In Vitro Evaluation of Bioceramic Sealer Penetration in Radicular Dentin: A Confocal Microscopy Study

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Abstract:

Objective: To assess the area of sealer penetration into dentinal tubules in different sections of the root canal using a confocal laser scanning microscope after irrigation with NaOCl and followed by ultrasonic activation. **Materials and Methods:** Mandibular premolars were selected according to the inclusion criteria which are: single straight root canal or slightly curved, fully developed apex with patent foramina, no obstruction in the canal system or indication of internal and/or external resorption or signs of crack or fracture, then the teeth were prepared, irrigated using NaOCl, activated, and then filled with Meta Biomed CeraSeal Bioceramic sealer using a single cone obturation technique. Rhodamine B was added to the sealer. Specimens were sectioned and divided into coronal sections, middle sections, and apical sections. The percentage of the sealer's penetration area was determined for each root level using a 10x confocal laser microscope. **Results:** The results were displayed as mean ±SD. A statistically significant difference was found between the studied sections ($P < 0.001$). Coronal sections presented the largest sealer penetration (35.19%), while apical sections showed the lowest sealer penetration. **Conclusions:** Penetration is affected by root dentine level as bioceramic sealer shows inferior penetration at smaller distances from the apex.

Introduction:

Successful root canal treatment is mostly dependent on eradicating pulp space infection, which may be accomplished by proper shaping and cleaning of all the pulp space and complete obturation of these spaces with a biocompatible obturating material.[1]

It is vital to have a sound knowledge of the internal anatomy of the teeth. Studies have shown that differences in canal morphology had a great influence on the alterations occurring during preparation, emphasizing the importance of canal anatomy.[2]

The hard tissue that surrounds the dental pulp may possess a diversity of forms and geometries. Several anatomic features and tissue remnants may be seen at the root apex. Intercanal connections may be revealed, and a single foramen may advance into multiple foramina. If this changed morphology is not identified, planned for, prepared, and obturated, treatment outcomes will usually become poor. This sequence of events supports the relationship between the root canal system's anatomic complexity and periradicular pathosis persistence.[3]

Irrigation is a crucial factor in effective endodontic treatment since it accomplishes numerous mechanical, chemical, and (micro) biological functions.[4] Root canal irrigation's goal is the disruption of microbial biofilms, the elimination of microorganisms, disintegration of the essential and necrotic pulp tissue, and hard tissue debris removal during instrumentation.[5] Sodium hypochlorite (NaOCl), ethylene diamine tetra acetic acid (EDTA), and chlorhexidine (CHX) are the most routinely employed irrigating solutions.[5]

The most efficient irrigant in endodontic treatment is sodium hypochlorite (NaOCl). It is the only solution currently in use that can dissolve organic debris in the canal. As a result, the application of hypochlorite is critical in eliminating necrotic tissue residues as well as biofilm.[4] The impact of irrigation on the smear layer has received a lot of attention in endodontic irrigation studies. Although smear layer removal is rather simple when the proper methods are followed.[4]

Sonic and ultrasonic irrigation uses acoustic streaming which seems to improve root canal cleanliness.[3] If the solution properly hydrates the pulp tissue debris and/or the smear layer and exposes it to ultrasonic agitation, the capacity of irrigating solutions with better wetting ability to dissolve tissue may increase. Ultrasounds, when used in conjunction with other irrigant treatments, facilitate the removal of the smear layer.[6]

Filling of the root canal system is critical to the success of the endodontic treatment where a three-dimensional fluid-tight seal throughout the whole length of the canals is the main cornerstone for its success.[7]

Due to the superior qualities of calcium silicate-based cement, endodontic sealers based on calcium silicate compositions have been launched in recent years.[8] A radiopaque, hydrophilic calcium silicate bioceramic sealer that creates hydroxyapatite on setting and binds to the root canal dentine wall. Because the sealer demonstrates no shrinkage and slight expansion, it is

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indicated for use with a single cone hydraulic condensation technique.[1]

Materials and Methods:

Sample collection: Analysis of the data from Donnermeyer etal.[9] research using G*Power 3.1 revealed that the size of the sample of each group needs to be at least 11. Therefore, 15 canals were selected for each test group.

Fifteen extracted, single-rooted human teeth were obtained from the oral and maxillofacial surgery department and approved by the institutional ethics committee of (approval number M 05150620) Faculty of Dentistry, Mansoura University.

The teeth were selected according to the following inclusion and exclusion criteria.

Inclusion criteria:
-Single straight root canal or slightly curved.
-Fully developed apex with patent foramina.
Exclusion criteria:
-Obstruction in the canal system.
-Internal or external resorption.
-Deep carious lesions.

Sample preparation: Decoronation of the teeth under study was performed to obtain standardized 16 mm roots. The patency of the canal was checked. A no.15 K file (Dentsply Maillefer, Ballaigues, Switzerland) was utilized to establish the working length. A radiograph was taken to ensure that the working length was adequate.

Race rotary files FKG (FKG Dentaire Sàrl, Le Crêt-du-Locle, Switzerland) were used to instrument the root canals to a size of 50/.04 according to the manufacturer’s instructions. Before the insertion of each file, A 30-gauge side-vented needle (NaviTip; Ultradent South Jordan, UT, US) was adjusted to be 1mm shorter than the working length and used to irrigate the canals with 1 mL 5.25% NaOCl (FIPCO, New Borg Elarab city, Egypt). The specimens were then finally irrigated with NaOCl and activated using Eighteeth ultra-x ultrasonic activator device for 30 seconds.

Sample obturation: Root canal filling was accomplished using the single cone technique. A radiograph was taken to guarantee appropriate master cone, the canals were then properly dried with paper points (Meta Biomed, Chungcheongbukdo, Republic of Kore).

CeraSeal Bioceramic Sealer (Meta Biomed, Chungcheongbukdo, Republic of Korea) was modified with 0.1% wt. Rhodamine B dye. The gutta-percha cone was then introduced into the canal to the working length which was then cut at the orifice level by a heated condenser before vertical light packing. After filling the canal, the canal entrance was sealed[1] and the roots were stored in an incubator for 7 days.

Sample sectioning: The roots were mounted inchemically cured acrylic resin and then cross-sectioned perpendicular to the root long axis with IsoMet 4000 micro saw (Isomet, Buehler, USA) mounting diamond disc 0.6 mm thick at 2500 rpm, and 10 mm/min feeding rate under cooling with water. An approximately 2.0 mm thick segment was taken from each third of the root to obtain three 2.0 mm thick specimens from each tooth. The specimens were divided into coronal, middle, and apical sections.

Confocal laser scanning microscopy (CLSM) analysis: After polishing, the specimens were mounted on glass slides. A CLSM (LSM 800 with Airyscan; Carl Zeiss Microscopy GmbH, Jena, Germany) with a 10x magnifying lens was used to inspect the samples. The resulting images were analyzed using VideoTest Morphology® software (iMicroTec, St.-Petersburg, 190000 Russia).

The total area with sealer penetration was delineated and measured by subtracting the root canal space from the entire area of the sealer penetration in square micrometers (μm^2)[10]. The percentage of sealer penetration (%) was calculated by the division of the area of sealer penetration by the circumference of the root canal wall.[10]
Statistical analysis and data interpretation: Data were entered into the computer and processed using IBM SPSS (IBM SPSS Statistics for Windows, Version 22.0, IBM Corp., Armonk, NY.). To describe qualitative data, numbers and percentages were employed. After determining normality using the Shapiro-Wilk test, quantitative data for parametric data were presented using mean and standard deviation. The significance of the acquired findings was determined at the 0.05 level. The ANOVA test was employed to compare more than two independent groups, whereas the Post Hoc Tukey's test was employed to find pair-wise comparisons.

Results:

The mean and standard deviation (SD) values of the percentage of the sealer penetration area in the three sections are shown in Table and Figure.

For the percentage area: ANOVA and Tukey’s test displayed a statistically significant difference among the examined sections ($P < 0.001$), Table. Coronal sections obtained the greatest percentage area of35.19% while, apical sections revealed the least percentage area of 24.94%, Table.

Discussion:

The current in vitro study aimed to evaluate the impact of various anatomical structures of dentin along the root canal system on Bioceramic sealer penetration. In this study, Meta Biomed CeraSeal Bioceramic sealer was used with a single cone technique as radicular dentine weakening and damage

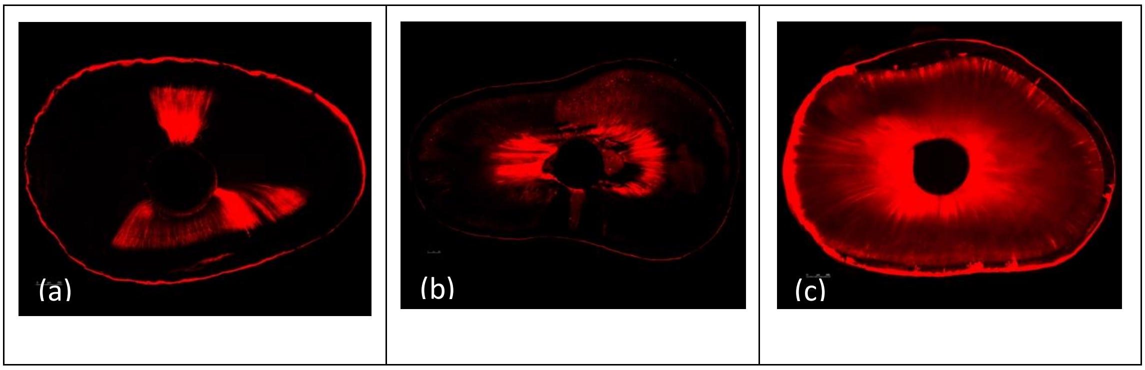

Figure: Shows the sealer penetration depth in (a) apical, (b) middle, and (c) coronal sections.

Table: Comparison of mean area percentage between different surfaces

Group no.	Group 1	Group 2	Group 3	Test of significance
Area percentage	35.19±0.68^{A}	34.03±1.82^{B}	24.94±0.37^{C}	F=362.46 P<0.001*

Dissimilar superscripted letters denote significant differences between groups within the same row, parameters described as mean ±SD.

were observed in vitro in both cold lateral compaction and warm vertical compaction. So, this technique is a less destructive option.[1]

NaOCI irrigation was paired with ultrasonic activation to remove the smear layer without the use of chelating agents to avoid any interaction with the used sealer. The CLSM has been utilized to reveal root canal sealers within dentinal tubules as it permits the identification of sealer penetration throughout the canal circumference of each sample using fluorescence.[11] Rhodamine B has been used as an indicator under CLSM because it allows for the identification of sealers within the dentinal tubules[12]and does not affect the physical properties of the sealers, as long as a small amount of dye (less than 0.2%) is mixed with the sealers.[13]

In the current study, for the three groups, the mean of the penetration area was measured and found to be higher at the coronal level (35.19%) compared to the middle level (34.03%), and both of these results were higher compared to the apical level (24.94%). This indicates that the use of ultrasonic helped in the removal of the smear layer in both the coronal and the middle thirds of the root canal system which comes in agreement with results from Ahmad et al.[14] who found that ultrasonic instrumentation with 1 percent NaOCl efficiently eliminated the debris and smear layer.Although Generali et al.[15] found no difference in sealer penetration at different levels, most likely because a chelating agent was used,[10] the results of this study were found in agreement with results from McMichael et al.[16] and El Hachem R[17], who found

that the BC Sealer penetration depth was significantly higher at the coronal and middle levels compared to the apical ones.

One explanation may be that the number and diameter of dentinal tubules decrease apically in the root canal. Furthermore, some areas in the apical third are dentinal tubule free, and tissue-like cementum can contour the apical root canal wall, obstructing tubules.[18] This irregular structure in the apical area complicates root canal treatment and may explain the lower penetration depths in the apical region.[3] Another explanation may be the better removal of the smear layer in the coronal region than or more difficult irrigant access in the apical one.[17]

This study did not examine the interface between the gutta-percha and dentin wall. Indeed, the incidence of voids in root canal filling material can result in the proliferation of residual microorganisms and may jeopardize the treatment outcome. Further studies are necessary to analyze the interfacial adaptation of these sealers to root canal walls.

Conclusions:

Penetration is affected by root dentine as bioceramic sealer shows inferior penetration at smaller distances from the apex.

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