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Effect of Cerium Oxide Nanoparticles on Sealer Penetration in Endodontics using Confocal Laser Scanning Microscopy: An *in-vitro* Study

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AIM : To evaluate the effect of cerium oxide nanoparticles on penetration of root canal sealers in endodontics.

MATERIALS AND METHODLOGY:
Extracted human mandibular premolars wereused for the study. The samples were decoronated below the cemento-enamel junction to standardised length of 14mm .The root canal preparation was done and irrigation protocols were followed .Following the completion of the above procedure, canals were dried using sterile paper points and the samples were be divided into groups for different sealers with rhodamine B dye for staining . The obturated teeth were stored for one week at 100% humidity and a temperature of 37°. The teeth samples were sectioned and analysed under confocal Laser Scanning Microscope for sealer penetration .

GROUPS:
Group A: Gutta percha with Epoxy resin sealerandCerium oxide
Group B: Gutta percha with Bioceramic sealer and Cerium oxide
Group C : Gutta percha with Epoxy resin based sealer
Group D : Gutta percha with Bioceramic sealer
Group E: Gutta percha with Cerium oxide

Results
The penetration depth in Bio-C + Cerium oxide was statistically significant than all other groups.($p<0.05$).

Conclusion
In conclusion, the present study demonstrated that addition of cerium oxidenanoparticles to the root canal sealers has enhanced the penetration of sealers inside the dentinal tubules.

I. INTRODUCTION

A successful endodontic treatment is one that involves removing diseased tissue, preventing bacteria contamination, and forming a three-dimensional root canal filling.[1]A three-dimensional and leak-proof canal filling can be accomplished by using gutta-percha and canal sealer together.The degree of sealer penetration into dentinal tubules varies depending on several factors, including presence of a smear layer, viscosity, surface activity, particle size of the sealer, the contact angle of the dentin, and the number and diameter of dentinal tubules.The number of dentinal tubules decreases with advancing age. [2]

Penetration can only occur if the particle size of the root canal sealer is smaller than the dentinal tubule diameter. Furthermore, using a canal sealer with low surface activity or containing sufficient surface-active reagents increases the penetration of the sealer into the dentinal tubules. The thickness of the film layer formed by the canal sealer and the working and hardening time also affect penetration.

AH Plus root canal sealer is considered as the gold standard for endodontics thanks to its many positive features.[3] Mineral trioxide aggregate (MTA) containing canal sealers have also recently begun to be used in root canal treatment due to their fluency, adhesion, and adequate hardening time, as well as superior biological properties.[4]Root ST, is a calcium silicate-based canal sealer with a hardening time of two hour. It is a root canal sealer in a premixed injectable form containing zirconium oxide, calcium silicate, filler, and hydrophilic thickening agents.[5]

Cerium oxide nanoparticle is an lanthanum based nanoparticle , which has antibacterial and anti-inflammatory effect. In dentistry , it is used as root canal disinfection, pulp capping agent and remineralising agent. It enhances bond strength to dentin.[6]

The access to areas such as isthmuses, ramifications, deltas, accessor and lateral canals are difficult andbacteria are most often located there, due to the communication of accessory canals with the periodontal membrane, a potential periodontic-endodontic pathway for bacterial penetration to and from the periodontium can be created.[7]Therefore the penetration of sealer into these areas might have a role in the eradication of bacteria from the dentin tubules. The analysis of the dentin/sealer interface can be done using confocal laser scanning microscope[8]. In comparison to conventional scanning electron microscope, using confocal laser scanning microscope provides the advantage of detailed information about the presence and distribution of sealers inside dentinal tubules at a relatively low magnification through the use of fluorescent Rhodamine-marked sealers.[9,10]

The purpose of this study is to evaluate the effect of cerium oxide nanoparticles on penetration of root canal sealers into the dentinal tubules under the confocal Laser Scanning Microscopy (CLSM).

II. MATERIALS AND METHODOLOGY

Pure SMV powder was procured (Sigma Aldrich Industries Pvt. Ltd., Germany).Forty (n=40) single rooted extracted human mandibular premolars free from dental caries that had completed root maturationwereselected. Teeth with previous root canal treatment, calcified canals, the curved roots, internal or external root resorption, decayed, cracked, or fractured teeth were excluded. The crowns of teeth selected were removed with a diamond disc under water cooling and standardized to 14 mm root length.

Chemo mechanical preparation of root canals

A number 15 K-type file was advanced into the canal until it emerged from the apical foramen. After shaping the canal with 15, 20, and 25 K files40/0.06 apical diameters and tapers were formed using Neo Endo S rotary file system. During preparation, irrigation was performed with five mL of 5.25%. After the shaping process, five mL 17% EDTA and five mL 5.25% NaOCl solution were used at a 2.5 mL/ min rate for final irrigation. Finally, the root canals were washed with five mL of distilled water and dried with sterile paper points.

The teeth were randomly divided into five groups, each consisting of eight teeth:

Group 1: Gutta percha with Epoxy resin sealer plus Cerium oxide

Group 2: Gutta percha with Bioceramic sealer plus Cerium oxide

Group 3 : Gutta percha with Epoxy resin based sealer

Group 4 : Gutta percha with Bioceramic sealer

Group 5: Gutta percha with Cerium oxide

To obtain fluorescent images using ConFocal Laser Scanning Microscope,the sealers were mixed with trace amounts of 0.1% rhodamine B. Single cone obturation technique was used for root canal obturation. The gutta-percha master cone was placed in the root canal with a canal sealer mixed with rhodamine B. The gutta-percha was placed in the root canal with a canal sealer mixed with rhodamine B. After the root canal filling, radiographs were taken to check for the existing gap. Access cavities were sealed with a cotton pellet and temporary cement barrier and stored in incubator in 37°C at 100% humidity for one week.Each specimen was sectioned perpendicular to its long axis with diamond disc at slow speed under constant water cooling to avoid friction. Roots were sectioned at middle third s and disk of diameter two mm was obtained.The coronal facing surface of each section was polished . The polished sections were imaged using a confocal laser scanning microscopy.

Penetration depth measurement

Images of 512 × 512 resolution were obtained by CLSM under ×10 using an argon laser light source . The images were transferred to ImageJ software to measure the total dentinal tubule penetration, using a calibrated measuring tool.

III. RESULTS:

This study evaluated the dentinal tubule penetration depth of different obturation materials using confocal laser scanning microscopy (CLSM), followed by quantitative assessment with ImageJ software, as images are given below

TABLE 1 REPRESENTS THE MEAN VALUE OF THE PENETRATION DEPTH OF THE SEALERS

GROUPS	DEPTH OF PENETRATION	MEAN VALUE
GROUP 1	1102.542 µm	1158.833
	1129.057 µm	
	1244.901 µm	
GROUP 2	1556.167 µm	1436.227
	1414.708 µm	
	1337.768 µm	
GROUP 3	788.341 µm	726.348
	690.122 µm	
	700.583 µm	
GROUP 4	863.591 µm	939.105
	988.268 µm	
	965.458 µm	
GROUP 5	446.527 µm	385.287
	387. 901 µm	
	321.435 µm	

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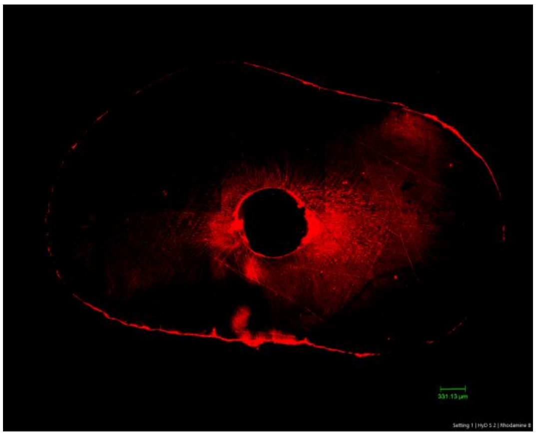
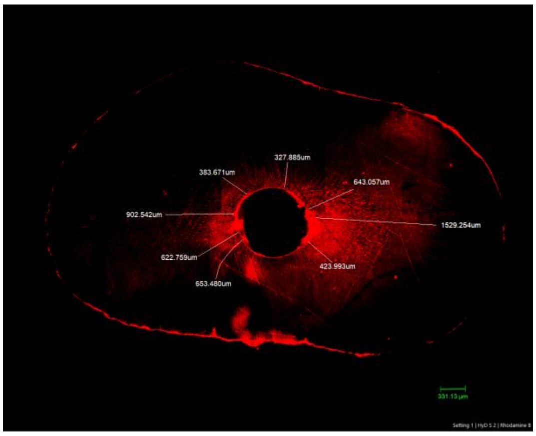
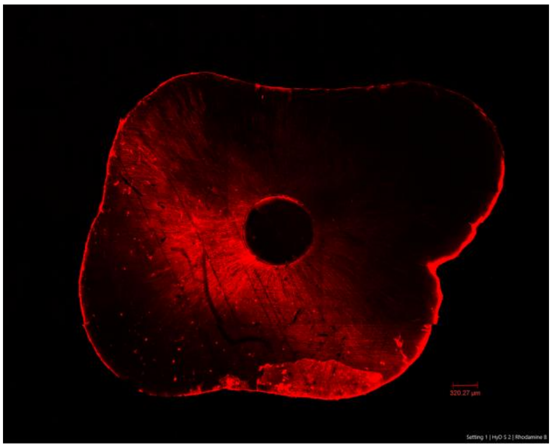
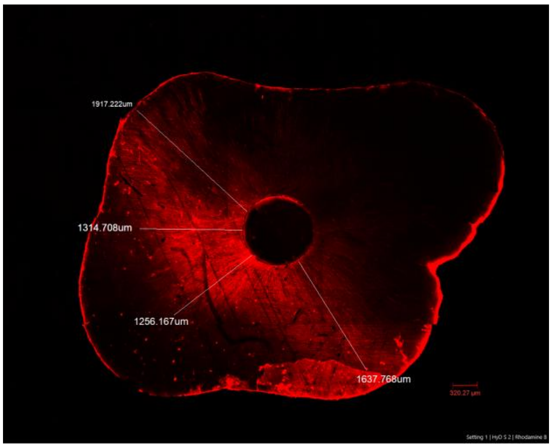
Group 1:



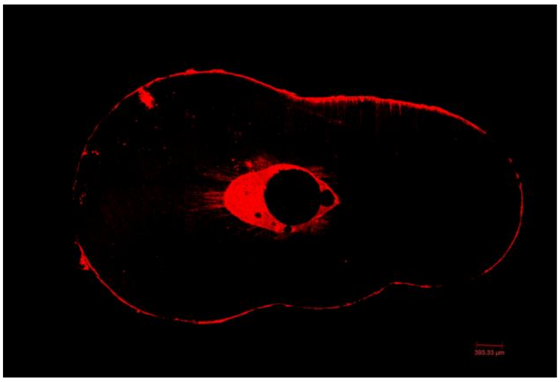


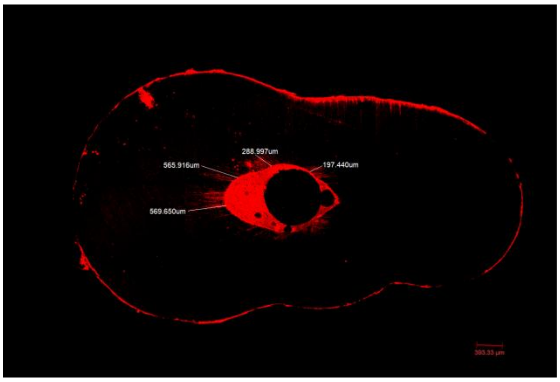
Group 2:





Group 3:





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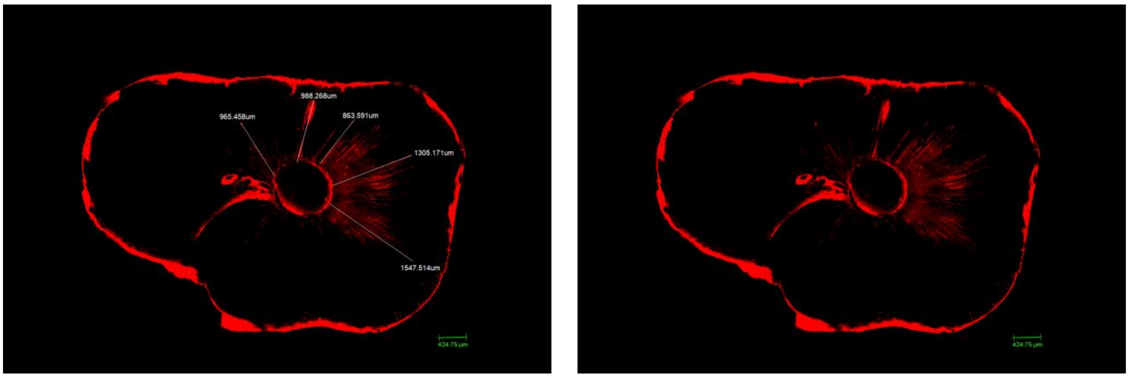
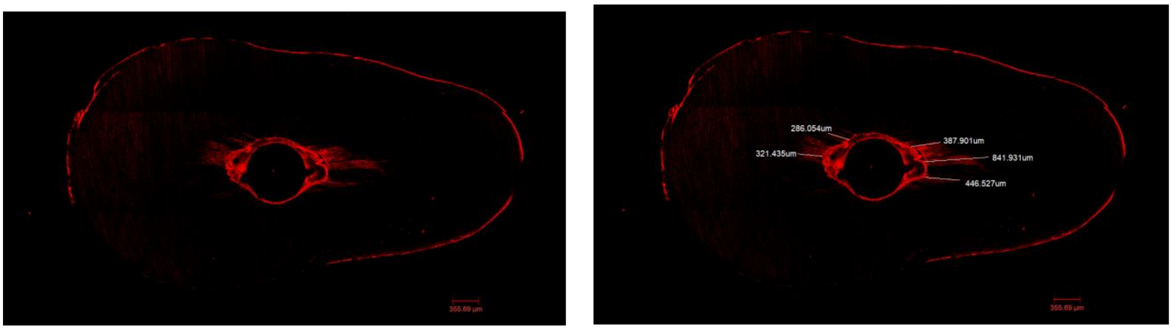
Group 4:



Group 5:



Group 2 (Gutta-percha with Bioceramic Sealer and Cerium Oxide) demonstrated the highest depth of sealer penetration with a mean value of **1436.227 µm**, followed by **Group 1** (Gutta-percha with AH Plus Sealer and Cerium Oxide) with a mean of **1158.833 µm**, and **Group 4** (Gutta-percha with Bioceramic Sealer) with **939.105 µm**. **Group 3** (Gutta-percha with AH Plus Sealer) showed moderate penetration (**726.348 µm**), while the least penetration was observed in **Group 5** (Gutta-percha with Cerium Oxide alone) with a mean value of **385.287 µm** [Table/Fig-2].

Among all the groups, **the addition of cerium Oxide to either AH Plus or Bioceramic sealers (Groups 1 and 2)** significantly enhanced the penetration depth compared to their counterparts without Cerium Oxide (Groups 3 and 4). **Group 2** showed the **deepest penetration**, indicating superior tubular infiltration capability, while **Group 5** showed minimal penetration, suggesting limited sealing ability when used alone.

These findings suggest that combining cerium oxide with conventional and bioceramic sealers enhances the sealer's ability to penetrate dentinal tubules, potentially improving the sealing efficacy of root canal obturation

IV. DISCUSSION

The complete sealing and obturation of the cleaned and shapedroot canal system are important steps that can affect thelong-term success of root canal treatment.[11]

Root canal sealers fill the gaps between the gutta-perchamaterial and root canal, and root canal dentin irregularities.The sealers penetrate the lateral and accessory canalsand dentine tubules.[12] When the smear layer is removedwith irrigation solutions, the canal sealer shows greaterpenetration. It is recommended that NaOCl and chelationagents be used together to remove the smear layer.

In previous studies examining canal sealer penetration intodentin tubules, horizontal and longitudinal sections wereobtained from teeth.Longitudinal sections have somedisadvantages: the dentin around the canal wall cannot bemeasured, and some areas of penetration depth may beunobserved. In our study, as in many studies, the sectionswere obtained perpendicular to the long axis of the toothfor more accurate measurement of these three parameters.[13,14]

Light microscopy, scanning electron microscopy (SEM), andusing confocal laser scanning microscope have been used to examine dentin tubule penetrationof the root canal. The samples are subjected to a series ofdamaging special processes so that images can be obtainedwith the

SEM scanning. The sample is not damaged as theuse of using confocal laser scanning microscope requires no special procedure to examine thesample.[15]In this examination method, the sealer penetrationinto the dentinal tubules is observed more clearly becausea fluorescent dye is used.In a study comparing the dentinpenetration of epoxy resin-based sealer (AH Plus) andcalcium silicate-based (Total Fill BC Sealer, BioRoot RCS)root canal sealers, the authors found that rhodamine Bpassively penetrates the dentinal tubules independently ofthe sealers.[16]

The ideal outcome in root canal obturation is to have high volume of gutta-percha and optimal volume of sealer within the root canal space and enhanced penetration into the canal irregularities and dentinal tubules. [17]The removal of the smear layer of the root canal walls is considered to be fundamental to allow sealer penetration into dentinal tubules irrespective of the root canal sealer used. Bacteria may penetrate into dentinal tubules up to 100–1,000 µm. Sealer penetration into the dentinal tubule would serve as a reasonable blocking agent that may prevent bacterial repopulation and can maintain their bactericidal action, which is favorable for healing periapical lesions and preventing reinfection. [18]

Cerium oxide nanoparticles (CeO_2 NPs) have emerged as promising additives in endodontic sealers due to their unique physicochemical properties that can enhance sealer penetration into dentinal tubules.[6]Their nanoscale size allows for deeper infiltration into the microstructures of dentin, promoting superior adaptation and sealing ability. Additionally, the surface activity and biocompatibility of these nanoparticles contribute to improved bonding between the sealer and dentin, which is critical for preventing microleakage and ensuring long-term endodontic success. The antioxidant and potential antimicrobial effects of cerium oxide further support its application in root canal therapy by contributing to a more stable and infection-resistant environment.

Group 2 (Gutta-percha with Bioceramic Sealer and Cerium Oxide) demonstrated the highest depth of sealer penetration.The hydrophobic property of AH Plus also prevents good adaptation to the incompletely dried canal wall. Whereas, Bio-C Sealer has higher flowability and smaller particle size related to the dentinal tubule penetration property of sealer.[19,20]Bio-C Sealer also has hydrophilicproperty and low contact angle which enable the sealer tospread easily over the dentinal wall and penetrate into thetubules and irregularities of radicular dentine. In addition,the moisture remaining in the dentinal tubules triggers itssetting reaction with the production of hydroxyapatite,thereby creating the chemical bond with root dentine.[21]

To achieve tubule penetration, the particle size of the material must be smaller than the tubule diameter; it can be assumed that because the particles for bioceramic sealer are is 0.2 µmin diameter, they will be well suited for tubule penetration. The small particle size, hydrophilicity, and low contact angle of bioceramic sealer explains why it penetrated the deepest .[22]AH Plus contains larger calcium tungstate particles with an average size of 8µm and zirconium oxide particles with a size of 1.5 µm, which might not enter easily into the smaller tubules

V. CONCLUSION:

Within the limitations of this study ,itcan be concluded that Cerium oxide nanoparticles addition to the root canal sealers had enhanced their penetration into the dentinal tubules.

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