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Original Research

Comparative evaluation of penetration depth of three different root canal sealers in the apical third using ultrasonics- a study using confocal laser scanning microscope

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ABSTRACT:

Background and objectives: The penetration of sealer into dentinal tubules is considered to be a desirable feature as sealers prevent the colonization of residual bacteria into the dentinal tubules because of their antibacterial properties. This study's objective is to assess the efficacy of ultrasonic agitation on the penetration depth of three different root canal sealers in the apical third. **Materials and methods**: 36 extracted human mandibular premolars teeth were collected and divided into three groups based on type of sealer used, Group I: AH Plus (Densply maillefer, Switzerland), Group II: GuttaFlow 2 (Coltene/Whaledent, Alstatten, Switzerland), Group III: Bio C Sealer (Angelus, Londrina, Brazil). Each sealer was incorporated with rhodamine B dye and subjected to ultrasonic activation. The teeth were obturated and coronally sealed with cavit. Horizontal sections are obtained 3mm from the apex and the depth of sealer penetration will be measured by confocal laser scanning microscope. **Results**: Values obtained were tabulated and subjected to statistical interpretation with Kruskal Wallis test (pair wise comparisons), Mann-Whitney test. Statistically significant difference exists between the three experimental groups**. Interpretation and conclusion:** Bioceramic sealer has better depth of penetration when subjected to ultrasonic agitation in the apical third when compared to other sealers. With in the limitations of the study it is concluded that by ultrasonic agitation, bioceramic sealers have a better penetration ability in the apical thirds of the root canal than Epoxy and Silicone based sealers.

Key words- Ultrsonics, Bioceramicselars, AH Plus, Gutta flow 2, Rhodamine B dye, confocal laser scanning microscope.

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INTRODUCTION

During endodontic therapy, obturation of the root canal system is crucial for stable, long-term results following ideal root canal preparation, cleaning, and disinfection.[1] Although root canal filling procedures have advanced, several issues persist, including poorly prepared areas that impact the adaption of obturation material, apical extrusion of gutta-percha and sealer.[1] Due to the technique's difficulty, the absence of hermetic sealing that creates ideal conditions for bacterial colonization, and the difficulty of treating obturations in the face of various anatomical variations within endodontic spaces, from lateral canals and isthmuses to oval and curved canals, all these will contribute to obturation failure.[2]An optimal root canal treatment is achieved by a number of crucial elements, including appropriate instruments, biomechanical preparation, obturation and post-endodontic restoration.[3] Furthermore, a root canal sealer is necessary to improve the seal during compaction and to reach small, typically inaccessible locations, such as the dentinal tubules, due to the size of the tubules and the existence of canal abnormalities. For several reasons, the penetration of sealer cements into dentinal tubules is regarded as a favorable result, by increasing the material-dentin interface, it will improve sealing capabilities and material retention, which can be enhanced by mechanical locking.[4] Thus, the removal of

microorganisms from the dentin tubules may be aided by the sealer's penetration into these regions.[5]

In endodontic therapy, many root canal sealants have been employed. High physical qualities and favourable manipulation characteristics characterize AH Plus (Dentsply DeTrey, Konstanz, Germany), as a popular epoxy resin-based sealant. It is regarded as the gold standard for usage as a control material in the majority of sealer research.[6] Calcium silicate-based sealers have been increasingly popular in recent years because of their exceptional physicochemical qualities, bactericidal activity, tiny particle size, and biocompatibility. Furthermore, as demonstrated by their setting expansion and chemical attachment to dentine created by the production of hydroxyapatite in the contact zone between the sealer and dentine, these materials have high sealing capabilities.[7] Gutta Flow (ColteneWhaledent, Altstatten, Switzerland) is a cutting-edge product that combines gutta-percha powder and silicone-based sealant. This product was modified further recently to create Gutta Flow 2, which has good physicochemical qualities, minimal cytotoxicity, and good dentin adhesiveness.[8]

An important consideration for choosing a root canal obturation material is the sealer's capacity to penetrate the dentinal tubules persistently and effectively.[9] Sealer cement penetration into dentinal tubules depends on several aspects, including as filling technique, dentine permeability, and smear layer removal.[10]

Human agitation techniques and machine-assisted agitation instruments have been created to improve its effectiveness. Examples include brushes, hand-activated files or gutta-percha cones, sonic systems, ultrasonic systems, and laser activation systems.[11]

In 1957, Richman first suggested using Ultrasonics in endodontics. Ultrasound is defined as sound energy with a frequency higher than 20 kHz, which is the range of human hearing. The frequencies employed by the first ultrasonic units were between 25 and 40 kHz. Then came the development of low-frequency ultrasonic handpieces, which operate between 1 and 8 kHz and generate reduced shear stresses, resulting in less surface modification of the tooth. [12]

The concept of passive ultrasonic irrigation, or PUI, was first presented by Weller and colleagues. During PUI, a small file is positioned in the middle of a root canal that has already been sculpted and activated to create audio streaming. The fluid moves in tiny, intense circles around the instrument as a result of this streaming.[13] Root canal sealers may be more likely to penetrate the dentinal tubules when activated, increasing their sealability and antibacterial properties. Not enough research has been done on how ultrasonic activation of the sealer into the root canal affects the quality of the filling.[14]

Not enough research has been done on how ultrasonic activation of the sealer into the root canal affects the filling quality. The sealer/dentin interface is now analyzed using a variety of microscopy techniques, including stereomicroscopy, transmission electron microscopy, scanning electron microscopy (SEM), and confocal laser scanning microscopy (CLSM).[15] Confocal laser scanning microscopy (CLSM) was better at giving detailed information on the presence of sealer and its distribution throughout the dentinal tubules uptoa magnifications of 100x.[16]

Recent research indicates that rhodamine B has no impact on sealer setting. Compared to methylene blue, it has more molecules that are surface-active and smaller particles. It was found that the 0.1% rhodamine-coated sealer did not show any changes in flow in accordance with the American Dental Association's (ADA) guidelines. Therefore, a 0.1% concentration of rhodamine B dye was used in our investigation.[17]

MATERIALS AND METHODOLOGY

Study Design: Study Design: This study was reviewed and approved by the Institutional Ethical Committee of Meghna Institute of Dental Sciences, Nizamabad, India in 2024,(approval number: 2024/MIDS/MDS/CONS/IEC/06). Power analysis was performed using G*Power software [alpha (α) = 0.05; power (1-β) = 0.80]. It indicated a requirement of 36 samples.

Inclusion criteria: Recently extracted single rooted mandibular premolars.

Exclusion criteria: Tooth having calcifications, fractures, resorptive abnormalities, or open apices.

Procedure: The armamentarium includes, the collected samples, the sealers, Protaper universal files, Ultra x for ultrasonic activation, temporary sealing material and glass slides for carrying the samples and miscellaneous. The samples were collected and cleaned for the removal of any soft or hard tissue debris. They were stored in a container with a lid containing sterile saline at room temperature until further processing. The samples were decoronated using a diamond disc to achieve a standard length of 15mm.The working length was determined by introducing a #10K file until it reached the apical foramen, subtracting 1 mm from this length. ProTaper rotary instruments (Dentsply Maillefer) were used to shape root canals up to F3.27G needle was used for irrigation, which was positioned 1 mm short of the working length. 3% sodium hypochlorite was used as an irrigant during instrumentation. Followed by instrumentation 3%NaOCl was ultrasonically activated in the canal, by placing the tip 1mm short of working length. To remove the smear layer, a final flush with 2 mL of EDTA was performed for 60 seconds following saline was used to clean the canals.Later paper points were used to dry them.

Depending on the sealer employed, the total 36 specimens were divided into three groups randomly:

Group I (n-12): AH-PLUS

Group II (n-12): GUTTA FLOW 2

Group III (n-12): BIO C

The sealers have been used in accordance with the guidelines provided by the manufacturer. In order to facilitate visibility with a confocal laser scanning microscope, fluorescent rhodamine B dye was incorporated with every sealer.
Paste carriers (MANI, PRIME DENTAL) were used to carry the sealers into the canals.
The activation in each group was carried out in two planes simultaneously because the ultrasonic oscillates in a single plane. In particular, as a normal procedure, for 20 seconds in the buccolingual direction and another 20 seconds in the mesiodistal direction of the root canal, which is 2 mm less than the working length.
Following activation, F3 gutta-percha was used to obturate each canal.Cavit, a temporary restorative substance, was used to seal the orifice.

Horizontal slicing of the specimens was done at a 3 mm level from the apical foramen after a week. These specimens are subjected to Confocal laser microscopic examination. The sections were examined with a 10x magnification. The wavelengths at which rhodamine B absorbed light and emitted light were determined to be 561 and 575 nm, respectively.

STATISTICAL ANALYSIS

SPSS software (version 21.0) was used to analyse the data that was obtained. The resultant values were tabulated and statistically interpreted using the Mann-Whitney test and the Kruskal Wallis test.

RESULTS

Mean depth of sealer penetration was higher for group III (BIO C)at 3 mm level (figure-1) and was statistically significant ($P < 0.05$) (table-1)

Table 1- Mean of penetration depth of different root canal sealers.

	Mean	Std. Deviation	Chi square	P value
AH PLUS(n=12)	5444.51	2065.1905	6.062	**0.048***
GUTTA FLOW 2(n=12)	4159.96	2515.3513		
BIO C(n=12)	6564.15	683.6669		
Total(36)	5132.05	2112.1457		

Kruskal Wallis test was employed, p<0.05 was considered statistically significant. The asterisk (*) indicates statistically significant findings.

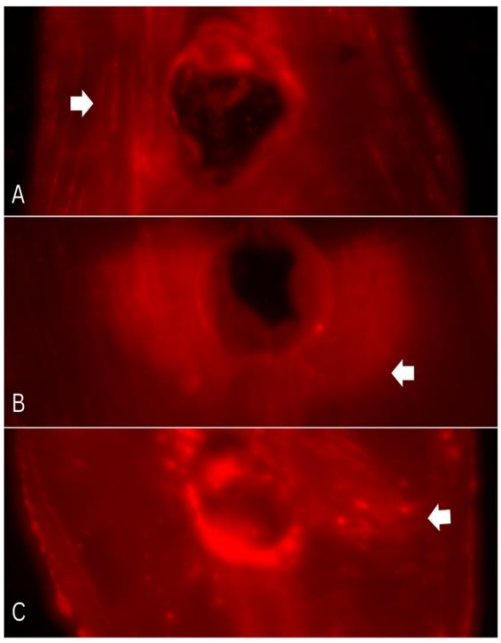


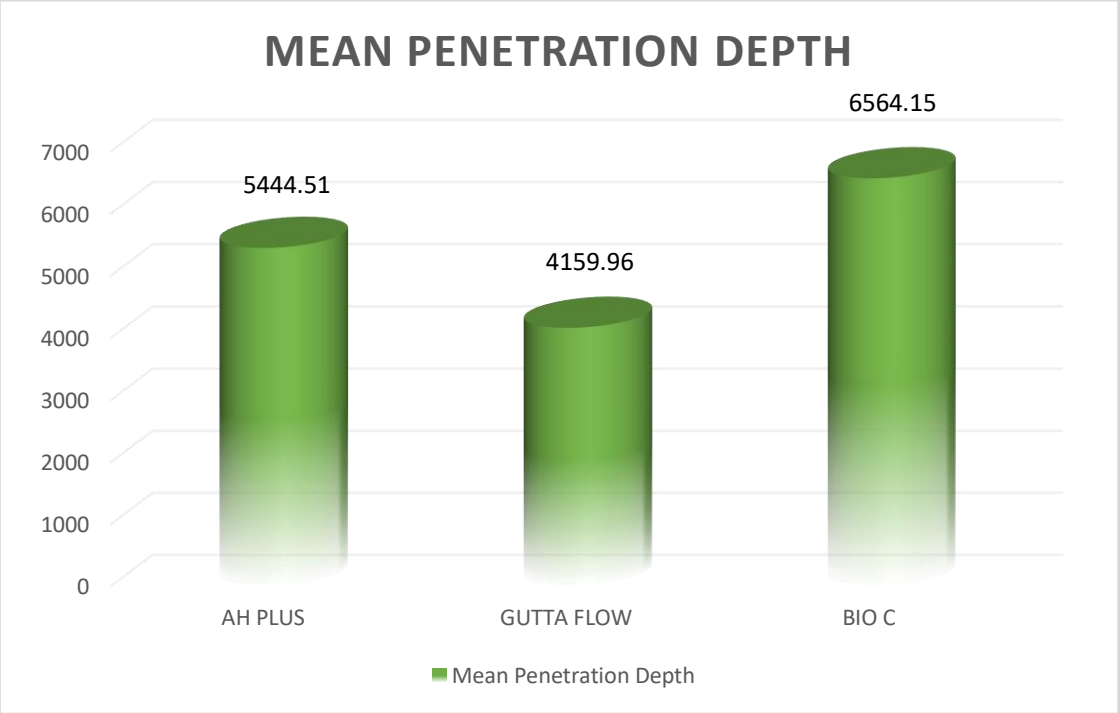
Figure 1 -representing the confocal images of the penetration depth of different sealers
A. AH Plus, B. Gutta flow, C. Bio C
The arrows indicate the depth of penetration in the horizontal section of the tooth

Table 2 Inter group comparisions

COMPARISON OF GROUPS	Mann-Whitney U	P value
AH PLUS VERSUS BIO C	51.000	0.225
BIO C VERSUS GUTTA FLOW2	64.000	0.644
AH PLUS VERSUS GUTTA FLOW 2	24.000	**0.006***

Mann whitney testwas employed, $p<0.05$ was considered statistically significant. The asterisk (*) indicates statistically significant findings.
No statistically significant difference exists between the penetration depth values of AH PLUS(GROUP I) vs BIO C(GROUP III) sealers of BIO C(GROUP III) vs GUTTA FLOW 2(GROUP II) sealer. Significant difference exists between the penetration depth values of AH PLUS(GROUP I) vs GUTTA FLOW 2(GROUP II) sealers ($p<0.05$). (table-2).

GRAPH 1 depicts the graphical representation of mean of penetration depth.



GRAPH 1: Mean Penetration Depth values of the three experimental groups values indicating BIO C has higher penetration depth.

DISCUSSION

The root canal system is protected from reinfection by encasing any residual bacteria and rendering them to be dormant. Endodontic sealers in conjunction with a solid core material, should fill irregularities alongside the root canal wall and penetrate the dentinal tubules to ensure, a long-lasting, bacteria-tight seal of the root canal system.[18]

During PUI, ultrasonic waves are used to transfer energy from a file or smooth oscillating wire to the irrigant. This causes the irrigant solution to cavitate and create a stream. The term "acoustic stream" refers to the rapid fluid flow in a circular or vortex pattern around the vibrating file.The formation of steam bubbles or the expansion, contraction, and/or deformation of already-existing bubbles in a liquid is known as cavitation.[19]Following ultrasonic activation, the physiochemical characteristics of sealers, such as setting time, flow, solubility, and radiopacity, were altered.[20]

The results obtained in this study showed that there is increased penetration of Bio C sealer followed by AH Plus and Gutta Flow 2. This may be due to the hydrophilic qualities of bioceramic sealants[7] and the Calcium phosphate, a component of bioceramic materials, improves their setting qualities and gives them a crystalline structure and chemical composition that resembles that of tooth and bone apatite materials. This improves the bonding between the sealer and the dentin at the root.The Bio-C Sealer penetrated the tubules smoothly and continuously, with very few gaps.[21] Its excellent flowability and reduced particle size are also a reason for this.[22]

This penetration's regularity could be related to both its hydrophilic content and premixed availability, containing the nanometric particles in its composition, enabling a more thorough examination and in a more uniform manner.

Conversely, epoxy-resin based sealers are hydrophobic despite having excellent fluidity and minimal polymerization shrinkage, so moisture could have a detrimental effect on their capacity to enter and adjust to the walls of dentinal tubules.AH-Plus exhibits less regularity, granularity, and intratubular gaps. These characteristics may be brought on by increased particle size, shrinkage during polymerization, or issues encountered during mixing.

These results are similar to the studies by Carolina CACERES et al. and Maria Rosa LARRAIN et al., who used a scanning electron microscope to measure the penetration of sealers in all the thirds of the root canal and found that Bio-C Sealer has better tubular

adaptation and greater penetration than AH-Plus in the apical thirds of the root canal.[7]

Studies by Arikatla et al. revealed, in contrast to the data above, that AH-Plus outperformed bioceramic sealers in terms of tubule adaptability, depth of penetration, and fewest gaps. Higher concentrations of epoxy resin, covalent sealer bonding with root dentin, and collagen can be the reasons behind AH Plus's higher penetration.[23]

Contradictory to these, research by Patri et al. showed that premixed bioceramic sealers were better adapted to dentine than epoxy-resin-based sealers.[24]

AH Plus sealer have a better adaptation than Gutta Flow 2. Due to the high surface tension pressures caused by the silicone content of Gutta Flow 2, it was challenging to propagate on the intratubular dentin. Additionally, the flow became more difficult because its film thickness was higher than AH Plus, resulting in a decrease of tubular penetration.[25] These are in accordance with the studies conducted by Sangham Madakwade er al.

LIMITATIONS

Sample size of the study is relatively small.

It was not possible to accurately replicate the oral state because this study was conducted in vitro.

To make a firm conclusion about the long-term effects of ultrasonics on different sealers and the sealer's penetration depth into root dentin, more research is needed.

CONCLUSION

The location of the lateral canals and the type of root canal sealer used will influence the penetration depth. Within the study's constraints, it is determined that bioceramic sealers, as opposed to epoxy and silicone-based sealers, have a greater capacity to penetrate the apical thirds of the root canal by ultrasonic agitation.

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