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Depth of Penetration of Sealer into Radicular Dentinal Tubules using Ultrasonic Activation: An in vitro Confocal Laser Scanning Microscopic Evaluation

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Running Title: Penetration of sealer into radicular dentinal tubules

Abstract: ***Aim: Comparative evaluation of sealer penetration into dentin by ultrasonic sealer activation modality using confocal laser scanning microscope. Objectives:To evaluate the depth of sealer penetration into dentinal tubules by ultrasonic activation modality. Methodology: Fouty freshly extracted human permanent single rooted premolars extracted for orthodontic reasons with mature apex were used. The teeth were decoronated, working length established and prepared uptoprotaper F5 rotary file. Ah-Plus sealer ws mixed with rhodamine B dye and 0.5ml of sealer was placed in the canals by tuberculin syringe. Sealer was activated using proultra ultrasonic file, canals were dried with paper points and obturation was done using lateral condensation technique. Roots were sectioned using hard tissue microtome. These sections were examined under confocal laser scanning microscope. Results: Statistical analyses of the data were performed using ANOVA and Tukey Multiple comparison test. Statistically significant difference was found among all the three zones (p=0.0001). Sealer penetration was maximum in coronal zone followed by middle followed by apical. Conclusion: Ultrasonic activation favoured the depth and penetration of sealer in the dentinal tubules at all the zones.***

Keywords: Ah- Plus sealer penetration, Ultrasonic activation, Apical third

1. Introduction

Complete obliteration of root canal system with development of a fluid tight seal is one of the foremost important factor for a successful endodontic treatment.[1]Common failure of the root canal obturation process mainly occurs because of gaps and porosities which are usually present between the sealer and dentinal tubules.[2]

The penetration of sealer cements into dentinal tubules will increase the interface between material and dentin thus improving the sealing ability. It will also improve the retention of the material by mechanical locking. Sealer cements inside the dentinal tubules may also entomb any residual bacteria within the tubule extension and the chemical components of sealer cements may exert an antibacterial effect that will be enhanced by closer approximation to the bacteria. [3]

Complete coverage of dentinal walls should take place after the placement and activation of the sealer within the entire root canal system.[4]

The analysis of the dentin/sealer interface allows the determination of which materials and filling techniques could obturate the root canals with fluid tight seal without any gaps and voids.

Several microscopy techniques are currently used to evaluate the sealer/dentin interface, including stereomicroscopy, scanning electron microscopy (SEM), transmission electron microscopy, and confocal laser scanning microscopy (CLSM). Confocal lasers scanning microscopy provides detailed information about the presence and allows distribution of sealers or dental adhesives inside dentinal tubules in the entire circumference of the root canal walls at relative low magnification as 100X through the use of fluorescent rhodamine marked sealers.[3]

Hence, the purpose of this study was to evaluate sealer penetration into dentinal tubules at coronal, middle and apical zone through ultrasonic sealer activation method using confocal laser scanning microscope microscope.

2. Materials and Methods

Specimen selection

Freshly extracted human permanent single rooted premolars extracted for orthodontic reasons with mature apex were collected. Fourty teeth free of cracks, caries, resorption, calcification, previous endodontic treatment, and less than 10° root curvature were selected and stored in distilled water until use.

Shaping and cleaning of root canal system

For standardization of the root length (14 mm), the crowns were resected with a diamond disc in a slow speed straight handpiece under constant water cooling. The working length was determined by inserting # 15 K-file (Dentsply Maillefer) into the canal until it was just seen at the apical foramen and then 0.5 mm was subtracted from this length. The roots were instrumented by using the ProTaper Universal root canal files (Dentsply Maillefer) in a sequential manner from S1 till F5. Canals were irrigated between files with 2 mL of 3%

NaOCl (vishal's Chemicals limited, India). To eliminate the smear layer 2 mL of 17% ethylenediaminetetra acetic acid (EDTA) (pH 7.7) for 3 min was used, followed by a final rinse of 2 mL distilled water. Each root canal was dried with absorbent points.

Sealer preparation and activation

AH plus sealer was mixed according to the manufacturer instructions and to allow analysis under the CLSM, each sealer was labelled with Rhodamine B (Mayor Diagnostics, Mumbai, India) to an approximate concentration of 0.1%(by weight). A 1-mL tuberculin syringe was used to dispense 0.05 mL inside each canal. No additional sealer was used.The ultrasonic unit (woodpecker) was used with a Pro-ultra tip (DentslyMaillefer, USA). The ultrasonic file was placed into the canal 3mm short of the apex and activated for 30 seconds.

Then a standardized protaper gutta-percha cone was placed to length, laterally condensed with finger spreader, and accessory cones were added as needed. Extruded sealer from apical foramen, if present, was wiped off with moist cotton. Three millimeter of gutta-percha and sealer was removed with a heated plugger from the coronal end of root canal and Cavit was placed. All The teeth were stored in an incubator at 37ºC at 100 % relative humidity, and the filling material was allowed to set for 48 hours.

Sectioning of samples: Horizontal sections were done at the 2, 4 and 6 mm levels from the apical foramen using a safe sided diamond disc. Each segment was standardized to a size of 2mm by reducing coronally and apically by using a series of silicon carbide disks (3M ESPE, Seefeld, Germany).

The segments of the root canal in which sealer penetrated into dentinal tubules were analyzed under Confocal laser scanning microscope (ZEISS, LSM 510 Meta Germany) to evaluate the depth of penetration of sealer. The measurement was done using digital measuring ruler present in LSM image browser software (LAS-AF, Leica)

Data was collected and samples were subjected to statistical analysis.

Observation and results (Table 1 & Graph 1)

Mean depth of sealer penetration at coronal zone was 1050.80±64.78, at middle zone it was 806.80±123.90, and at apical zone it was 226.40±105.55. By using one way ANOVA statistically significant variation was found in mean sealer penetration of three zones(F=180.16,p-value=0.0001).

By using Tukey Multiple comparison test statistically significant difference was found among coronal and apical zone (p=0.0001), coronal and middle zone(p=0.0001) and middle and apical zone(p=0.0001). Sealer penetration was maximum in coronal zone followed by middle followed by apical.

3. Discussion

The success of root canal treatment depends greatly on the preparation of the canal, and the removal of complete pulp tissue. [5]The importance of thorough cleaning & impervious filling of apical part of the canal for successful healing of the periapex was highlighted analogically as early as 1939, by Kronfeld. Thus, a fluid tight seal should be an unavoidable objective to be achieved at sealer and dentin interface.

In the present study, the depth and penetration of epoxy-resin based sealer activated by ultrasonics was evaluated using Confocal laser scanning microscopy.

Passive ultrasonic irrigation was first described by Weller *et al*. **(1980)**[6] It relies on the transmission of acoustic energy from an oscillating file or smooth wire by means of ultrasonic waves and can induce acoustic streaming and cavitation of the irrigant. The cleaning efficacy of passive ultrasonic irrigation implies the effective removal of dentin debris, microorganisms (planktonic or in biofilm), and organic tissue from root canal.[6]

The morphology of apical third varies tremendously, from the early work of Hess &Zurcher (1925) to the most recent studies demonstrating anatomic complexities like multiple apical foramina, fins, delta, loops, furcation accessory canal, & more, it has been established that the root with graceful, tapering canal & a single apical foramen is an exception rather than a rule (Hess W,1925) [7].

Most of the accessory lateral canals and apical ramifications are present in the apical 3mm of the root canal. Thus, in the present study depth of penetration of sealer in the apical area was taken in account.

In the present study, mean depth of sealer penetration at coronal zone was 1050.80±64.78, at middle zone it was 806.80±123.90, and at apical zone it was 226.40±105.55. Statistically significant difference was found among coronal and apical zone (p=0.0001), coronal and middle zone (p=0.0001) and middle and apical zone(p=0.0001).

Sealer penetration was maximum in coronal zone followed by middle followed by apical.

The results of the present study are in agreement with **Saman R. Gharib, Patricia A. Tordik,, Glen M. Imamura, Thomas A. Baginski, and Gary G. Goodell, in 2007** [8] suggested a greater percentage of sealer-dye penetration and greater depth of sealer-dye penetration in the coronal and middle thirds compared with the apical third of the root. **Saurabh S. Chandra, Padmanabhan Shankar, and Rajamani Indira in 2012**[9] showed that the maximum penetration of the 4 resin sealers was seen in the coronal third, followed by the middle third and least in the apical third.

The apical dentin has been shown to exhibit sclerosis of dentin, which may prevent deeper penetration of irrigating solutions and root canal sealers. The dentinal tubules are tapered structures measuring approximately 2.5 mm in diameter near the pulp, 1.2 mm in the mid-portion of the

dentin, and 900 nm near the dentino-enamel junction (DEJ). They range in size from approximately 1–3 mm in diameter. Major branches occur in the root dentin than in the coronal dentin.

Though the amount of sealer penetration in the coronal and middle third was maximum, ultrasonic activation favoured the penetration of sealer in the apical third as well. **Duarte MA, Balan NV, Zeferino MA in 2012** suggested that ultrasonic activation favored a higher pH level and calcium release describing that ultrasonic activation could promote a greater tubular penetration of the calcium hydroxide pastes.[4]

Guimaraes BM *et al*. 2014stated that the use of ultrasonic activation of an epoxy resin-based sealer promoted greater dentinal sealer penetration and less presence of gaps. This may be explained by **t**he oscillating files in ultrasonics transmits the acoustic microstreaming energy and cause a greater depth of dentinal sealer penetration and coverage of root canal walls in the same manner as it promotes the penetration of irrigants in an area of anatomic complexities and the dentinal tubules.[4]

Confocal microscopy offers several advantages like the ability to control depth of field, elimination or reduction of background information away from the focal plane (which leads to image degradation), and the capability to collect serial optical sections even from thick specimens. **Picoh et al** (1991) [10] reported that when using CLSM, artifacts could practically be excluded.

4. Conclusion

Under the parameters of present study, it can be concluded that epoxy resin sealer exhibited better percentage and depth of penetration in the radicular dentinal tubules. Ultrasonic activation of pseudoplastic sealers can significantly increase its percentage and depth of penetration and level of root canal can affect both, percentage, and depth of penetration of sealers in the dentinal tubules.

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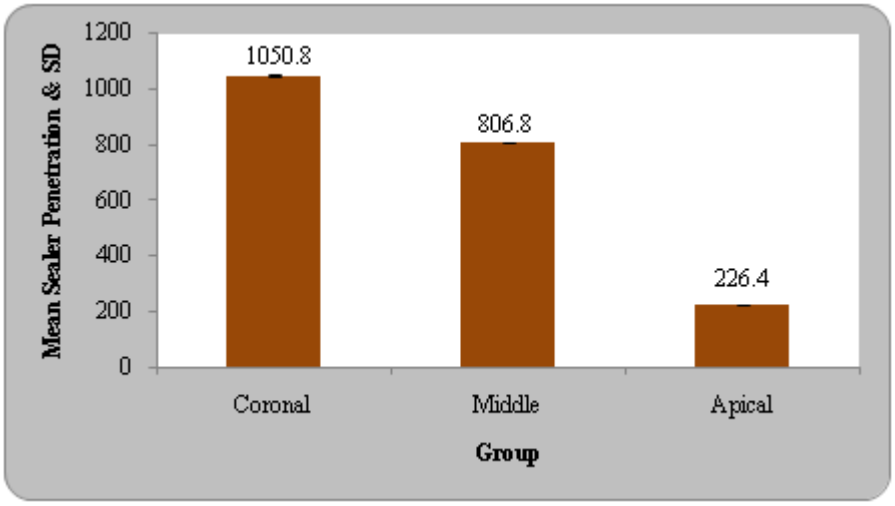
Table 1: Multiple Comparison: Tukey Test
Comparison of depth and percentage of sealer penetration at coronal (6mm), middle (4mm) and apical (2mm) levels

roup		Mean Difference (I-J)	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Coronal	Middle	244.00	44.61	0.0001 S,p<0.05	133.37	354.63
	Apical	824.40	44.61	0.0001 S,p<0.05	713.77	935.03
Middle	Apical	580.40	44.61	0.0001 S,p<0.05	469.77	691.03



Graph 1: Comparison of depth of sealer penetration into dentinal tubules by ultrasonic modality at three different zones (µm)