Confocal Laser Scanning Microscopy Evaluation of The Effect Of Lentulo Spiral And Ultrasonic Activation on Intra-Tubular Sealer Penetration

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Abstract:

Objective: To evaluate the effect of lentulospiral and ultrasonic activation on the intra-tubular AH plus sealer penetration using confocal laser scanning microscopy. **Materials and Methods:** Twenty extracted single-rooted teeth were collected; teeth were prepared up to ProTaper finishing file F4 (size 40). Teeth were randomly divided into two main groups (n=10 teeth) according to activation techniques. The AH plus sealer with rhodamine B was activated by lentulospiral in group 1 and Irrisafe ultrasonic tip in group 2. The root canals were obturated with a single gutta-percha cone (size F4). The roots were sectioned at 3, 6, and 9 mm levels from the apex and examined with confocal laser scanning microscopy. Statistical analyses were performed at a 5% significance level. **Results:** At the cervical third, the lentulospiral group had significantly higher means of maximum depth (828.2±274.1 μm) and percentage (49.72±8.96 %) and area (2774.9±908.6 μm^2) of sealer penetration ($P<0.05$), while the Irrisafe group had significantly higher means of maximum depth (486.9±269.9 μm) and percentage (24.34±13.21 %) sealer penetration at the apical third ($P<0.05$). There was a positive correlation between the maximum depth (μm) and the area (μm2) of sealer penetration ($P < 0.01$ and confidence of 99 %). **Conclusions:** Regarding sealer activation, lentulospiral performed better in the cervical region while activated Irrisafe ultrasonic tip performed better in the apical region.

Introduction:

The main goal of endodontic therapy is to prevent inflammation in the peri-apical area by decreasing the microbial load inside the root canal system. Therefore, following cleaning and shaping procedures, three-dimensional filling of the root canal system and its ramifications is essential to prevent microbes' migration and proliferation in the root canal and surrounding periodontium.[1]Gutta-percha, one of the most commonly used root canal filling materials, is compactable, inert, radiopaque, tissue tolerant, dimensionally stable, can be softened, and can be easily removed from the canal when necessary. However, it lacks adhesion to root canal walls. Consequently, root canal sealers are necessary for the filling of root canals, apical deltas, ramifications, and dentinal tubules. Penetration of sealers into the dentinal tubules is considered to be a key factor associated with the outcome of endodontic treatment.[2,3]Sealers should provide dimensional stability, good adaptation, and penetration into the dentinal tubules.[4,5] This will help in reducing the incidence of microleakage formation and enhance the contact area between dentin and root fillings, which will increase the sealing capacity of the whole root canal system, also may prevent bacteria from penetrating the dentinal tubules and provide an anti-microbial effect when become in close contact with microbes. Moreover, sealer penetration helps in increasing fracture resistance of root canals.[6-9]To improve the quality of obturation, sealers are mandatory to be used. Sealer placement into the root canal system should be predictable and provide complete coverage to the dentinal walls. Its placement inside the root canal can be done by many methods including the use of gutta-percha, endodontic files or reamers, paper points, and lentulospiral and ultra-sonic powered tips.[10,11]

Ultrasonic activation (UA) of sealer inside the root canal has been showing promising results, as it improves sealer penetration to areas of anatomical complexities, more deeply into the dentinal tubules with less gap formation. The activation occurs by using ultrasonic tips connected to handpieces with high frequencies of vibration enhancing acoustic transmission and cavitation.[12] Assessment of inter-facial adaptation and dentinal tubule penetration depth of root canal sealers can be done by several microscopy techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), Stereomicroscopy, and confocal laser scanning microscopy (CLSM). Compared to SEM, confocal laser scanning microscopy provides more detailed information about the intratubular sealer penetration by mixing fluorescent Rhoda mine dye with the sealers.[13]Therefore, the purpose of this study is to evaluate the effect of lentulospiral and ultrasonic activation on the intratubular AH plus sealer penetration using confocal laser scanning microscopy.

Materials and methods:

The research protocol was submitted to the Institutional ethics committee at the Faculty of Dentistry-Mansoura University and accepted with approval record

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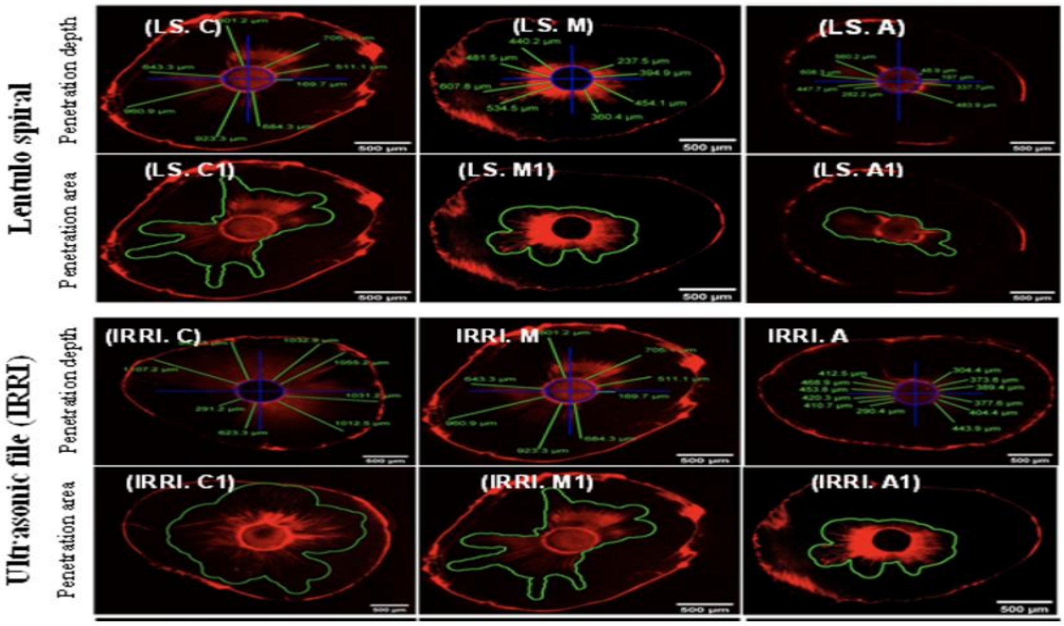

Figure: Confocal laser scanning microscopy (CLSM) images for AH plus sealer penetration in the groups: Ultrasonic (IRRI), and Lentulo spiral (LS). Maximum penetration depth in the coronal, middle, and apical thirds is denoted by the symbols C, M, and A, respectively. The penetration area in the coronal, middle, and apical thirds is denoted by the symbols C1, M1, and A1, respectively.

(M06150620). Twenty freshly extracted single-rooted teeth (with intact roots) were collected from Thedepartment of oral surgery. The teeth were disinfected in 5.25% sodium hypochlorite (NaOCl) for one hour and then rinsed under tap water. Roots were cleaned of tissue with an ultrasonic scaler to remove calculus and remaining soft tissue. The teeth were examined radiographically using a digital periapical radiograph and clinically examined using a dental operating microscope (Leica, M320 Germany) at 25X magnification. Teeth with previous endodontic treatment, severe curvature, or calcified canals were excluded.

The teeth were decoronated by using a diamond disk under constant irrigation. The roots were standardized to 13-mm length. A size 10 K-File (Dentsply Sirona, Ballaigues, Switzerland) was inserted until being visible at the apical foramen and the working length determined by reducing 1mm from root length, to be 12mm. A smooth reproducible glide path was confirmed with a size 15 stainless steel K-file. The root canals were enlarged to an apical size of 40 using ProTaper Universal instruments (Dentsply Sirona, Ballaigues, Switzerland) in agreement with the manufacturer's recommendations. After each instrument, a size 10 K-file was used to ensure patency and the canals were irrigated with 3 ml of 2.5 % NaOCl solution delivered using a plastic syringe with a 30-gauge side-vented needle. The irrigating solution has been activated using EDDY Sonic-powered tips for 30 seconds. The smear layer was removed with 17% ethylene diamine tetra acetic acid-based solution (EDTA) activated with EDDY Sonic powered tips then the canals were finally rinsed with distilled water. After root canal preparation, the canals were dried with ProTaper sterile paper points (Dentsply Sirona, Ballaigues, Switzerland). To allow analysis under confocal laser scanning microscopy, the sealers were mixed with rhodamine B (Sigma-Aldrich, St. Louis, MO) for fluorescence, and the mixing ratio of the sealer and rhodamine B was 100:1 by weight. Gutta-percha cone size F4 (ProTaper Universal; Dentsply Sirona) was inserted in the root canal to the working length. A radiograph was taken to make sure that the cone reached the working length. The sealer was placed inside the canal with a size 40 gutta-percha cone. The teeth were randomly divided into two groups (n=10 teeth) according to sealer activation techniques as follows:

Group 1: A #25 Lentulo spiral (Dentsply Sirona, Ballaigues, Switzerland) was used at 300 rpm in small pecking movements inside the canal for at least 5 seconds reaching the working length.
Group 2: A 25 Irrisafe ultrasonic tip (Satelec, Acteon, France) mounted on a P5 ultrasonic device marked at power 5. The ultrasonic tip was manipulated by using in-and-out motions up to the most apical extent where it could vibrate freely for about 5 seconds. The canal was obturated with a single gutta-percha cone size F4 (Dentsply Sirona). The quality of obturation was evaluated by radiographic imaging. The obturation was considered satisfactory when the filling was seen adapted to the canal walls with no incidence of voids after obturation, specimens were stored in an incubator at 37°C and 98% humidity for 7 days to allow the sealer to set.
Confocal laser scanning microscopy assessment: The teeth were sectioned at 3-, 6- and 9-mm levels from the apex. Specimens of each experimental group were prepared for examination using CLSM (Leica, DMI8, Germany) at wavelengths of 540 nm and 590 nm, via a 10X lens. The specimens were mounted on a glass slap

using cyanoacrylate glue and they were coded for identification. Images obtained from CLSM were analyzed using Image software (version 1.53a National Institutes of Health, USA). The maximum depth of penetration was measured by measuring the deepest penetration from the canal wall to the point of maximum sealer penetration with the measuring tool in Image J. The root canal wall circumference was outlined and measured by using the measuring tool available in the software. The maximum depth of penetration was divided by the total canal circumference to calculate the Penetration Percentage (%) of the sealer.[13-16] The sealer penetration area was calculated by subtracting the amount of root canal space from the total area of the sealer penetration.[16,17]

Statistical analysis: Statistical evaluation was performed using the statistical software SPSS (version 23, IBM Co. USA). The Shapiro–Wilk test was used for testing the normality of data. The data were normally distributed. Student t-test (for intergroup comparison), one-way ANOVA, and post hoc Tukey test (for intra-group comparison between the different levels) in addition to the Pearson Correlation coefficient test were used for statistical analyses of the data. The significance level was set at 5%.

Results:

Maximum depth, percentage, and area of sealer penetration in different regions are shown in Figure and, Table. A positive correlation was found between maximum depth of sealer penetration (µm results and the sealer penetration area (µm2) results (Pearson's correlation value > 0.75), and this correlation is statistically significant (P < 0.01& confidence 99 %). Also, there was a positive correlation between penetration percentage (%) results and the sealer penetration area (µm2) results (Pearson's correlation value > 0.75), and this correlation is statistically significant (P < 0.01& confidence 99 %).

Table: Mean±standard deviation of maximum depth, percentage and area of sealer penetration in different thirds

	Group	Cervical third	Middle third	Apical third	P- value
Maximum depth of sealer penetration (µm)	Lentulo spiral	828.2±274.1 $^{A a}$	350.3±147.2 $^{A b}$	52.8±19.1 $^{A c}$	< 0.0001*
	Irrisafe	498.8±127.7 $^{B a}$	564.2±129.7 $^{A a}$	486.9±269.9 $^{B a}$	0.322
P- value		< 0.0001*	0.078	< 0.0001*	
Penetration Percentage of sealer(%)	Lentulo spiral	49.72±8.96 $^{A a}$	26.30±12.02 $^{A b}$	13.99±8.47 $^{A b}$	0.015 *
	Irrisafe	32.53±10.30 $^{B a}$	37.24±9.59 $^{A a}$	24.34±13.21 $^{B a}$	0.093
P- value		0.027 *	0.146	0.011 *	
Penetration area of the sealer (μm^2)	Lentulo spiral	2774.9±908.6 $^{A a}$	542.2±120.3 $^{A b}$	536.2±146.6 $^{A b}$	< 0.0001*
	Irrisafe	1264.5±562.4 $^{B a}$	1703.2±407.5 $^{B a}$	743.6±317.4 $^{A b}$	0.001*
P- value		< 0.0001*	< 0.0001*	0.205	

Different superscript uppercase letters indicate significance in the same column (p < 0.05) and different superscript lowercase letters indicate significance in the same row (p < 0.05).

Discussion:

Root canal 3-D obturation is the final objective of orthograde root canal treatment. Gutta-percha is the most used core-filling material. However, it lacks adhesion to canal walls. The root canal sealer is necessary to improve the seal during compaction and to penetrate canal irregularities and normally inaccessible areas such as dentinal tubules. The intra-tubular sealer penetration is regarded as an advantageous outcome because it will increase the interface between dentin and material, therefore, enhancing the seal, and retention of the material may be increased by mechanical interlocking. Moreover, Intra-tubular sealers may entomb any residual bacteria within the dentinal tubules and the sealer chemical components could provide an antibacterial effect. Consequently, it is crucial for the degree of intra-tubule sealer penetration and the percentage of the dentin/sealer interface covered by the sealer to be as large as possible in all cases.[1-4]Different observational approaches can be used to examine the sealers' interaction with the dentine substrate. Scanning electron microscopy (SEM) can be used to evaluate the intratubular sealer penetration, adhesive interface integrity, and topography of samples.[18,19]

However, there are a few important aspects to consider. Dehydration, demineralization, polishing, observation under a high vacuum, and other methods that are used to prepare samples for SEM analysis may cause artifacts, leading to artificial gaps, which inhibit the correct study of samples.[20,21] As a result, analyzing samples using a method that does not require pre-preparation would be an alternative to avoid the previous problem.[20] The CLSM was used to investigate the root canal filling tomographic features and the adhesive interface because it permits measuring samples in-depth, even in a wet environment.[13,15] To study the root canal with CLSM, the sealer must be marked with a fluorescent dye such as Rhodamine B. The addition of Rhodamine B with concentrations lower than 0.1 % sealers enables the marked structure to be excited by certain wavelengths and become into a visible spectrum, but any concentration greater than 0.1% will lead to excessive fluorescence that makes the specimens difficult to visualize.[18,22] Some previous studies have concluded that both assessing methods, CLSM and SEM, provide high-quality images of the examined structures.[15,23] Nevertheless, the best approach for evaluating the intra-tubular sealer penetration, is still a controversial point among researchers.[18-20] Some studies still favor SEM for the interface analysis, while others have reported the advantages of CLSM.[18,19,21,24]The present study aimed to evaluate the effect of lentulospiral and ultrasonic activation on the intra-tubular penetration of the AH plus sealer by using confocal laser scanning microscopy. The irrigation protocol using 17% EDTA as a final rinse was to remove the smear layer and leave exposed collagen of the dentin to interact with the epoxy resin of sealers increasing the effectiveness of resin-based sealers.[9,25] The epoxy resin forms sealer tags thus bonding to the root canal walls.[26]In the current study, the lentulo spiral produced significantly greater intra-tubular sealer penetration than the Irrisafe ultrasonic tip in the cervical third. This may be attributed to the more efficient rotary motion of the lentulo spiral in a relatively wider cervical region where the smear layer is more effectively removed. Dash et al.[27] reported that Lentulo spiral technique had a mean penetration depth and percentage higher than the ultrasonic technique because the lentulo spiral forces the sealer along the instrument length, whereas ultrasonic files move the sealer centrifugally.The intra-tubular sealer penetration findings showed that the activated Irrisafe ultrasonic tip performed better than lentulo spiral in the apical region in agreement with previous studies.[24,28] These findings might result from the transmission of acoustic microstreaming energy generated by the ultrasonic tip, which probably forced the sealer into these areas. These effects frequently occur in the irrigants during passive ultrasonic irrigation. On the other hand, these findings are in disagreement with Dash et al. [27] who used ultrasonic tip at lower power than that in the current study.

Conclusions:

Under the conditions of the current study, regarding intra-tubular sealer penetration, lentulospiral performed better in the cervical region while activated Irrisafe ultrasonic tip performed better in the apical region

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