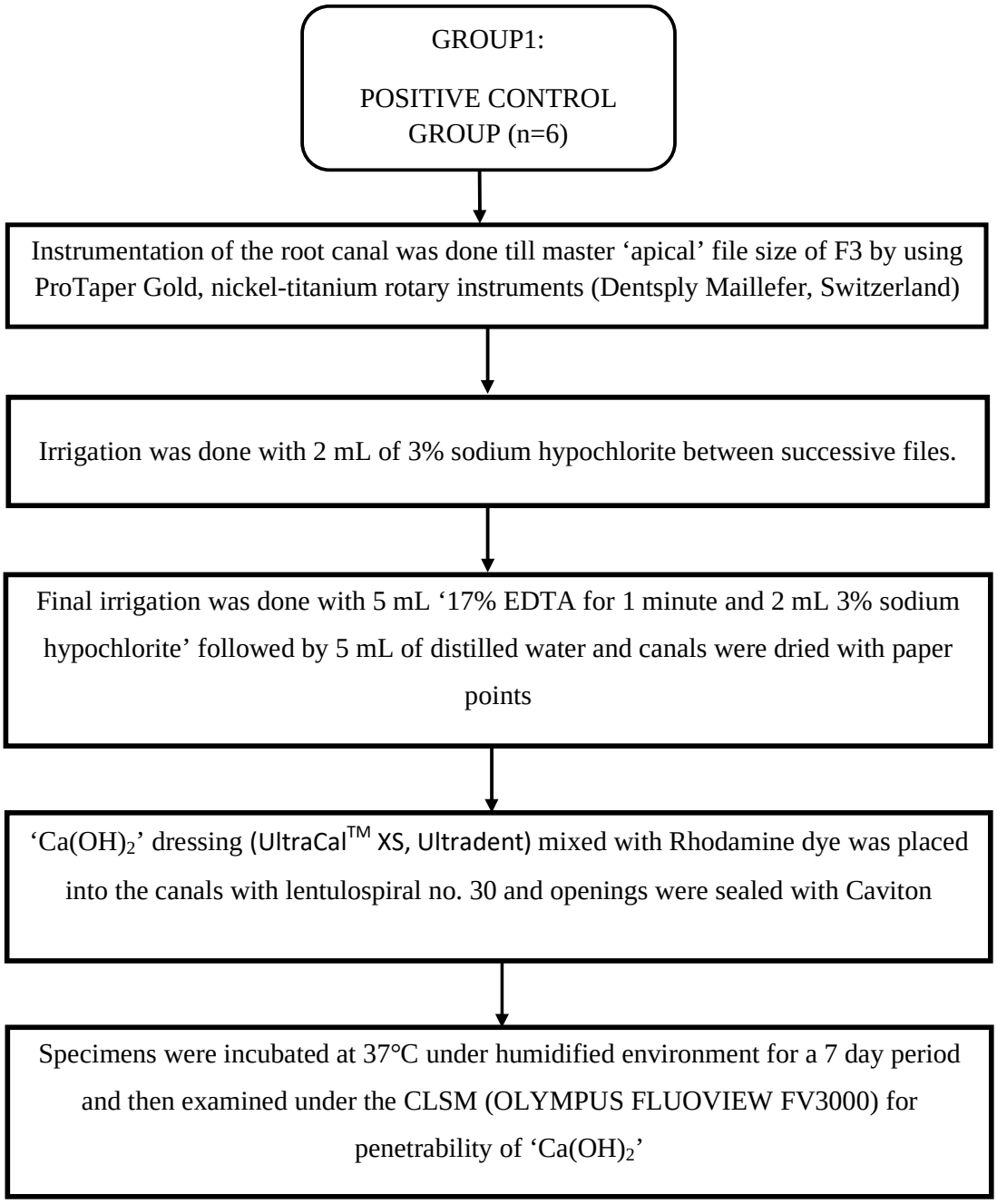
- 'AH Plus' sealer (Dentsply, Germany)
- 'Bio-C' sealer (Angelus, Londrina, Brazil)
- Gutta-percha points (Diadent Group International)
- Caviton (GC Corporation, Japan)

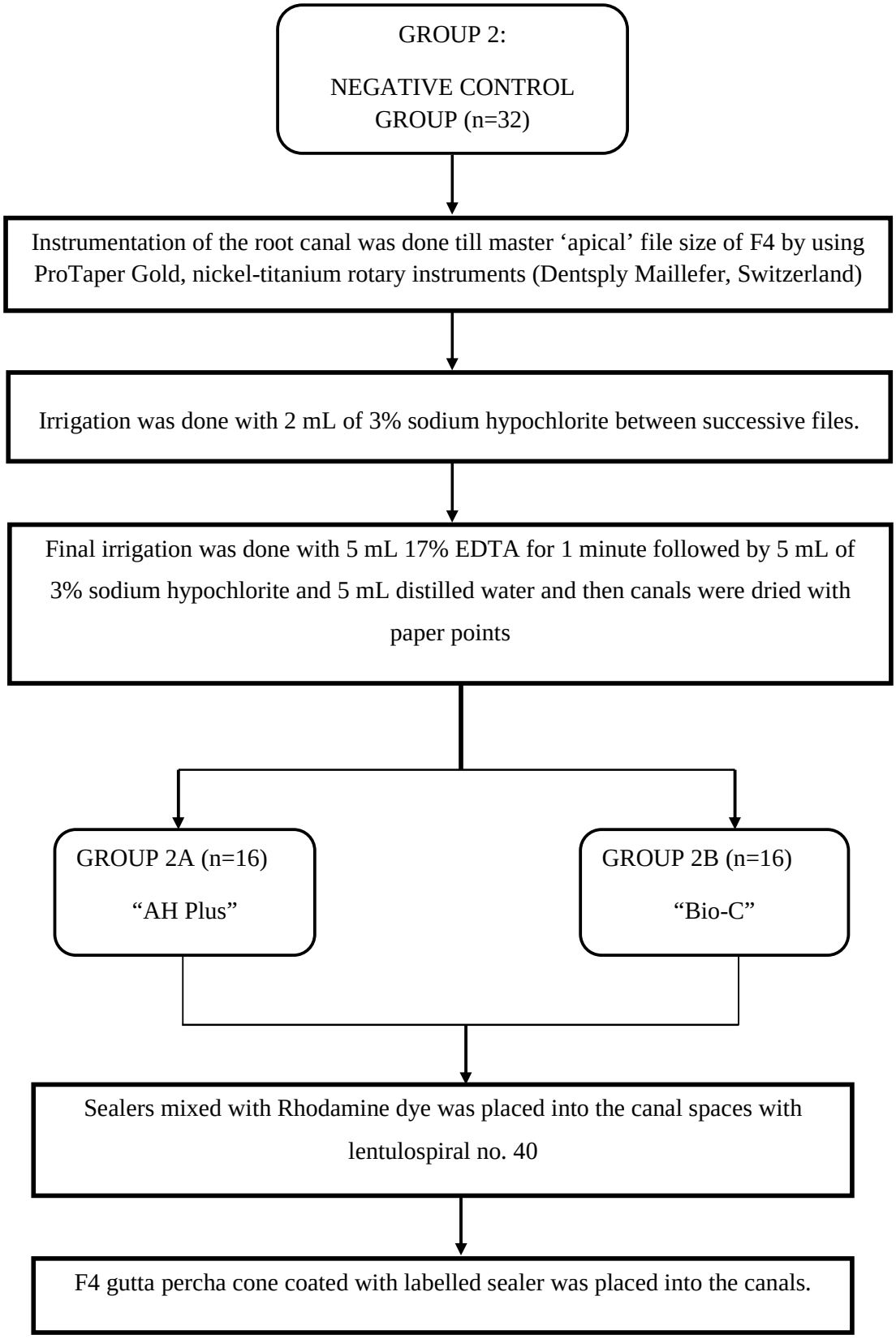
ARMAMENTARIUM:

- Micromotor (NSK, Japan)
- K Files (10-40) (Mani Inc, Japan)
- ProTaper Gold nickel-titanium files (Dentsply Maillefer, Switzerland)
- Endomotor (E-Connect Pro, Eighteeth)
- 27-gauge syringe (UNOLOK)
- Ultrasonic system handpiece and files (Satelec)
- Lentulospirals (Mani Inc, Japan)
- Diamond disks
- Confocal laser scanning microscope (Olympus fluoview FV3000)

METHODOLOGY WITH FLOWCHART:




GROUP1:
POSITIVE CONTROL GROUP (n=6)
Instrumentation of the root canal was done till master 'apical' file size of F3 by using ProTaper Gold, nickel-titanium rotary instruments (Dentsply Maillefer, Switzerland)
Irrigation was done with 2 mL of 3% sodium hypochlorite between successive files.
Final irrigation was done with 5 mL '17% EDTA for 1 minute and 2 mL 3% sodium hypochlorite' followed by 5 mL of distilled water and canals were dried with paper points
'Ca(OH)2' dressing (UltraCal™ XS, Ultradent) mixed with Rhodamine dye was placed into the canals with lentulospiral no. 30 and openings were sealed with Caviton
Specimens were incubated at 37°C under humidified environment for a 7 day period and then examined under the CLSM (OLYMPUS FLUOVIEW FV3000) for penetrability of 'Ca(OH)2'


GROUP 2:
NEGATIVE CONTROL GROUP (n=32)
Instrumentation of the root canal was done till master 'apical' file size of F4 by using ProTaper Gold, nickel-titanium rotary instruments (Dentsply Maillefer, Switzerland)
Irrigation was done with 2 mL of 3% sodium hypochlorite between successive files.
Final irrigation was done with 5 mL 17% EDTA for 1 minute followed by 5 mL of 3% sodium hypochlorite and 5 mL distilled water and then canals were dried with paper points
GROUP 2A (n=16)
"AH Plus"
GROUP 2B (n=16)
"Bio-C"
Sealers mixed with Rhodamine dye was placed into the canal spaces with lentulospiral no. 40
F4 gutta percha cone coated with labelled sealer was placed into the canals.

The root canal was obturated and excess gutta-percha was removed at orifice and condensed with a plugger.

Teeth were then sealed with Caviton and incubated at 37℃ under humidified conditions for a 7-day period.

Specimens were sectioned at 3,7 and 11mm from the apex to represent the 'apical', 'middle' and 'coronal' thirds respectively to obtain 1 mm thick sections and were then examined under the confocal laser scanning microscope (OLYMPUS FLUOVIEW FV3000) for 'dentinal tubule penetration' of sealers.

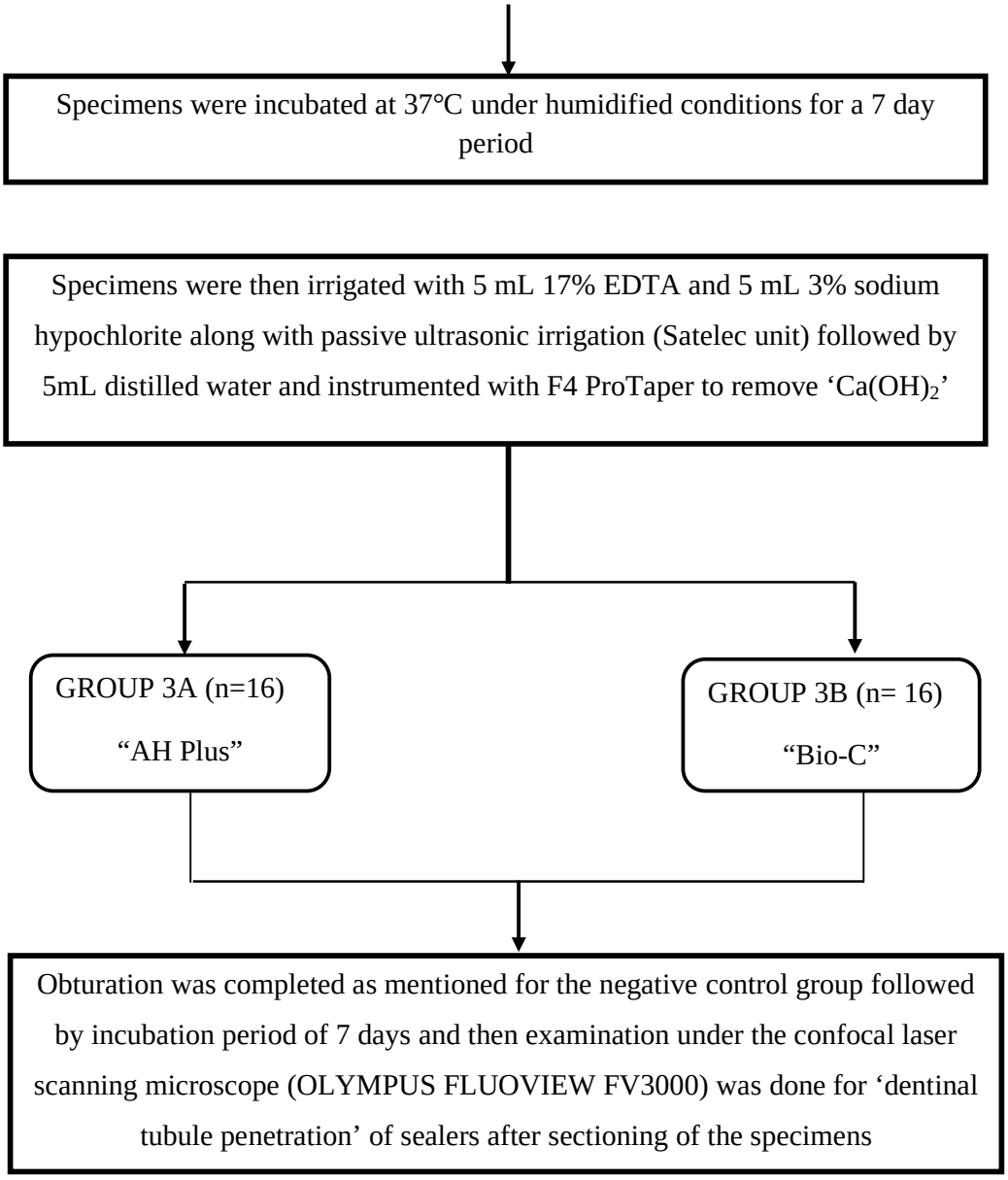
GROUP3:

EXPERIMENTAL GROUP (n=32)

Instrumentation of the root canal was done till file size F3 using ProTaper Gold, nickel-titanium rotary instruments (Dentsply Maillefer, Switzerland) before '$Ca(OH)_2$' placement and irrigation was done with 2 mL 3% sodium hypochlorite between successive files.

Final irrigation was done with 5 mL '17% EDTA for 1 minute and 2 mL 3% sodium hypochlorite' followed by 5 mL of distilled water and canals were dried with paper points

'$Ca(OH)_2$' dressing (UltraCal^{TM} XS, Ultradent) was placed in all the specimens and the teeth were sealed with Caviton


Specimens were incubated at 37°C under humidified conditions for a 7 day period
Specimens were then irrigated with 5 mL 17% EDTA and 5 mL 3% sodium hypochlorite along with passive ultrasonic irrigation (Satelec unit) followed by 5mL distilled water and instrumented with F4 ProTaper to remove 'Ca(OH)2'
GROUP 3A (n=16) "AH Plus"
GROUP 3B (n= 16) "Bio-C"
Obturation was completed as mentioned for the negative control group followed by incubation period of 7 days and then examination under the confocal laser scanning microscope (OLYMPUS FLUOVIEW FV3000) was done for 'dentinal tubule penetration' of sealers after sectioning of the specimens

DETAILS OF THE PROCEDURES CONDUCTED DURING THE RESEARCH:

Seventy extracted human permanent mandibular premolars with single root and single canal were used for the study. They were then disinfected in 0.1% thymol solution, cleaned of debris and calculus and stored in 0.9% saline solution till use. All teeth were radiographed and were selected as per the inclusion and exclusion criteria.

All teeth were decoronated using a diamond disk under continuous water cooling to obtain a standardized root length of 14 ± 1 mm. 10 K-file was used to determine WL by inserting into root canal till it was seen at the 'apical foramen' and then 1mm was subtracted from the recorded length

Six specimens were chosen randomly to represent the "positive control group" and the remaining specimens were allocated to "negative control group" and experimental group.

"Positive control group" was used to check for the penetrability of '$Ca(OH)_2$' and the "negative control group" was used to test the sealer penetration without '$Ca(OH)_2$' in order to see the difference in sealer penetration in the experimental group after using '$Ca(OH)_2$'.

Specimens in the "positive control group" were instrumented till master 'apical' file size of F3 using ProTaper Gold, nickel-titanium rotary instruments (Dentsply Maillefer, Switzerland) without applying apical pressure to the established working length.

During instrumentation, the canals were irrigated with 2 mL of 3% sodium hypochlorite between successive files. Final irrigation was done with 5 mL 17% EDTA for 1 minute and 2 mL 3% sodium hypochlorite followed by 5mL of distilled water for 1 minute. The canals were then dried with sterile absorbent paper points following which the canals were filled with '$Ca(OH)_2$' (UltraCal^{TM} XS, Ultradent) mixed with Rhodamine dye with no. 30 lentulospiral. The 'coronal' openings were sealed with Caviton and were incubated at 37ºC under humidified conditions for 7 days. The specimens were then examined under the CLSM (Olympus Fluoview FV3000) for penetrability of '$Ca(OH)_2$'.

In the "negative control group", the canals were instrumented upto F4 ProTaper and 2 mL of 3% sodium hypochlorite was used between successive instruments. 'Final rinse' with '5 mL 17% EDTA for 1 min and 5 mL 3% sodium hypochlorite' followed by 5 mL of distilled water was done.

In the experimental group, the canals were instrumented upto F3 ProTaper and filled with '$Ca(OH)_2$' intracanal medicament (UltraCal^{TM} XS, Ultradent) as in the "positive control group" followed by a 7 days incubation period. After this, specimens were irrigated with '5 mL 17% EDTA and 5 mL 3% sodium hypochlorite' along with passive ultrasonic irrigation (Satelec unit) followed by 5mL of distilled water and instrumentation was done with F4 ProTaper Gold file to remove the '$Ca(OH)_2$'.

All the root canals of the specimens in the "negative control" and experimental groups were irrigated with 5 mL distilled water and then dried with absorbent paper points.

Preparation of the sealers:

To facilitate fluorescence under the CLSM, each sealer was mixed with "Rhodamine B dye" during manipulation in an approximate ratio of 0.1% (weight)

The specimens of both the "negative control group" and experimental group were divided into 2 subgroups based on "root canal sealer" used.

"Group 2A": "AH Plus sealer"

"Group 2B": "Bio-C sealer"

"Group 3A": "AH Plus sealer"

"Group 3B": "Bio-C sealer"

Each sealer was prepared according to the manufacturer's instructions. The root canal walls were coated by sealers with lentulospiral no. 40 after which the master cone gutta percha was entirely coated with the labelled sealer and inserted in the canal up to the working length. The root canal was then obturated and excess gutta-percha was removed at orifice and condensed with a plugger.

The teeth were sealed with Caviton and incubated at 37°C under humidified conditions for a 7-day period.

Preparation of samples:

Samples were prepared by sectioning them horizontally at the 'coronal' (11mm from the apex), 'middle' (7mm from the apex) and 'apical' third (3mm from the apex) of each root to obtain 1mm thick sections. The specimens then were examined using CLSM (OLYMPUS FLUOVIEW FV3000) for "dentinal tubule penetration" of sealer. Epifluorescence was obtained using excitation and emission wavelengths of 514 and 561 nanometers respectively for "Rhodamine dye".

Calculation of 'dentinal tubule penetration':

Images analysis was done using the Fiji Image J software and longest depth of sealer penetration for each specimen was measured. 'The depth of penetration was measured from the canal wall to the point of maximum sealer penetration using the measuring tool in the Image J software'. Analysis was done by a single operator and each measurement was repeated twice in order to ensure reproducibility and consistency.

STATISTICAL ANALYSIS:

'One-way ANOVA test' was used for 'statistical analysis' for maximum 'tubular penetration' depth and Tukey's multiple post-hoc test was used for pairwise comparison among the four groups.

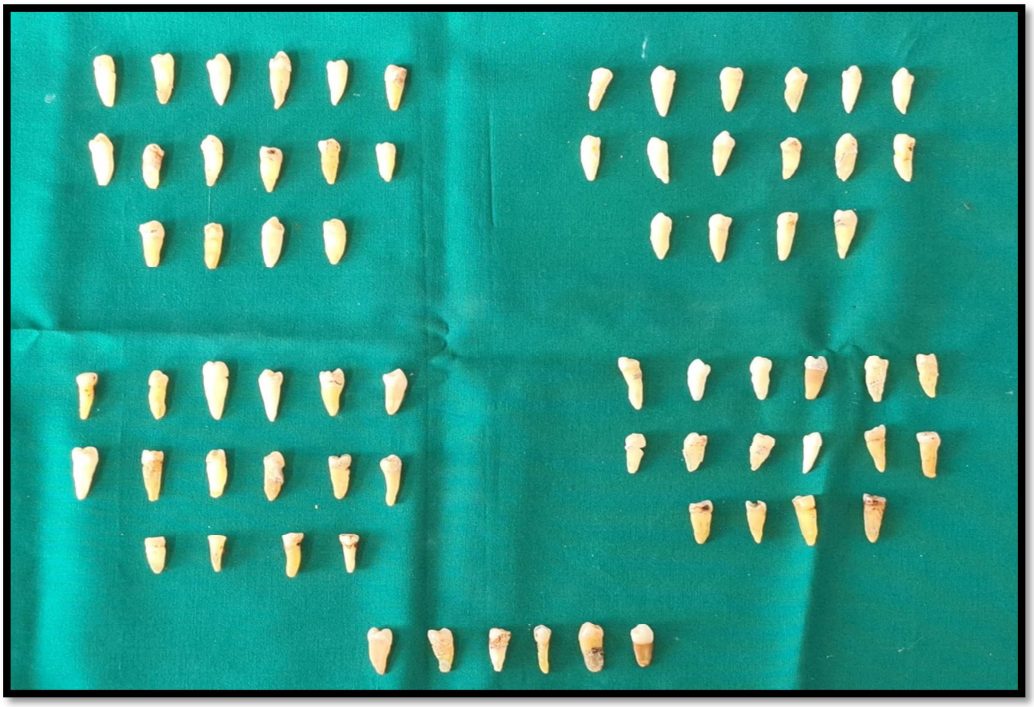


Fig 1: Human Mandibular Premolars

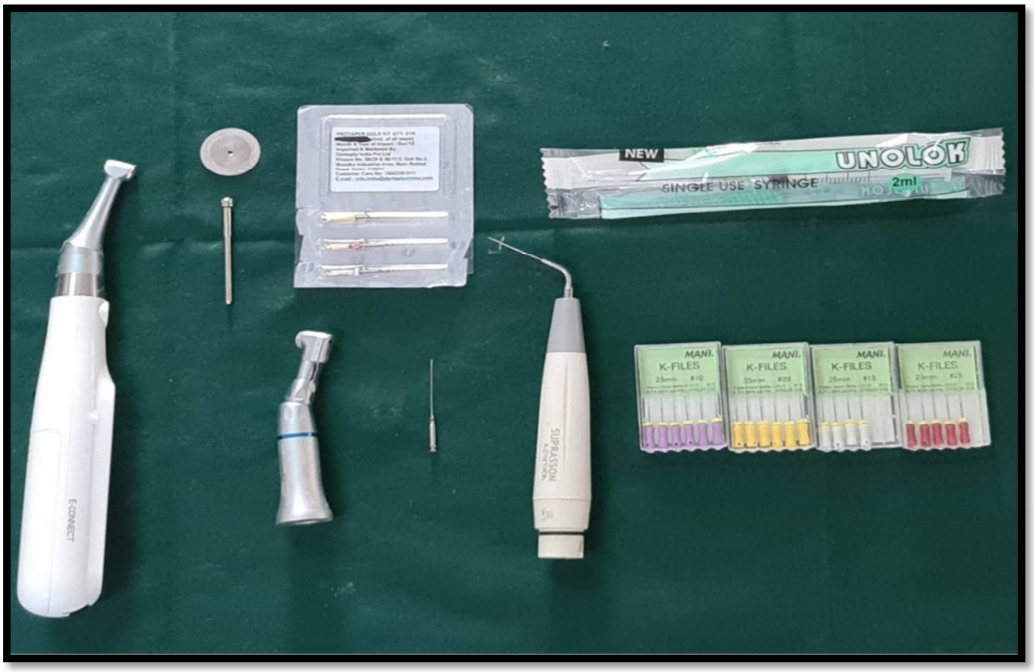


Fig 2: Armamentarium

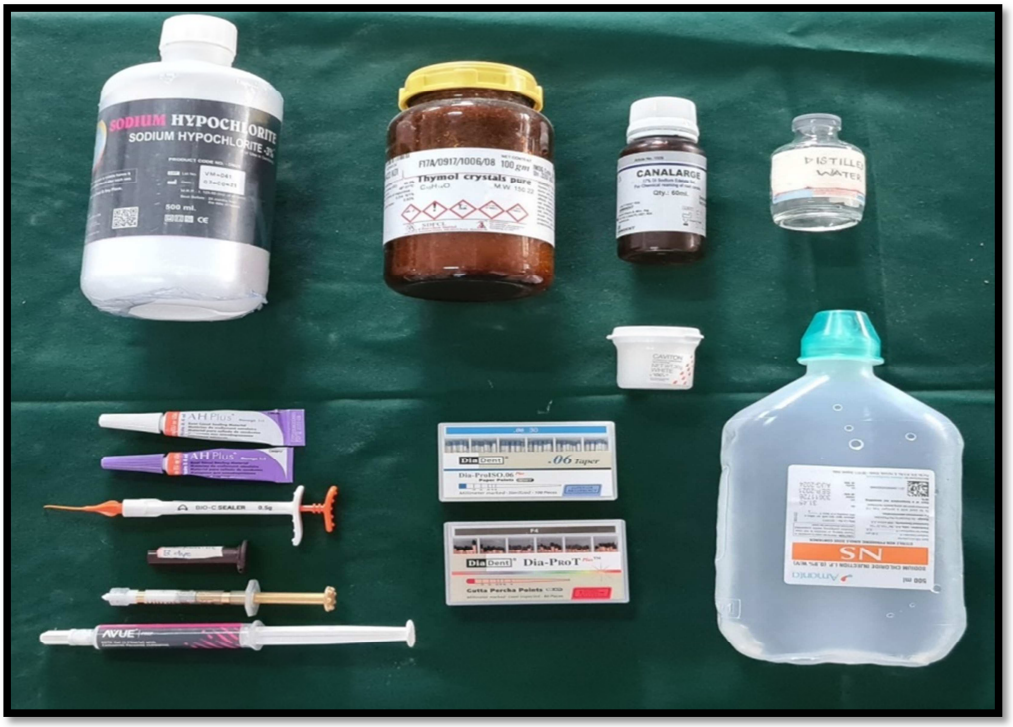


Fig 3: Materials

Fig 4: Debris Removal

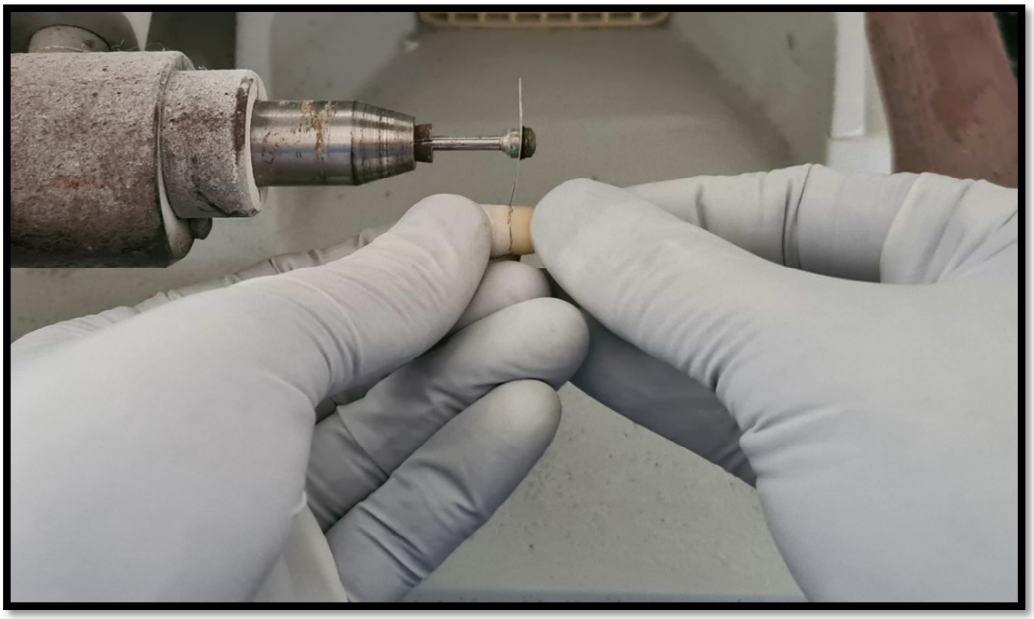


Fig 5: Decoronation

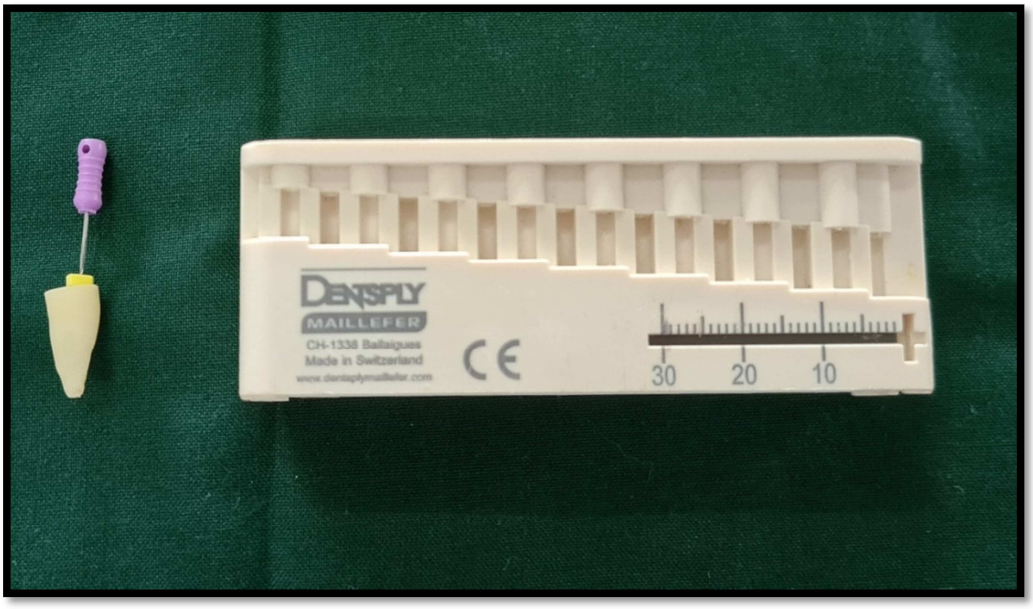


Fig 6: Working Length Determination

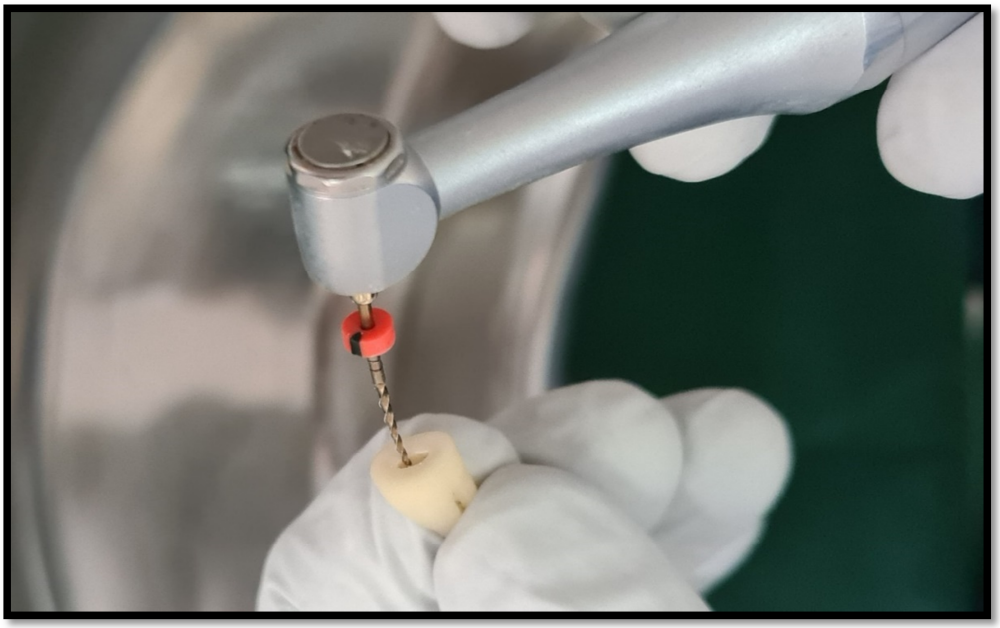


Fig 7: Bio-Mechanical Preparation

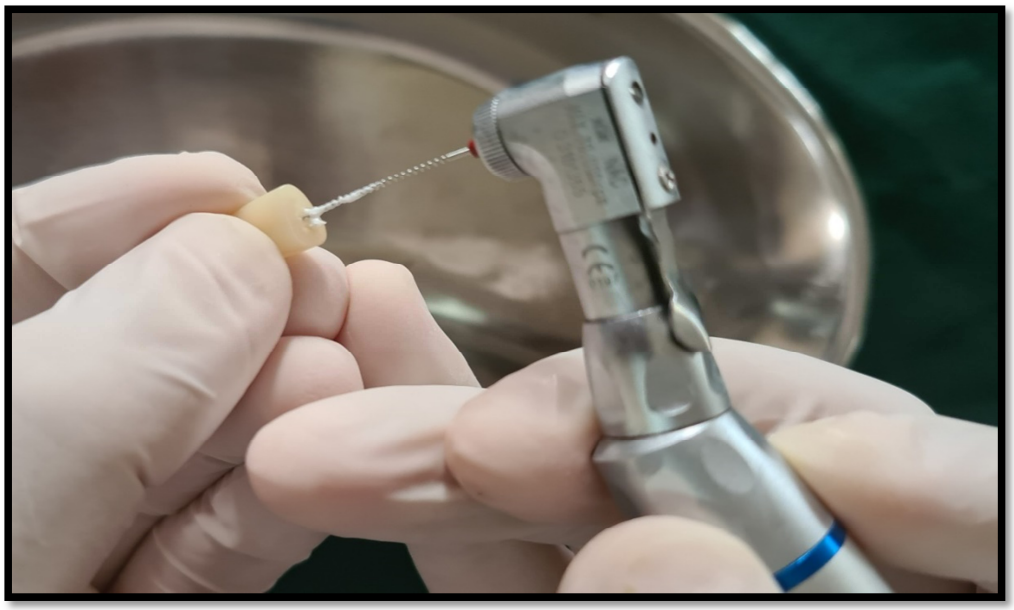


Fig 8: Calcium hydroxide placement using lentulospiral

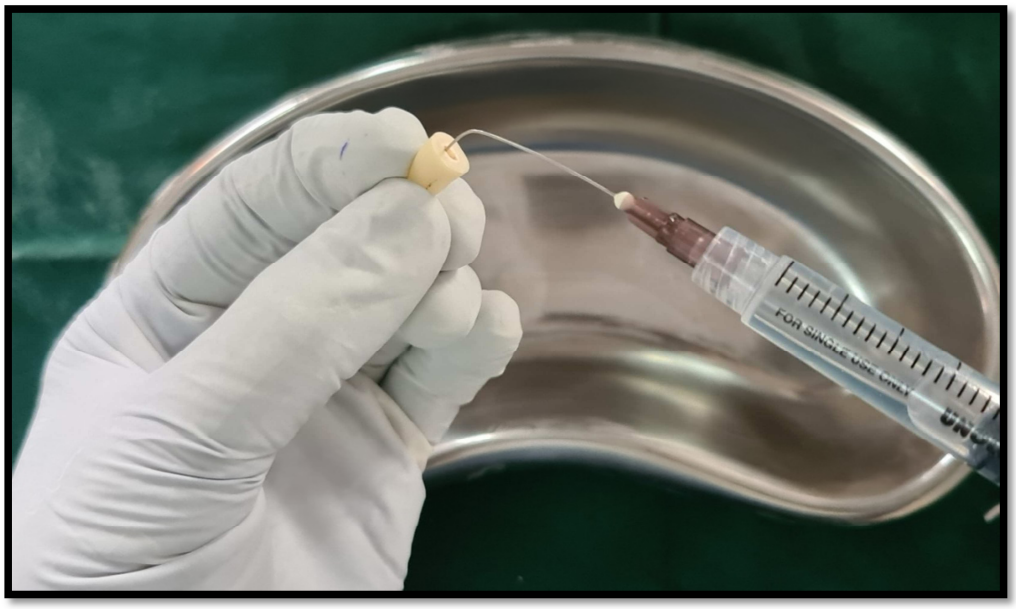


Fig 9: Irrigation

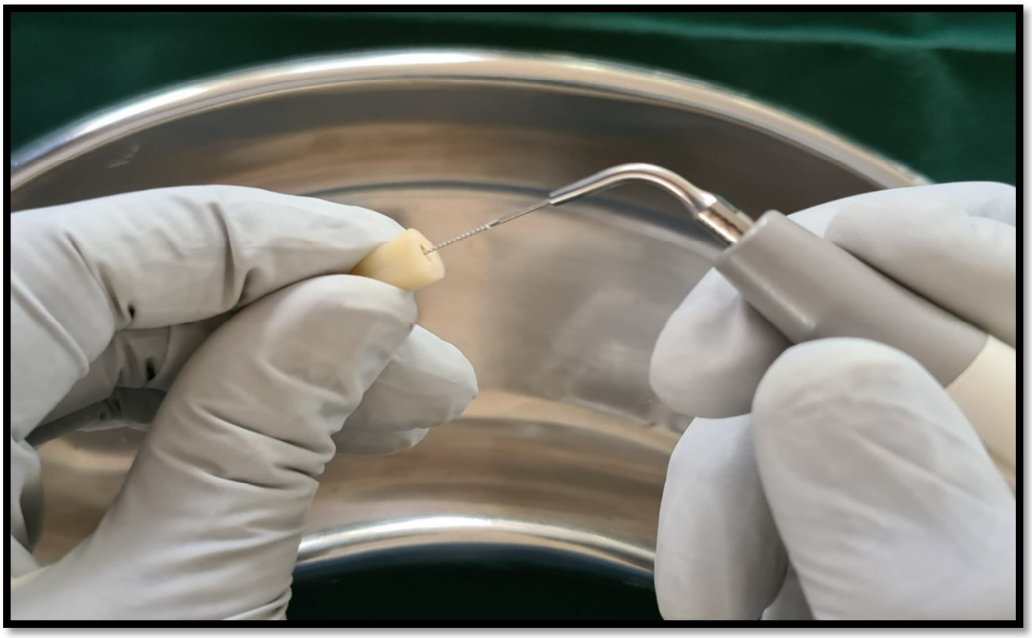


Fig 10: Passive Ultrasonic Irrigation

Fig 11: Dye Incorporation in Sealers

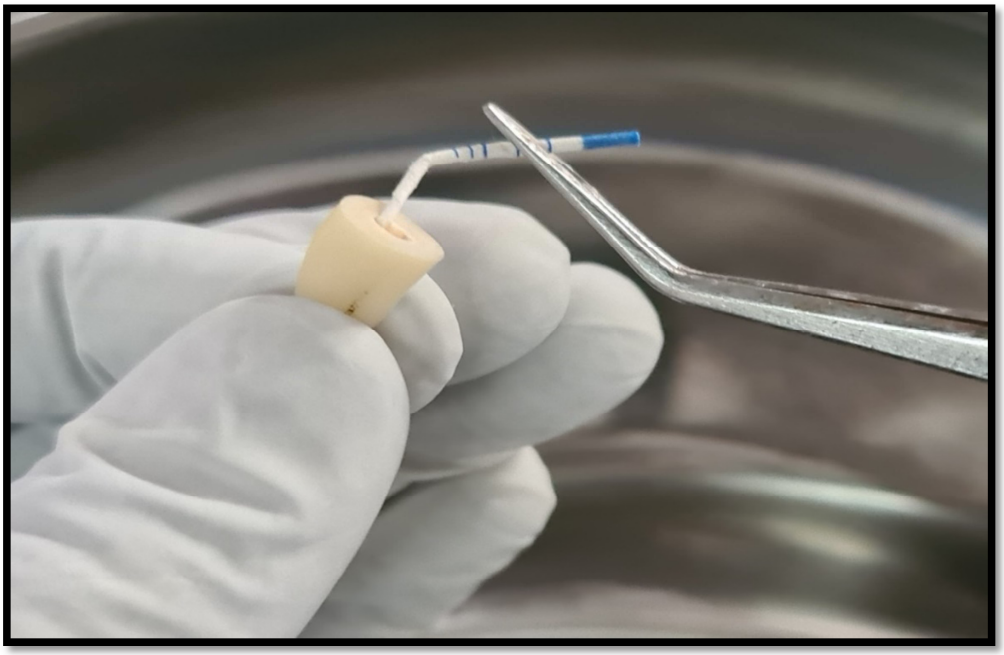


Fig 12: Drying Canals with paper points

Fig 13: Obturation

Fig 14: Incubation

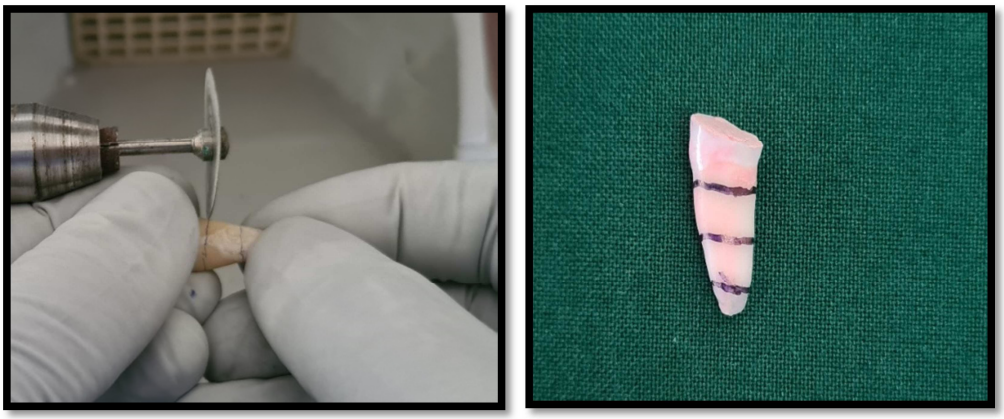


Fig 15: Sectioning

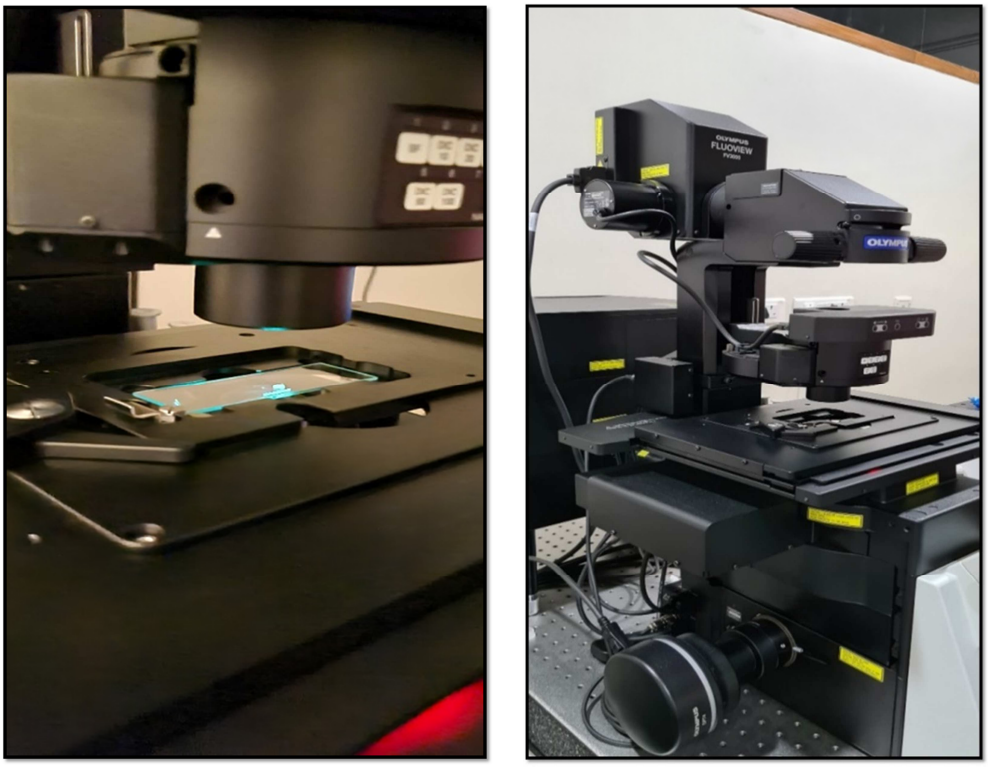


Fig 16: Confocal Laser Scanning Microscopy

RESULTS

In all, 16 samples in each subgroup were analyzed for dentinal 'tubular penetration' of sealer in 'coronal', 'middle' and 'apical' third sections with and without the use of '$Ca(OH)_2$' as an intracanal medicament.

Table 1 shows the mean depth of penetration, standard deviation and statistically significant p-value in 'coronal' section of the four groups containing 16 samples each by One-way ANOVA. The highest mean depth of penetration in the 'coronal' section was seen with 'Bio-C' sealer after '$Ca(OH)_2$' removal (Group 3B) being 1713.26 µm and the results were shown to be statistically significant (P=0.0001). Samples filled with "AH Plus" sealer after '$Ca(OH)_2$' (Group 2B) showed the least penetration, i.e., 1084.90 µm. [Table 1 and Graph 1]

Tukey's multiple post-hoc test was done for pairwise comparison of the four groups in the 'coronal' section and highly significant difference was seen between "AH Plus" (Group 2A) and 'Bio-C' after '$Ca(OH)_2$' removal (Group 3B). Statistically significant difference (p=0.0120) was seen between "Bio-C" sealer penetration with (Group 3B) and without '$Ca(OH)_2$' (Group 2B), thereby indicating that '$Ca(OH)_2$' showed a positive influence on the sealer penetration. Without '$Ca(OH)_2$', "AH Plus" showed a higher penetration clinically, however no 'statistically significant difference' was found (p=0.0670). [Table 2 and Graph 4]

A statistically significant difference (p=0.0001) was observed in relation to the depth of 'tubular penetration' in the 'middle' section among all the groups and "Bio-C" sealer after '$Ca(OH)_2$' removal (Group 3B) showed the highest penetration

(1914.62 µm). Lowest penetration was seen for "AH Plus" without '$Ca(OH)_2$' (Group 2A) which was 848.37 µm. [Table 3 and Graph 3]

For the 'middle' third, pairwise comparison showed that "Bio-C" sealer had the highest penetration compared to all the other groups irrespective of the placement of '$Ca(OH)_2$' ($P<0.05$). After '$Ca(OH)_2$' removal (Group 3B) "Bio-C" showed a highly statistically significant difference than without 'Ca(OH)2' (Group 3A). In the negative control group, i.e., without '$Ca(OH)_2$', "Bio-C" sealer (Group 2B) showed higher penetration than "AH Plus" sealer (Group 2A) with $P=0.0001$. After '$Ca(OH)_2$' removal, "AH Plus" did not show a statistically significant difference in penetration than without '$Ca(OH)_2$'. [Table 4 and Graph 4]

In the 'apical' third, highest depth of penetration was seen with "Bio-C" sealer without '$Ca(OH)_2$' (Group 2B) being 1482.82 µm and least for "AH Plus" without '$Ca(OH)_2$' (Group 2A), i.e., 281.69 µm with a statistically significant difference ($P= 0.0001$) among all the groups according to the One-way ANOVA test. [Table 5 and Graph 3].

Pairwise comparison for the 'apical' third sections showed statistically significant difference between all the groups ($P<0.05$). "AH Plus" showed a decreased "tubular penetration" compared to "Bio-C" sealer irrespective of the placement of '$Ca(OH)_2$' whereas "Bio-C" penetrated deeper without '$Ca(OH)_2$' (Group 2B) than after '$Ca(OH)_2$' removal (Group 3B). [Table 6 and Graph 4]

Comparison of the "tubular penetration" within the three sections of the four groups indicated a statistically significant difference ($P=0.0001$) in three groups, that is, "AH Plus" without '$Ca(OH)_2$' (Group 2A), "AH Plus" after '$Ca(OH)_2$' removal

(Group 3A) and Bio- C after '$Ca(OH)_2$' removal (Group 3B). The 'coronal' section showed the highest penetration for "AH Plus" (Group 2A and Group 3A) whereas for "Bio-C" after '$Ca(OH)_2$' removal (Group 3B), highest penetration was seen in the 'middle' third. [Table 7 and Graph 5,6,7,8]

Pairwise comparison showed significant difference between all the three sections for "AH Plus" without '$Ca(OH)_2$' (Group 2A) and 'Bio-C' after '$Ca(OH)_2$' removal (Group 3B). When "AH Plus" was used after '$Ca(OH)_2$' (Group 3A) there was no significant difference ($P=0.194$) between the 'coronal' and 'middle' third but a highly significant difference ($P<0.05$) was noted between the 'middle'/ 'apical', and 'coronal'/ 'apical thirds'. No 'statistically significant difference' between either of sections for 'Bio-C' sealer without '$Ca(OH)_2$' (Group 2B) was noted. [Table 8 and Graph 9]

Table 1: Comparison of four groups (2A, 2B, 3A and 3B) on coronal section with mean depth of tubular penetration by One way ANOVA

Groups	Mean	SD	F-value	P-value
Negative control (AH Plus) Group 2A	1280.31	151.30	23.8860	0.0001*
Negative control (Bio-C) Group 2B	1465.37	291.90		
Experimental (AH Plus) Group 3A	1084.90	207.14		
Experimental (Bio-C) Group 3B	1713.26	203.04		

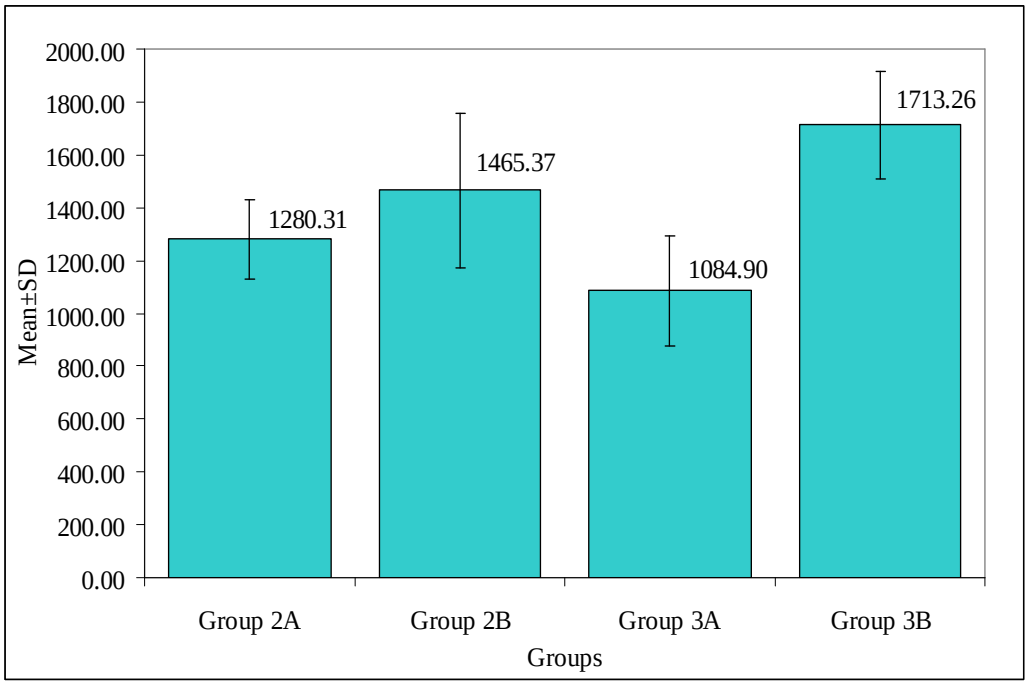
*$p<0.05$ indicates significant result

Table 2: Pair wise comparison of four groups (2A, 2B, 3A and 3B) on coronal section with mean depth of tubular penetration by Tukeys multiple posthoc procedures

Group (I)	Group (J)	Mean difference (I-J)	SE	p-value
Negative control (AH Plus) Group 2A	Negative control (Bio-C) Group 2B	-185.06	77.51	0.0900
	Experimental (AH Plus) Group 3A	195.41	77.51	0.0670
	Experimental (Bio-C) Group 3B	-432.96	77.51	0.0001*
Negative control (Bio-C) Group 2B	Experimental (AH Plus) Group 3A	380.47	77.51	0.0001*
	Experimental (Bio-C) Group 3B	-247.90	77.51	0.0120*
Experimental (AH Plus) Group 3A	Experimental (Bio-C) Group 3B	-628.36	77.51	0.0001*

Statistically significant $p<0.05$

Graph 1: Comparison of four groups (2A, 2B, 3A and 3B) on coronal section with mean depth of tubular penetration



Negative control (AH Plus)-Group 2A; Negative control (Bio-C)-Group 2B; Experimental (AH Plus)-Group 3A; Experimental (Bio-C)-Group 3B

Table 3: Comparison of four groups (2A, 2B, 3A and 3B) on middle section with mean depth of tubular penetration by One way ANOVA

Groups	Mean	SD	F-value	P-value
Negative control (AH Plus) Group 2A	848.37	281.36	70.6220	0.0001*
Negative control (Bio-C) Group 2B	1629.76	301.99		
Experimental (AH Plus) Group 3A	982.23	91.03		
Experimental (Bio-C) Group 3B	1914.62	241.06		

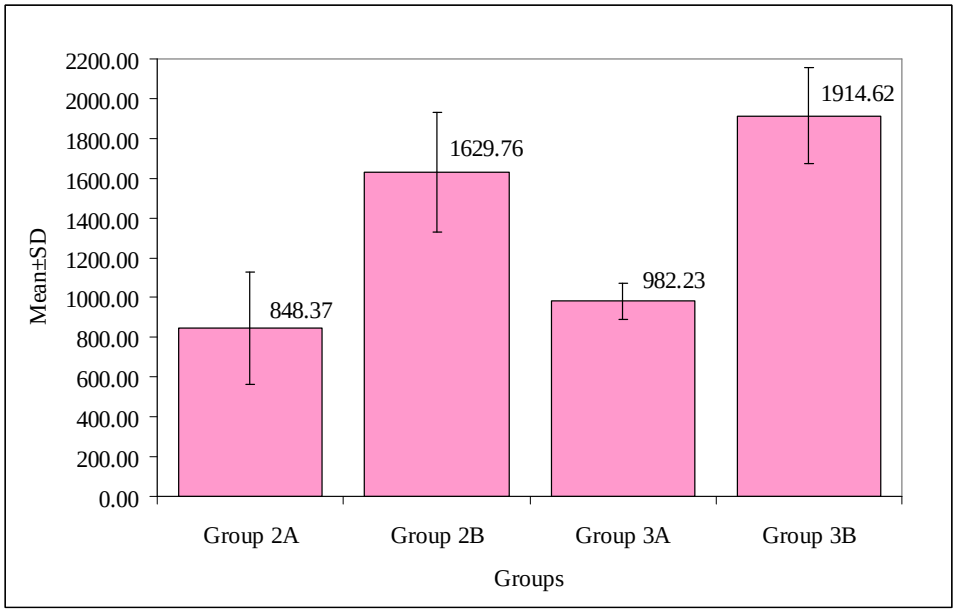
*p<0.05 indicates significant values

Table 4: Pair wise comparison of four groups (2A, 2B, 3A and 3B) on middle section with mean depth of tubular penetration by Tukeys multiple posthoc procedures

Group (I)	Group (J)	Mean difference (I-J)	SE	p-value
Negative control (AH Plus) Group 2A	Negative control (Bio-C) Group 2B	-781.39	86.02	0.0001*
	Experimental (AH Plus) Group 3A	-133.86	86.02	0.4110
	Experimental (Bio-C) Group 3B	-1066.25	86.02	0.0001*
Negative control (Bio-C) Group 2B	Experimental (AH Plus) Group 3A	647.53	86.02	0.0001*
	Experimental (Bio-C) Group 3B	-284.86	86.02	0.0080*
Experimental (AH Plus) Group 3A	Experimental (Bio-C) Group 3B	-932.38	86.02	0.0001*

*$p<0.05$ indicates significant values

Graph 2: Comparison of four groups (2A, 2B, 3A and 3B) on middle section with mean depth of tubular penetration



Negative control (AH Plus)-Group 2A; Negative control (Bio-C)-Group 2B; Experimental (AH Plus)-Group 3A; Experimental (Bio-C)-Group 3B

Table 5: Comparison of four groups (2A, 2B, 3A and 3B) on apical section with mean depth of tubular penetration by One way ANOVA

Groups	Mean	SD	F-value	P-value
Negative control (AH Plus) Group 2A	281.69	82.77	84.0780	0.0001*
Negative control (Bio-C) Group 2B	1482.82	331.86		
Experimental (AH Plus) Group 3A	802.25	173.77		
Experimental (Bio-C) Group 3B	1048.12	209.73		

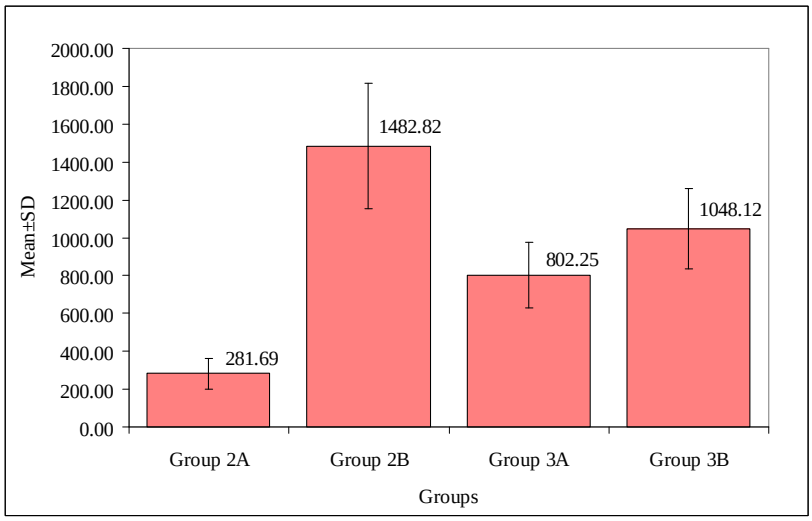
*p<0.05 indicates significant results

Table 6: Pair wise comparison of four groups (2A, 2B, 3A and 3B) on apical section with mean depth of tubular penetration by Tukeys multiple posthoc procedures

Group (I)	Group (J)	Mean difference (I-J)	SE	p-value
Negative control (AH Plus) Group 2A	Negative control (Bio-C) Group 2B	-1201.13	77.29	0.0001*
	Experimental (AH Plus) Group 3A	-520.57	77.29	0.0001*
	Experimental (Bio-C) Group 3B	-766.43	77.29	0.0001*
Negative control (Bio-C) Group 2B	Experimental (AH Plus) Group 3A	680.57	77.29	0.0001*
	Experimental (Bio-C) Group 3B	434.70	77.29	0.0001*
Experimental (AH Plus) Group 3A	Experimental (Bio-C) Group 3B	-245.87	77.29	0.0120*

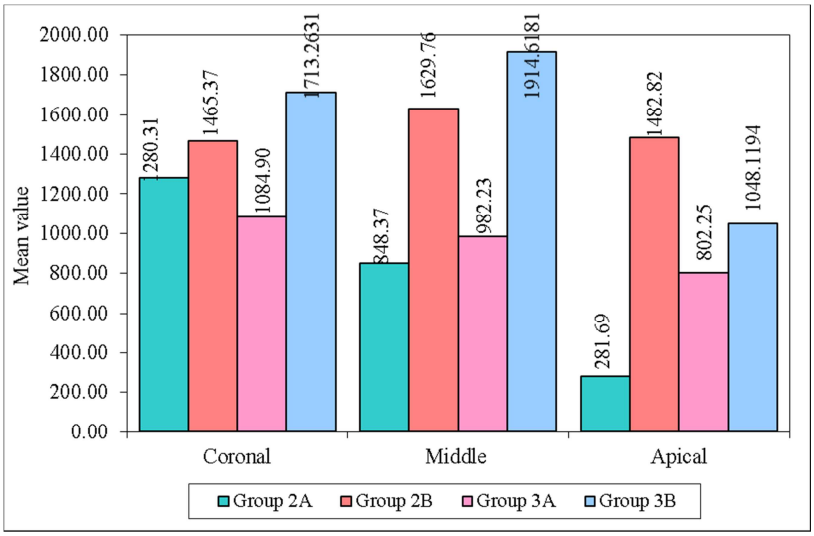
*$p<0.05$ indicates significant results

Graph 3: Comparison of four groups (2A, 2B, 3A and 3B) on apical section with mean depth of tubular penetration



Negative control (AH Plus)-Group 2A; Negative control (Bio-C)-Group 2B; Experimental (AH Plus)-Group 3A; Experimental (Bio-C)-Group 3B

Graph 4: Comparison of four groups (2A, 2B, 3A and 3B) on coronal, middle and apical sections with mean depth of tubular penetration



Negative control (AH Plus)-Group 2A; Negative control (Bio-C)-Group 2B; Experimental (AH Plus)-Group 3A; Experimental (Bio-C)-Group 3B

Table 7: Comparison of three sections (coronal, middle and apical) in four groups (2A, 2B, 3A and 3B) with mean depth of tubular penetration by one way ANOVA

Groups	Sections	Mean	SD	F-value	P-value
Negative control (AH Plus) Group 2A	Coronal	1280.31	151.30	110.5480	0.0001*
	Middle	848.37	281.36		
	Apical	281.69	82.77		
Negative control (Bio-C) Group 2B	Coronal	1465.37	291.90	1.3660	0.2660
	Middle	1629.76	301.99		
	Apical	1482.82	331.86		
Experimental (AH Plus) Group 3A	Coronal	1084.90	207.14	12.0720	0.0001*
	Middle	982.23	91.03		
	Apical	802.25	173.77		
Experimental (Bio-C) Group 3B	Coronal	1713.26	203.04	68.8690	0.0001*
	Middle	1914.62	241.06		
	Apical	1048.12	209.73		

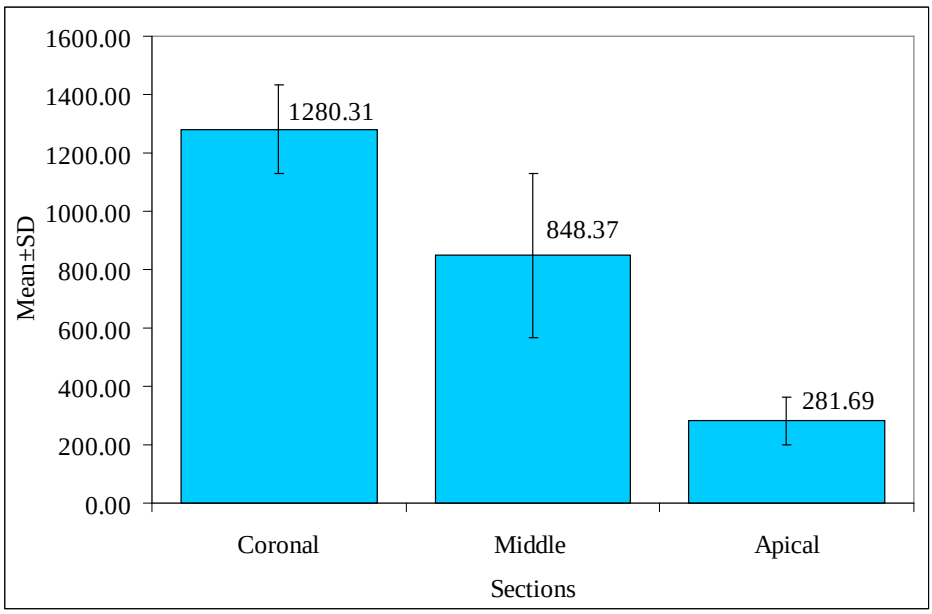
*$p<0.05$ indicates significant values

Table 8: Pair wise comparison of three sections (coronal, middle and apical) in four groups (2A, 2B, 3A and 3B) with mean depth of tubular penetration by Tukeys multiple posthoc procedures

Groups	Section (I)	Section (J)	Mean difference (I-J)	SE	p-value
Negative control (AH Plus) Group 2A	Coronal	Middle	431.94	67.36	0.0001*
		Apical	998.62	67.36	0.0001*
	Middle	Apical	566.68	67.36	0.0001*
Negative control (Bio-C) Group 2B	Coronal	Middle	-164.40	109.27	0.2980
		Apical	-17.46	109.27	0.9860
	Middle	Apical	146.94	109.27	0.3780
Experimental (AH Plus) Group 3A	Coronal	Middle	102.67	58.24	0.1940
		Apical	282.65	58.24	0.0001*
	Middle	Apical	179.98	58.24	0.0090*
Experimental (Bio-C) Group 3B	Coronal	Middle	-201.36	77.28	0.0330*
		Apical	665.14	77.28	0.0001*
	Middle	Apical	866.50	77.28	0.0001*

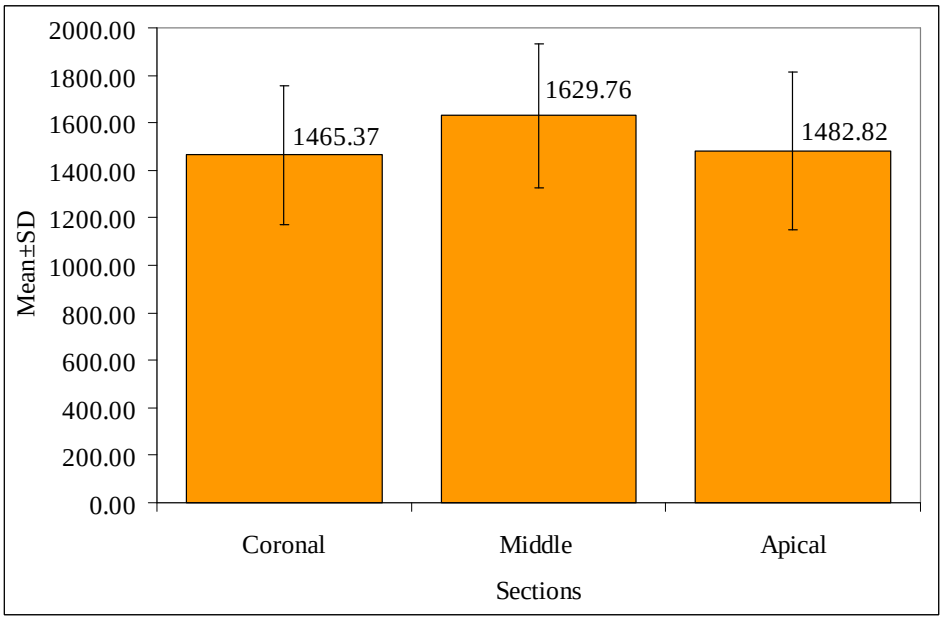
*$p<0.05$ indicates significant values

Graph 5: Comparison of three sections (coronal, middle and apical) in group 2A with mean depth of tubular penetration



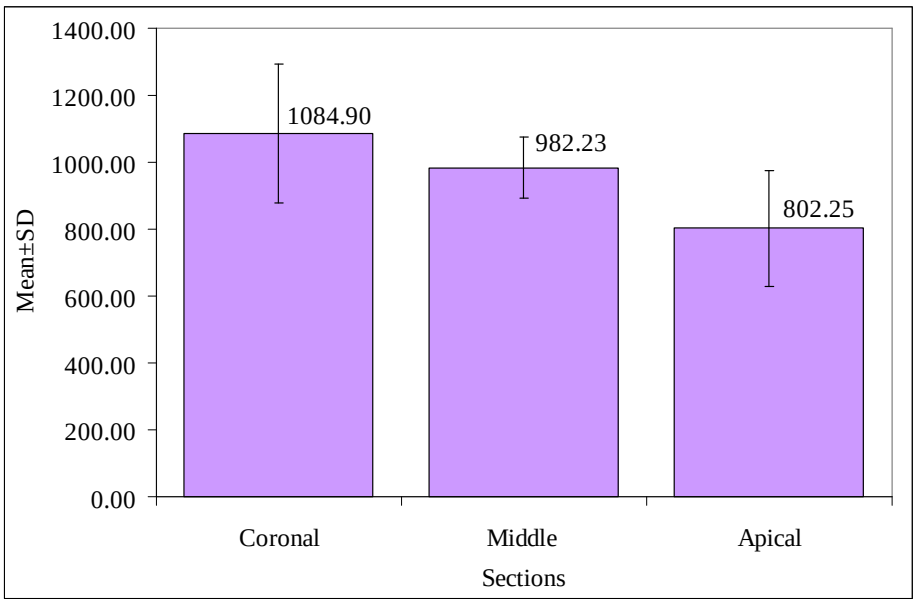
Negative control (AH Plus)-Group 2A

Graph 6: Comparison of three sections (coronal, middle and apical) in group 2B with mean depth of tubular penetration



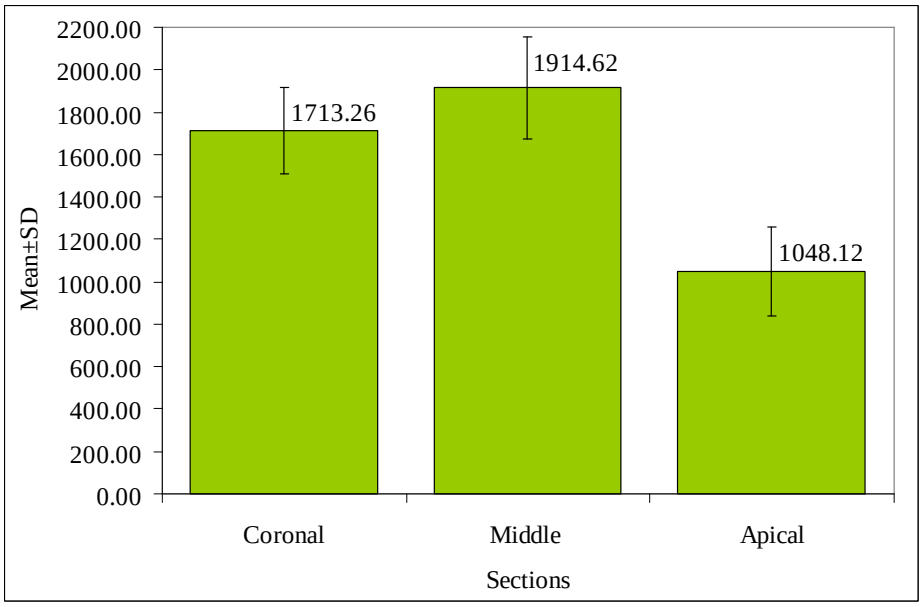
Negative control (Bio-C)-Group 2B

Graph 7: Comparison of three sections (coronal, middle and apical) in group 3A with mean depth of tubular penetration



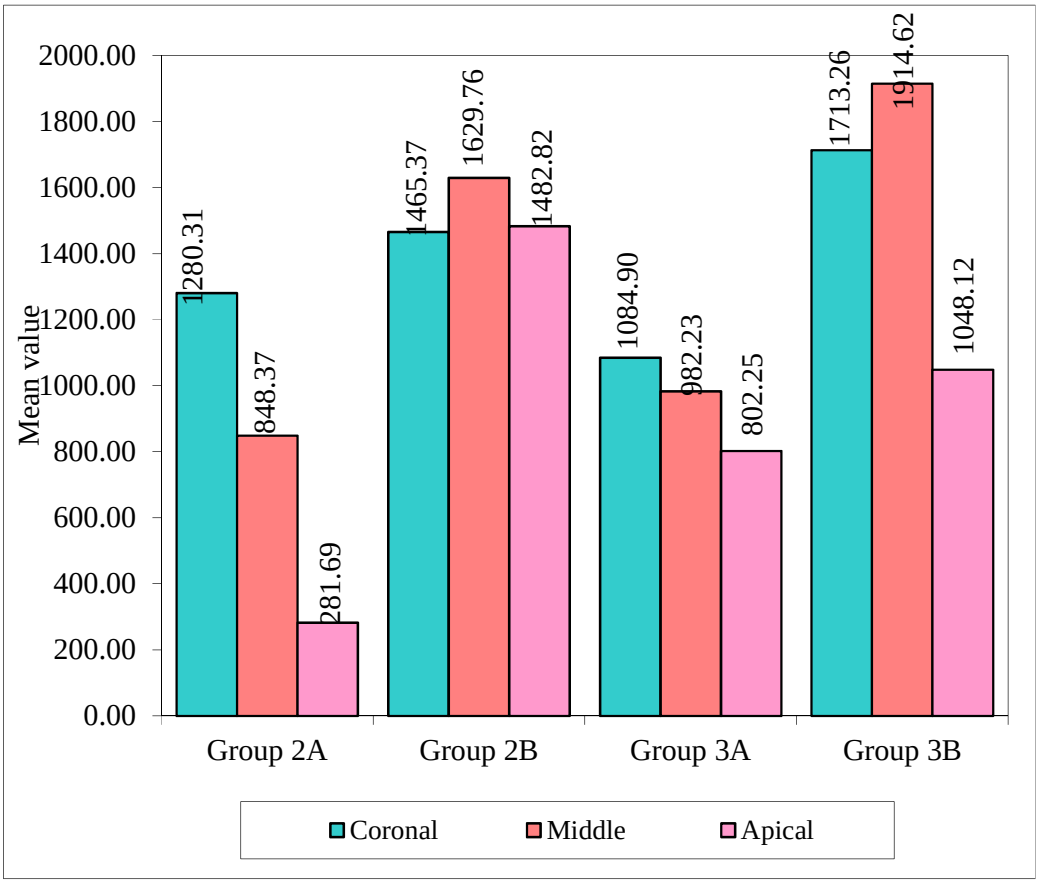
Experimental (AH Plus)-Group 3A

Graph 8: Comparison of three sections (coronal, middle and apical) in group 3B with mean depth of tubular penetration



Experimental (Bio-C)-Group 3B

Graph 9: Comparison of three sections (coronal, middle and apical) in four groups (2A, 2B, 3A and 3B) with mean depth of tubular penetration



Negative control (AH Plus)-Group 2A; Negative control (Bio-C)-Group 2B; Experimental (AH Plus)-Group 3A; Experimental (Bio-C)-Group 3B

CLSM Images depicting penetration of AH Plus without calcium hydroxide placement [Group 2A]

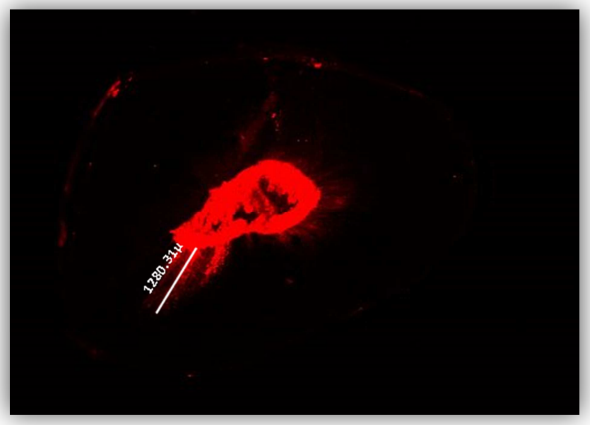


Fig 17 a: CORONAL

[Group 2A]

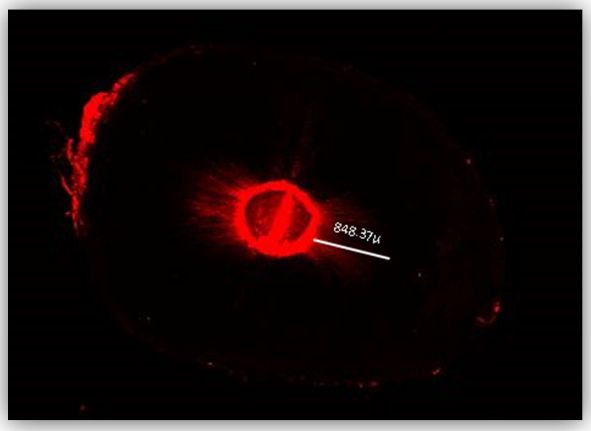


Fig 17 b: MIDDLE

[Group 2A]

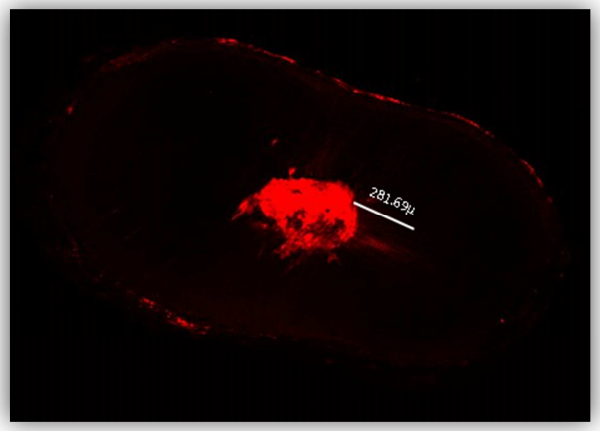


Fig 17 c: APICAL

[Group 2A]

CLSM Images depicting penetration of Bio-C without calcium hydroxide placement [Group 2B]

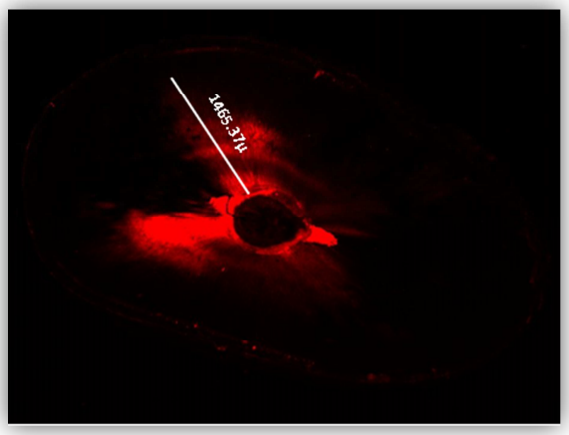


Fig 18 a: CORONAL

[Group 2B]

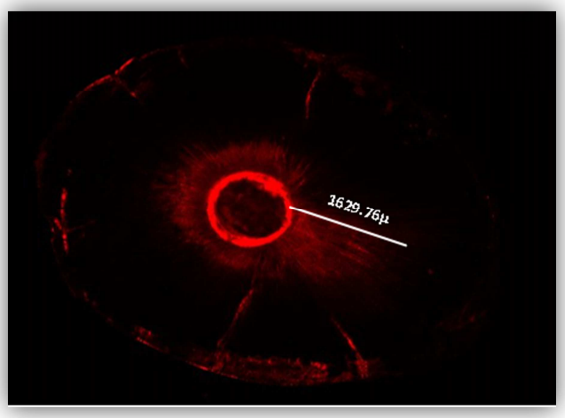


Fig 18 b: MIDDLE

[Group 2B]

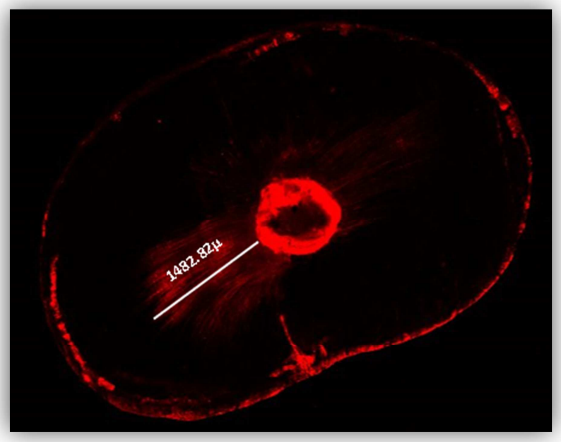


Fig 18 c: APICAL

[Group 2B]

CLSM Images depicting penetration of AH Plus after calcium hydroxide removal [Group 3A]

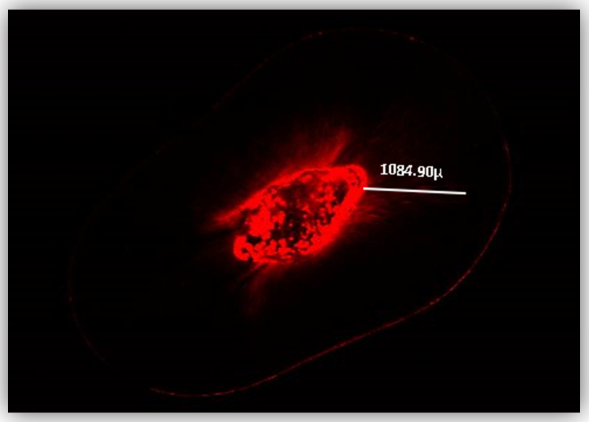


Fig 19 a: CORONAL

[Group 3A]

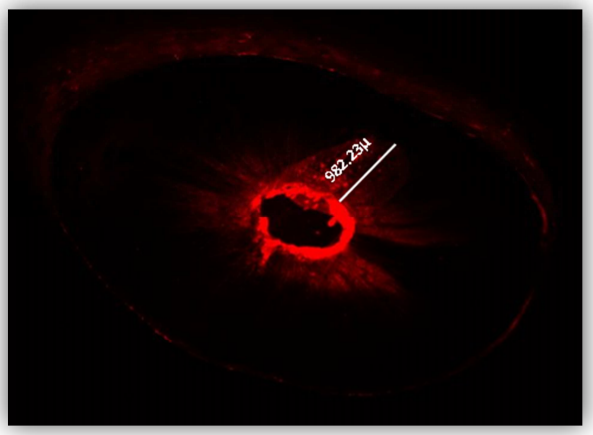


Fig 19 b: MIDDLE

Group 3A]

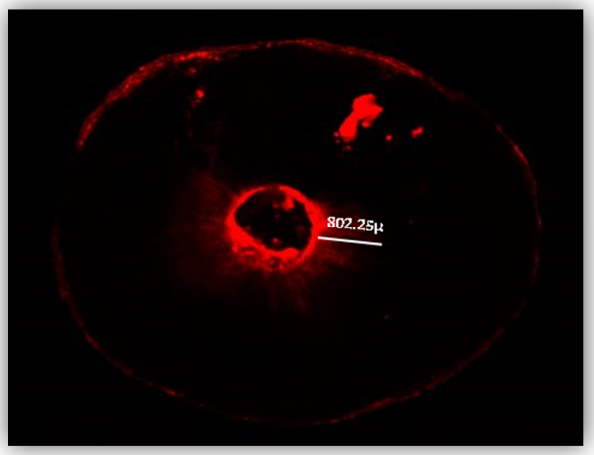


Fig 19 c: APICAL

[Group 3A]

CLSM Images depicting penetration of Bio-C after calcium hydroxide removal

[Group 3B]

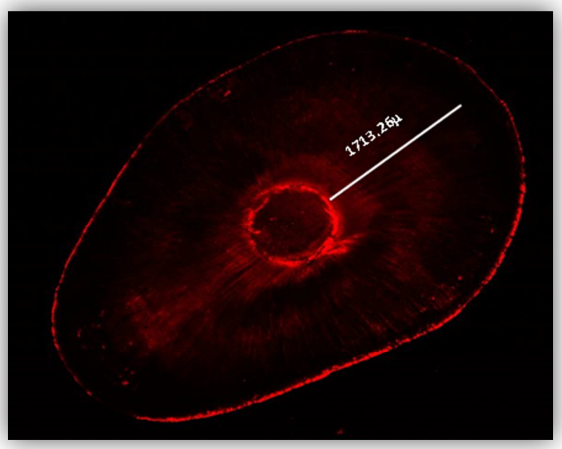


Fig 20 a: CORONAL

[Group 3B]

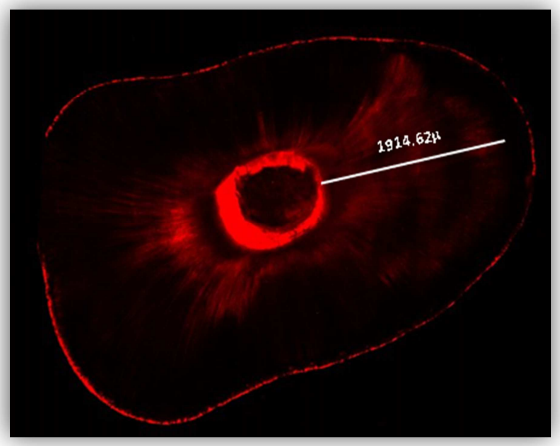


Fig 20 b: MIDDLE

[Group 3B]

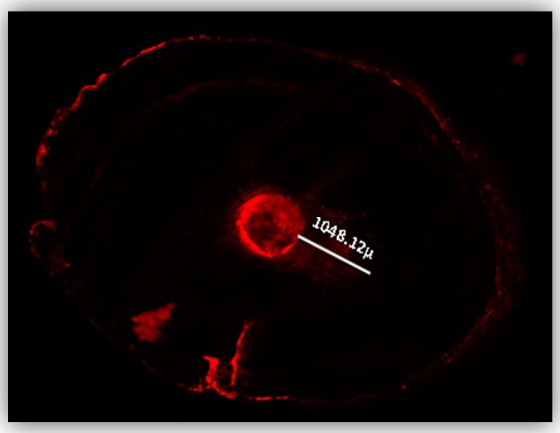


Fig 20 c: APICAL

[Group 3B]

DISCUSSION

One of the key elements for successful endodontic therapy is appropriate dentinal 'tubular penetration' of the sealer which is of utmost importance as it helps to improve the root canal filling retention. It also helps to entrap the residual 'bacteria' within the 'dentinal tubules' by acting like a barrier which has been observed to persist even after retreatment procedures. [34,35,36] The penetration of the sealer depends on multiple factors, such as, its physico-chemical properties, tubule density, tubule diameter, root canal dimensions and smear layer removal. [37]

A very important property of sealer that determines its penetration into the 'dentinal tubules and irregularities' within the canal system is 'Flow', which in turn is affected by the composition, particle size and film thickness of the sealer. [35] Bronzel et al (2019) demonstrated that "Bio-C" sealer, a premixed calcium silicate-based sealer had the highest flow rate as compared to "AH Plus", an 'epoxy resin-based sealer' and Totalfill BC which is also a calcium silicate-based sealer. [16] 'AH Plus' is considered as the 'gold standard' because of its good physicochemical properties such as low solubility and better handling characteristics against which all new sealers can be compared. [38]

A systematic review and meta-analysis by Falk Schwendicke et al (2017) on single versus multiple visit root canal treatment concluded that risk of flare -up was found to be higher with single visit treatment and hence it was recommended to perform preferably multiple-visit treatment in teeth where the risk of complications was higher as in teeth with an already existing 'periapical' lesion. [39]

Calcium hydroxide is the most commonly used intracanal medicament in routine clinical practice owing to its excellent antibacterial and anti-inflammatory properties. An alkaline environment created by the '$Ca(OH)_2$' plays a positive role in 'apical' healing by inducing hard tissue formation. However, it has been observed that '$Ca(OH)_2$' cannot be completely eliminated from the 'root canal system', its residues thereby acting as a barrier for sealer penetration. [40,41] Till date, no technique has been able to completely remove the '$Ca(OH)_2$'. Routinely, conventional syringe irrigation was used but it was found to be less efficacious. Passive ultrasonic irrigation has been proved to be one of the best methods for '$Ca(OH)_2$' removal and hence was used in this study. [40,42] This drawback may be overcome by the use of calcium silicate based sealers which in the presence of this alkaline environment and '$Ca(OH)_2$' residues has shown better penetrability. [2]

Studies done by Wang et al (2018), El Hachem R et al (2019) and Akcay et al (2016) have shown 'bioceramic 'root canal sealers' to have a better 'tubular penetration' as compared to "epoxy resin-based sealer", "AH Plus". This can be attributed to their fluidity, hydrophilic nature and minute particle size, which enables better penetration and greater adaptation to the 'dentinal tubules'. [14,15,21]

Human mandibular premolars were selected for the study as they are most often encountered with anatomic variations which could be challenging and they are also commonly extracted for orthodontic treatment. [43] Specimens were stored in 0.1% thymol solution in order to prevent fungal growth. All the specimens were decoronated so that we could obtain a standardized root length of 14 ± 1 mm with a flat 'coronal' surface for equivocal reference evaluation and elimination of discrepancies due to variation in access cavity preparations. ProTaper Gold files were

used for bio-mechanical preparation as they possessed superior flexibility and greater resistance to cyclic fatigue. [44,45]

Standard irrigation protocol of 3% sodium hypochlorite was used between successive instrumentation due to its potential tissue dissolving and antimicrobial properties. Studies on whether the presence of smear layer interferes with 'dentinal tubule penetration' are still controversial and hence 17% EDTA was used after the use of NaOCl for smear layer elimination. Final irrigation was performed with distilled water to banish the effect of the remaining oxygen from NaOCl on the polymerization of the sealers. [46,47]

Cavit was used as a temporary restorative material over the gutta percha in order to prevent microleakage.[48] All the samples were incubated at 37°C in humidified conditions for a week to ensure complete setting and polymerization of the sealer and simulate the oral environment.

Confocal Laser Scanning Microscope was preferred for evaluation of the "tubular penetration" depth in this study over other methods such as SEM as it helped to visualize sections at different levels, creating a 3D image and also made depth measurement more precise. Additional step of sample preparation such as dehydration or gold spluttering was eliminated by using the CLSM, thereby preserving the integrity of the dentin and allowing the samples to be reused. One of the advantages of CLSM is that it avoids artifacts because the analysis is performed from the surface to 20-30 microns depth. The Rhodamine dye used to obtain the fluorescence was required in minute amount (0.1%) and hence the properties of the sealer remained unaffected. [49,50]

The results of this study show that 'Bio-C' sealer after '$Ca(OH)_2$' removal had higher 'tubular penetration' for the 'coronal' and 'middle third' of the root canal, whereas intergroup comparison for the 'apical' third showed 'Bio-C' without the use of '$Ca(OH)_2$' to have the highest penetration. Therefore, the null hypothesis that there would be no difference in penetration after the use of '$Ca(OH)_2$' was rejected. [Table7,8; Graph 9 and Fig 20 a, 20 b, 18 c]

Intragroup comparison for 'AH Plus' sealer revealed highest penetration in the 'coronal' third followed by 'middle' and least in the 'apical' third when used with and without '$Ca(OH)_2$'. This was in accordance with a number of previous studies that can be due to the small diameter of the 'apical' third with less number and density of dentinal tubules. Another reason can be due to inefficacious irrigant delivery and ineffectual "smear layer removal" in the 'apical region'.[51] [Table7,8; Graph 4 and Fig17, 19]

For 'Bio-C' sealer, highest penetration was noted in the 'middle' third, followed by 'coronal' and lowest in the 'apical' third regardless of the use of '$Ca(OH)_2$'. The less tubule density and inefficient smear layer removal from the 'apical' portion resulted in decreased penetration in the 'apical' third. Moreover, the 'apical' root dentin is poorly permeable due to the sclerotic dentin as compared to the 'coronal' and 'middle' third dentin.[52] [Table7,8; Graph 4 and Fig 18,20]

Intergroup comparison revealed "Bio-C" sealer performing better than "AH Plus" sealer in the negative control (without '$Ca(OH)_2$' as well as the experimental groups (After '$Ca(OH)_2$' as intracanal medicament) with a statistically significant difference. [Table7,8 and Graph 9] A study done by Carolina Caceres et al (2021) comparing the 'dentinal tubule penetration' and adaptation of 'Bio-C' and 'AH Plus' using SEM analysis showed results analogous to our study with 'Bio-C' showing a

better penetration and adaptation. It was also observed that 'Bio-C' tags had enhanced adaptation to the walls of the tubules in contrast to 'AH Plus' which may be associated with sealer's hydrophilic properties unlike 'Epoxy resin-based sealers' which are hydrophobic and have a high fluidity, thereby negatively affecting their penetrability and adaptation to the dentinal walls. [14,30,53]

Although studies have shown that '$Ca(OH)_2$' residues block the dentinal tubules and hamper sealer penetration, 'Bio-C' sealer in our study showed an even more enhanced penetration after the use of '$Ca(OH)_2$'. One of the reasons could be because of the alkaline environment created by '$Ca(OH)_2$'. Calcium silicate based sealers have a high pH and hence greater penetration in such an environment. Ozyurek in his study assessing impact of '$Ca(OH)_2$' on 'AH Plus' and BioRoot RCS penetration, also demonstrated similar results with BioRoot RCS having deeper penetration after '$Ca(OH)_2$' than 'AH Plus'. [2,54,55]

Similar results were also seen by Cruz et al, wherein MTA Fillapex had a higher penetration when '$Ca(OH)_2$' residues were present. This is attributed to kindred properties of the sealer, affinity betwixt the MTA compounds and the '$Ca(OH)_2$' residues in turn affecting the sealer penetration by a merge between these two compounds.[3,56]

This study illustrated good performance of 'Bio-C' sealer in terms of 'dentinal tubule penetration' when compared to 'epoxy resin-based sealer', 'AH Plus'. Further studies regarding push-out bond strength of 'Bio-C' sealer need to be done to evaluate the adaptation of the sealer. Research on this sealer is still scarce and hence further studies are needed in order to evaluate its long-term behavior to establish a strong evidence based literature.

CONCLUSION

It can be concluded that 'Bio-C sealer' showed better 'dentinal tubule penetration' both after and without the use of '$Ca(OH)_2$' as an intracanal medicament. However, persistence of $Ca(OH)_2$ medicament residues did not hinder but enhanced the tubular penetration of the 'Bio-C sealer'. Higher penetration was in "middle third" of the "root canal" for 'Bio-C' sealer whereas 'AH Plus' showed the greatest penetration within the 'coronal third'. Least penetration was seen in the 'apical third' for all the samples.

Future studies evaluating the other properties of the 'Bio-C' sealer and long-term studies need to be done in order to establish a strong literature base.

SUMMARY

Successful endodontic therapy is dependent on proper cleaning and debridement of the 'root canal system' and a fluid tight impervious seal. Various newer equipment and materials have been introduced in order to achieve a highly sterile environment within the canal resulting in successful outcomes. A number of "root canal sealers", especially bioceramic sealers are being launched into the market with improved properties such a good flow. 'Tubular penetration' of sealer is one important feature that helps to establish a fluid impervious seal and enhanced adaptation between root dentin wall and core material.

In this study, "dentinal penetration" after using '$Ca(OH)_2$' medicament has been evaluated as it has been observed that residues of this medicament could interfere with sealer penetrability. However, an association between these residues and the newer bioceramic sealers was found which led to the aim of this study.

70 extracted human mandibular premolars were decoronated to achieve a standardized root length of 14±1mm using a diamond disk. WL was determined using a 10 K-file and the samples were divide into 3 groups, Positive control group, Negative control group and experimental group. Biomechanical preparation was completed with ProTaper Gold files up to size F3 followed by '$Ca(OH)_2$' placement for the Experimental group and the negative control group was prepared up to size F4. After medicament removal using passive ultrasonic irrigation, preparation was enlarged to F4 for the experimental group. Irrigation was done using 17% EDTA and final rinse by distilled water, dried using paper points and divided into 2 subgroups based on the sealer type. Obturation was done using sealer labeled with "0.1%

Rhodamine dye" and the samples incubated for a week. Sectioning was done for CLSM evaluation and images obtained were analyzed using Image J software.

The 'null hypothesis' that '$Ca(OH)_2$' did not influence the sealer penetration was rejected.

Highly significant results were obtained with 'Bio-C' sealer after '$Ca(OH)_2$' medicament showing the highest penetration than "AH Plus". Among the sections of the 'root canal', 'AH Plus' exhibited greatest penetration in the 'coronal third' whereas for Bio-C, 'middle third' showed maximum penetration. Least penetration was seen with 'AH Plus' in 'apical third'.

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ANNEXURE – I

ETHICAL CLEARANCE CERTIFICATE

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CERTIFICATE

This is to Certify that the synopsis titled

EVALUATION OF EFFECT OF CALCIUM HYDROXIDE INTRACANAL MEDICAMENT ON THE DENTINAL TUBULE PENETRATION OF AN EPOXY RESIN BASED SEALER AND A BIOCERAMIC BASED SEALER USING CONFOCAL LASER SCANNING MICROSCOPY: IN VITRO STUDY *Submitted by*

Dr. SHEFALI PEDNEKAR *P. G. Student /*

Staff, Guided by DR. NEHA. DHADED *from Department of* CONSERVATIVE DENTISTRY & ENDODONTICS *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 16th December 2021

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ANNEXURE – II

BIOSTATISTIC CLEARANCE CERTIFICATE

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Biostatistics Clearance Certificate

This is to certify that the Biostatistics aspect of the Dissertation / Research work of **Dr. Shefali G Pednecar, Post Graduate Student**, under the guidance of **Dr. Neha Dhaded**$_{M.D.S}$, **Reader, Department of Conservative Dentistry and Endodontics**, entitled **"Evaluation of effect of Calcium Hydroxide intracanal medicament on the dentinal tubule penetration of an Epoxy resin based sealer and a Bioceramic based sealer using Confocal Laser Scanning Microscopy- An In-vitro study"** has been done under my guidance and considered satisfactory.

Place: Belagavi
Date: 9/12/2021

Name & Signature of Biostatistician
(Dr. S.B. Javali)

ANNEXURE – III

PLAGIARISM CHECK CERTIFICATE

Scientific Correspondence and Review Committee

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PLAGIARISM CHECK REPORT

Name of the Applicant : DR. SHEFALI PEDNEKAR
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Batch & Year : 2019-22
Department CONSERVATIVE DENTISTRY AND ENDODONTICS

The soft copy of Research Work / Manuscript by DR. SHEFALI PEDNEKAR entitled "EVALUATION OF EFFECT OF CALCIUM HYDROXIDE INTRACANAL MEDICAMENT ON THE DENTINAL TUBULE PENETRATION OF AN EPOXY RESIN BASED SEALER AND BIOCERAMIC BASED SEALER USING CONFOCAL LASER SCANNING MICROSCOPY – AN INVITRO STUDY" under the guidance of DR. NEHA DHADED has been submitted for Anti-Plagiarism check to the Scientific Correspondence & Review Committee of KLE VK Institute of Dental Sciences using "Turn-it-in" software.

The scan has been carried out and the scanned output reveals a Similarity Index of 7%, which is **within / not within** the acceptable limits of 10% as per the UGC guidelines.

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