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Effect of ultrasonic agitation on the penetration depth of different root canal sealers: An in vitro study

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Abstract---Aim: The present *in vitro* study was taken to compare penetration depth of five different root canal sealers to radicular dentin by confocal laser scanning microscope. Materials and Methods: 100 extracted intact single- rooted human teeth were collected. Teeth were decoronated, shaping and cleaning was done with rotary file

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system till #25 number file with 0.06% taper. Half of the samples (n=50) were irrigated without ultrasonic agitation while other half (n=50) samples were irrigated with ultrasonically agitation. Teeth were obturated and divided into five groups on the basis of five different sealer used (Group A: Zinc oxide eugenol sealer (control group), Group B: Apexit Plus, Group C: AH Plus, Group D: MTA Fillapex, Group E: Bio-C sealer). Samples were sectioned horizontally at 2mm, 4mm and 6mm from the apical region and penetration depth was observed under confocal laser scanning microscope. Data obtained was statistically analysed using Independent t test and Tukey Test. Results: Among the groups, Bio- C sealer (Group E) revealed greatest penetration depth at 2mm and 4mm while zinc oxide eugenol (Group A) had the least penetration depth. Among the subgroups, samples irrigated with ultrasonic agitation showed rise in penetration depth of the sealers. Conclusion: Ultrasonic agitation intensifies the kinetic energy, increased removal of smear layer, therefore causing high velocity and flow of sealer in the dentinal tubules.

***Keywords*---**AH Plus, Apexit Plus, Bio-C, confocal laser scanning microscope, MTA Fillapex, penetration depth, ultrasonic agitation, zinc oxide eugenol.

Introduction

Endodontic treatment relies completely on 3 phases- bacterial control, efficacious sealing of root canal, shaping and cleaning. Shaping and cleaning of the root canals consists of elimination of vital and necrotic pulp tissues, microbial irritants and their by- products. However the instruments whether hand or rotary cannot reach up to certain areas that include anatomical complexities.[1]

Several endodontic irrigants are employed for efficient removal of endotoxins, disinfection of anatomical complexities and root dentin. Success of every step reckon on the accomplishment of the final phase that is obturation and sealing.[1,2] Complete eradication of microbial entity and to consider any future propensity to reinfection is main goal of endodontic treatment.[3]

Current accepted system of obturation make use of solid or semisolid core for example gutta-percha and sealer.[4] Long lasting success of endodontic treatment depends on choice of root canal sealer as per its clinical use. Root canal sealers are classified on the basis of their setting reaction and compostition.[5]

Zinc oxide eugenol sealers have reduced sealing property due to its increased solubility hence causes poor bonding between gutta-percha and sealer. This sealer is also a source of disinfection of dentinal tubules.[6] Calcium hydroxide sealers are antimicrobial, encourage osteogenesis and cementogenesis with enhanced healing of periapical area.[5] Epoxy resin sealers have been widely used because of their decreased solubility, better apical seal, superior adhesion to root dentin, improved penetration to micro-irregularities.[3,4] MTA a bioactive material responsible for hard tissue conduction and induction, induces mineralization, promotes formation of apatite like crystals in apical and middle third of canal.[7,8] Bioceramic sealers are water-based, can either be bioactive or bio inert. Two main benefits are biocompatibility and presence of calcium phosphate as it promotes sealer to root dentin adhesion. [9, 10]

Depth penetration of sealers take on an important role and its extent into the dentinal tubules relies on the degree of removal of smear layer. Other factors which are responsible for good penetration are physical and chemical properties of root canal sealer, technique of obturation, flow of the sealer into complexities, particle size and setting time.[11] This in vitro study compared the penetration of five different sealers to radicular dentin under confocal laser scanning microscope.

Materials and Methods

The current study was approved by the institutional ethical committee. A total of 100 extracted, non-carious, single rooted human teeth with intact, fully formed apices, without any previous endodontic treatment, fracture, resorptive defects and calcifications were selected.

OSHA guidelines and recommendations were followed for cleansing of teeth and ultrasonic scalers was used for making them free of calculus and soft deposits. The teeth were stored in normal saline till usage and were radiographically verified for presence of single canal. Decoronation of the teeth was done with a diamond disc, under copious water irrigation and at slow speed to obtain the standard root length of 16mm (as shown in Figure I).

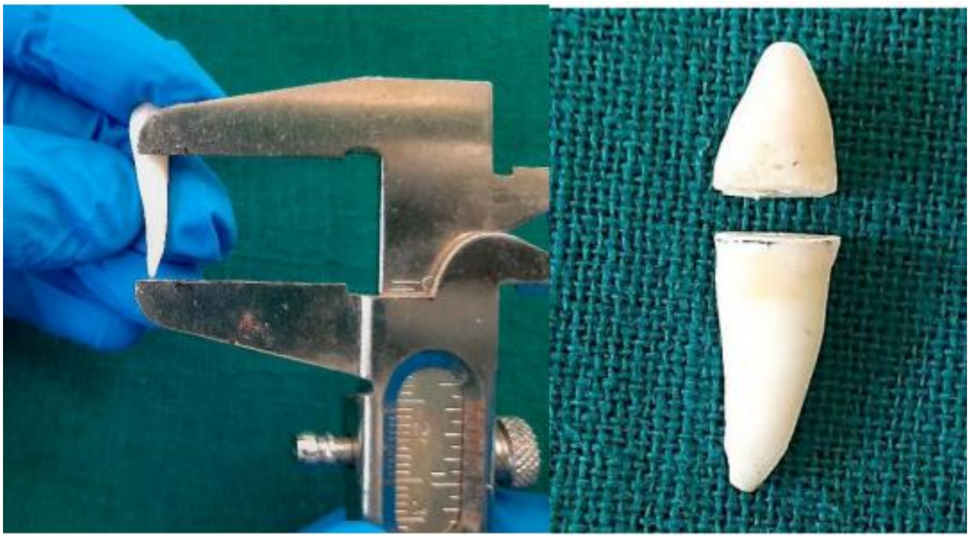


Figure I

A size #10 K-file (MANI, Prime Dental, India) was introduced in the canal and working length was determined 1mm short of the radiographic apex. Root canal shaping was done with Revo-S (Micromega, France) rotary instrumentation. Between every instrumentation, canals were irrigated with 3% sodium hypochlorite and 17% EDTA (Dental Avenue, Mumbai) was used for final irrigation of the canals. Half of the samples were ultrasonically agitated with ultrasonic files (MANI, Prime Dental, India) affixed with Satellac scaler in buccolingual and mesiodistal direction for 20 seconds each in first subgroup of every main group, 2mm short of working length. Samples were divided into five groups according to the different root canal sealers used (n = 20):

- Group A: Zinc oxide eugenol (Control group)
- Group B: Apexit Plus (Ivoclar, Vivadent)
- Group C: AH Plus (Dentsply)
- Group D: MTA Fillapex (Angelus)
- Group E: Bio- C sealer (Angelus)

Sealers were mixed with 0.1% (by weight) Rhodamine B dye in all the samples for better evaluation of penetration under confocal laser scanning microscope (Nikon) and applied using Lentulo-spiral (MANI, India). The samples were kept in incubator for 1 week at 37˚C for adequate setting of the sealer.

Sectioning and image evaluation

After incubation, the samples were sectioned horizontally by using diamond disc to obtain 2.0 mm thick sections at 2 mm, 4 mm and 6 mm level from the apex (as shown in Figure II) and were polished with sandpaper and then analysed under confocal laser scanning microscope (Nikon) to evaluate the penetration depth of different sealers.

Figure II

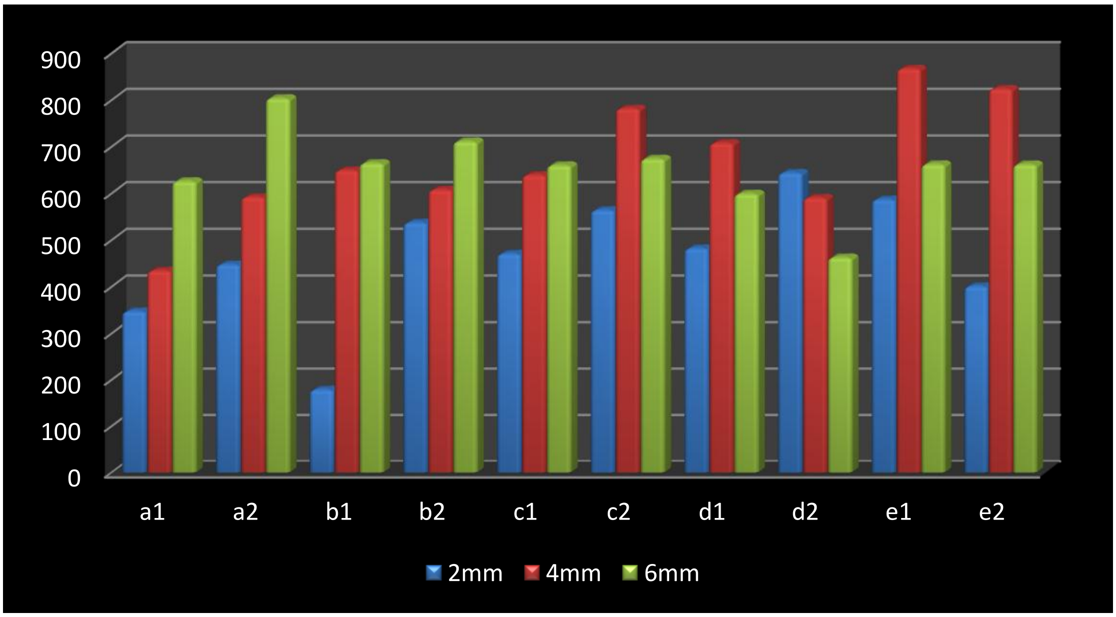
For accurate visualization of images, the samples were observed at 10X magnification. The emission wavelength for Rhodamine B dye was 561 nm and images were recorded at 100X magnification. Images were exported to Nikon Imaging Software and depth of penetration measured by Image J software.

Results

Data was analysed using software SPSS 23.0. Statistical analysis was done utilizing Tukey test.

Table 1
Group Statistics at 2mm (apical section), 4mm (middle section) and 6mm (coronal section)

	2mm		4mm		6mm	
	Mean	SD	Mean	SD	Mean	SD
a1	347.16	59.02	434.37	58.94	626.01	189.80
a2	447.55	21.97	591.85	50.19	803.91	15.20
b1	178.62	29.39	649.38	73.82	664.74	71.64
b2	537.31	156.73	607.65	13.09	711.43	58.09
c1	470.59	95.11	639.11	223.18	660.23	202.71
c2	564.05	230.53	781.66	337.36	674.27	57.02
d1	482.14	34.48	707.87	62.24	599.23	375.20
d2	644.01	86.66	590.45	157.29	462.64	166.73
e1	586.81	88.80	867.10	174.88	662.29	127.61
e2	400.86	41.79	823.86	183.15	662.08	192.36



Graph 1- Group Statistics

Table 1 and graph 1 shows mean penetration depth of all the samples (n = 100) of different subgroups. Mean penetration depth of sealer was greatest for subgroup

e1 (Bio-C sealer) at 2mm (apical section) and 4mm (middle section), subgroup b1 (Apexit Plus) at 6mm (coronal section) with ultrasonic agitation and results were statistically significant ($p \leq 0.05$). Despite of ultrasonic agitation, the results of mean penetration depth were:
Bio-C sealer (Group E) > AH Plus (Group C) > MTA Fillapex (Group D) > Apexit Plus (Group B) > Zinc oxide eugenol (Group A).

Discussion

Microorganisms are the prime cause of pulp degeneration which subsequently leads to apical periodontitis. Response of host to the bacterial irritants present lead to local inflammation, growth of pathologic entities and disruption of attachment apparatus. Eradication of these crucial pro-inflammatory microbial factors is the ambient motive of endodontic treatment.[12]

Materials and instruments are continuously integrated in the endodontic practice, leading to acquisition of new protocols and techniques.[13] Anatomy of the root canal might affect the quality of obturation. To reduce anatomical variations and to attain standardization, single rooted teeth with one canal were chosen for this study.[6]

Shaping and cleaning of the samples were carried out using rotary instrumentation as this is very common technique- it rationalizes the preparation procedure and gives a consistent preparation.[14] Instrumentation of the root canal system creates amorphous, uneven and granular layer that covers the dentinal tubules called as smear layer. Irrigation and lubrication plays a crucial role in endodontics to expedite the instrumentation, preventing accumulation of debris, removal of smear layer and bacteria.[15]

Agitation of irrigants enhances the sealing properties of filling material to attain impervious seal interface among core material and root canal wall.[16]In the present study, 17% EDTA was used as it owns low surface tension and would enlarge the porosity in endodontic treatment, eliminates inorganic part of smear layer, opens the dentinal tubules and also lateral canals.[14]

In first subgroup- ultrasonic agitation of sodium hypochlorite was performed. Intermittent flushing method of passive ultrasonic irrigation was incorporated. In this technique, the irrigant is refilled with fresh irrigant after each ultrasonic agitation cycle. A final irrigation was done by EDTA to remove effect of residual oxygen generated from sodium hypochlorite.[13, 17] Devarajan M et al stated that sealers penetration improves:

- Interface between gutta-percha and dentinal tubules by enhancing sealing ability
- Entomb the persisting microorganisms in the tubules
- Chemical composition of sealers create bactericidal effect
- Sealer plugs form mechanical interlock with the tubules hence increasing the retention of core material and decreasing chances of microleakage.
- Sealers were applied with Lentulo-spiral as it rotates, the spring part plunges the sealer centrifugally.[18]

An ideal sealer procure flow, low viscosity and superior wetting as key properties for better penetration in the dentinal tubules. Flow is defined as capability of sealer to penetrate into the anatomical complexities which is different for every sealer.[19]
Stereomicroscopy, Confocal laser scanning microscope and Scanning electron microscope are distinct microscopy techniques for assessing sealer/ dentin interface and their penetration. Confocal laser scanning microscopic images gives more elaborated details of the penetration of root canal wall at comparatively low magnification in collation with conventional SEM.[20] Therefore, the effect of ultrasonic agitation on penetration depth of different root canal sealers used in the study was evaluated using confocal laser scanning microscope.

The results of the current study reported penetration depth of Bio- C sealer greater than AH Plus, followed by MTA Fillapex, followed by Apexit Plus, followed by zinc oxide eugenol and are statistically significant ($p \leq 0.05$) (as shown in Graph 1 and Table 1). The results are in accordance with Upadhyay V et al (2011)[6], Arikatla EK et al (2018)[21], Zordan- Bronzel CL et al (2019)[22], Tanomaru-Filho M et al (2020)[23], Caceres et al (2021)[24]. This can be due to:

- Particle size of Bio-C sealer is <2 µm, had highest flow as this is most essential property to penetrate into the dentinal tubules.
- Bioactive sealers develop tag-like structure, hence resulting in fluid-tight seal with the root dentin. This is due to the hydrophilic nature and chemical bond formed by bioceramic sealer with dentinal wall, releasing hydroxyapatite as final product.
- Penetration of AH Plus sealer was more than MTA Fillapex as it sets up covalent bond amidst epoxy resin and collage, increasing the chemical bonding to dentin.[21,22,24]

In the present study, depth penetration of sealer is increased with ultrasonic activation at 4mm and 6mm (as shown in figure III and IV) and the results were statistically significant ($p \leq 0.05$).

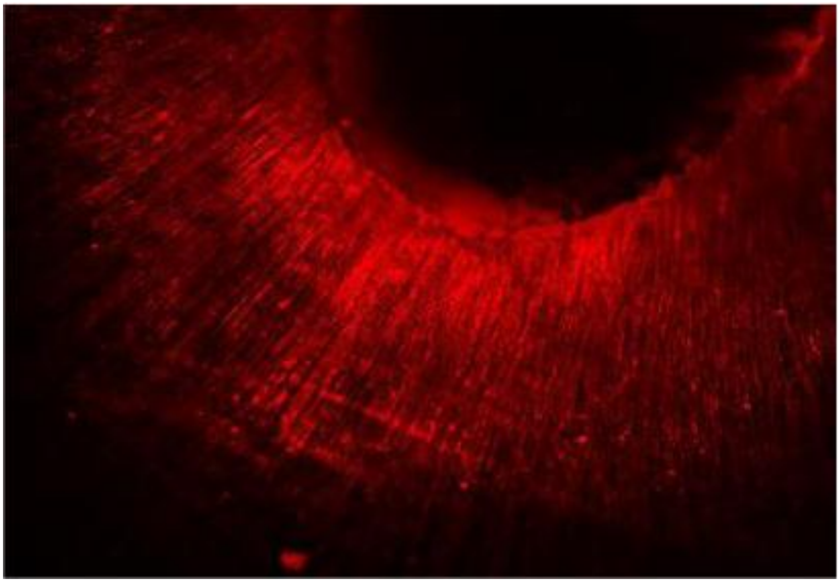


Figure III

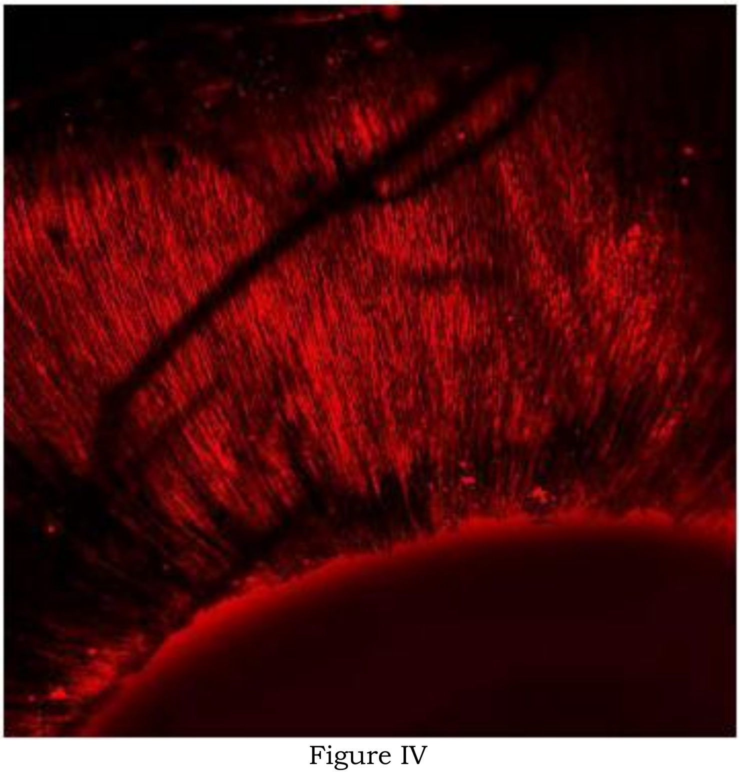


Figure IV

The findings are in accordance with Prasad PK et al (2018)[25] who assessed impact of ultrasonic activation of depth of penetration of three root canal sealers. This might be because ultrasonic agitation intensifies the kinetic energy, therefore causing high velocity and flow in the sealer and promoting penetration in the dentinal tubules.[25]

The difference in penetration at 4mm and 6mm was observed due to increased density and diameter of dentinal tubules at middle and cervical third of the root. Least penetration at apical region (2mm) (as shown in Figure V) could be due to presence of sclerotic dentin, number and diameter of dentinal tubules decrease as we approach towards apical region, uneven structure and density of tubules in apical third, accumulation of cementum- like tissue blocks the tubules and hinder penetration of sealer.[26]

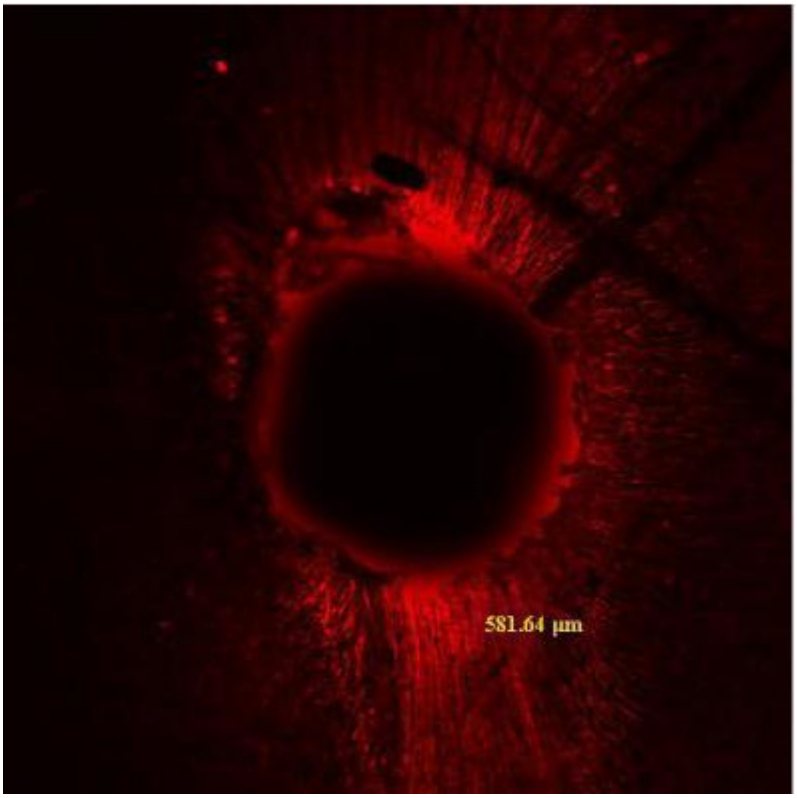


Figure V

In the confocal laser scanning images, increased penetration depth was observed in buccolingual direction than mesiodistal direction. This difference is in correlation with Kuci A et al due to the phenomenon of “butterfly effect”.[27] Confocal laser scanning microscope gives more elaborated details of the penetration of root canal wall at comparatively low magnification in collation with conventional SEM.[28]

Conclusion

It can be concluded from the present study that ultrasonic agitation positively intercept the intra-tubular penetration of root canal sealers. The ability of a sealer to infiltrate in the dentinal tubules helps in selecting the ideal sealer for obturation.

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