Original Article

Dentinal tubule penetration of a silicone-based endodontic sealer following N-acetyl cysteine intracanal medicament removal using ultrasonic agitation and laser activated irrigation – An *in vitro* study

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Abstract

Context: The removal of intracanal medicament is essential for sealer penetration and the success of endodontic therapy.

Aims: To evaluate and compare the dentinal tubule penetration of a silicone-based endodontic sealer following N-acetyl cysteine (NAC) intracanal medicament removal using ultrasonic agitation and laser-activated irrigation.

Materials and Methods: Eighty-one extracted single-rooted mandibular premolars were decoronated and prepared with ProTaper Universal rotary files up to MAF F3. To prepare medicament, NAC powder was mixed with propylene glycol in the ratio of 1:1, placed using a size #30 Lentulospiral, and specimens stored in an incubator for 14 days. The specimens were then instrumented with #30 Hedström and divided into three groups according to final irrigant activation techniques: Group I: Diode laser activation, Group II: Passive Ultrasonic agitation, Group III: No agitation (positive control). Canals were obturated with GuttaFlow bioseal sealer mixed with 0.1% Rhodamine B dye and gutta-percha cones and incubated for 7 days. The specimens were sectioned horizontally to obtain 1 mm thick sections from 2, 5, and 8 mm from the apex. Sections were examined under Confocal Laser Scanning Microscope to measure the depth of sealer penetration (in μm).

Statistical Analysis: One-way analysis of variance and Tukeys multiple *post hoc* test.

Results: The highest mean depth of penetration of 728.52 μm was seen with Group I, followed by Group II and least was seen in Group III.

Conclusions: Diode laser activation group was most effective in the removal of NAC intracanal medicament from all the three regions of the root canal.

Keywords: Confocal laser scanning microscope; conventional syringe needle irrigation; diode laser activation; guttaFlow bioseal; N-acetyl cysteine; passive ultrasonic agitation

INTRODUCTION

The efficacy of intracanal medicaments to disrupt biofilms and eliminate residual bacterial infections is extensively studied for effective chemo-mechanical preparation, a prerequisite for successful endodontic treatment.[1]

Different materials such as calcium hydroxide, chlorhexidine, steroids, triple, and double antibiotic

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pastes are effective intracanal medicaments but presented several disadvantages. Numerous natural extracts such as papain, *Aloe vera*, *Morinda citrifolia*, and turmeric have also been studied for their antimicrobial activity, but there is insufficient evidence on their efficacy.[2] N-acetyl cysteine (NAC), a thiol-containing anti-oxidant and mucolytic drug, breaks down di-sulfide bonds in mucous and lowers the viscosity of secretion.[3] NAC has demonstrated bactericidal activity against *Enterococcus faecalis*, present in 22%–77% of endodontic failed cases and is newly studied intracanal medicament.

The use of bioactive sealers, like calcium silicate-based sealers, has grown in popularity. Its greater dentinal tubule penetration can be explained by its hydrophilicity, low contact angle, and small particle size. A novel endodontic sealer, GuttaFlow bioseal, is a cold-filling sealer containing calcium silicate and gutta-percha.[4]

Removal of intracanal medicament from radicular dentin is crucial for improving sealer adaptation. Hence, the most effective final irrigation technique can specify the cleanliness of canals and ensure maximum depth of penetration of sealer into dentinal tubules.[5] The passive ultrasonic irrigation was formerly regarded as the gold standard and believed to be more efficient than self-adjusting file, syringe needle irrigation, EndoActivator, and CanalBrush in medicament removal.[6]

The 940-nm and 980-nm wavelength diode laser has also demonstrated encouraging outcomes in terms of smear layer reduction and root canal disinfection.[7]

This study investigated dentinal tubule penetration of GuttaFlow bioseal following NAC intracanal medicament removal using high-power diode laser activation and passive ultrasonic agitation.

METHODOLOGY

Eighty-one extracted human mandibular premolar teeth were selected, decoronated and root length of 12 mm was achieved. Working length was determined where 15 K (Mani, Japan) file exits foramen, 1 mm less than root length.

Cleaning and shaping up to MAF size F3, Protaper Universal rotary system (Dentsply Maillefer, Switzerland) and irrigation with 2 ml of 3% Sodium Hydrochlorite (Vishal, Dentocare) following each change of instruments was done. Final rinse with 5 mL of 17% ethylenediaminetetraacetic acid (EDTA) (Ammdent Canalarge) followed by 2 ml of saline (Sodium Chloride Saline Injection IP) solution for 1 min was done, canals were dried using paper points (Diadent). For preparation of medicament, NAC powder (Molychem) was mixed with propylene glycol in the ratio of 1:1 (2 g/ml) and was placed in the canal using a size #30 Lentulospiral (Mani, Japan), orifice was sealed with Cavit (Meta Biomed Md Temp Restorative), and specimens were incubated in 100% humidity at 37°C for 14 days.

Following this, specimens were instrumented with #30 Hedström files (Dentsply) supplemented with 5 ml of 3% NaOCl (Vishal, Dentocare) to remove the medicament. Then, according to irrigant activation techniques, the specimens were divided into three groups for medicament removal:

Group I: Diode laser activation - 970 nm high-power diode laser (Sirona SIROLaser) with peak power 2 watts (CW), was used and a 200 μm diameter fiber tip was placed 1 mm short of apex and was activated in helical motion for 20 s (5 s per cycle).

Group II: Passive ultrasonic agitation - passive ultrasonic agitation using no. 25 ultrasonic tip (Satellac) was done for 30 s cycle for five times.

Group III: No agitation (positive control) - As a positive control, no agitation was done for this group.

Final rinse was done with 5 mL of 17% EDTA (Ammdent Canalarge) followed by 2 ml of saline solution (sodium chloride saline Injection IP) for 1 min, canals were dried using paper points. Single cone obturation technique, with F3 gutta-percha cone (Diadent Group International, Korea) in conjunction with GuttaFlow bioseal sealer mixed with 0.1% Rhodamine B dye was done, orifice was sealed with Cavit (Meta Biomed Md Temp Restorative), and specimens were incubated in 100% humidity at 37°C for 7 days.

Using a diamond disc, the specimens ($n = 27$) were sectioned horizontally at 2, 5, and 8 mm from the apex to create 1 mm thick sections. Sections were examined under a confocal laser scanning microscope and analyzed using Image J software for determining the depth of sealer penetration (measured in μm) into dentinal tubules [Figure 1].

Statistical analysis

The data were analyzed using IBM SPSS Statistics 20.0 (IBM SPSS Inc., Chicago, IL, USA). The normality of the data was evaluated using Shapiro–Wilk test and it was found to be normally distributed. One-way analysis of variance test was carried out to evaluate statistically significant differences between the three groups followed by Tukey's multiple *post hoc* test to evaluate the pairwise comparison. The significance level was set at $P \leq 0.05$.

RESULTS

As shown in Table 1, on intergroup comparison, the highest

penetration depth of 728.52 µm was seen with Group I - diode laser activation group followed by Group II - Passive ultrasonic agitation followed by Group III - no agitation (positive control) ($P \leq 0.0001$). On intragroup comparison showed highest penetration depth in the coronal third region (738.85) followed by middle third (625.84) and least was seen in the apical third (541.29) ($P \leq 0.0001$) [Figure 2].

Table 2 depicts statistically significant difference seen between all three groups and regions.

DISCUSSION

The use of intracanal medicament with antimicrobial and anti-biofilm properties is one of the most practised means to disinfect root canals.[8]

NAC, a powerful anti-oxidant, is regarded as a nonantibiotic substance with antimicrobial qualities that prevent different bacteria from forming biofilms.[9] The sulfhydryl group of NAC is considered to have the ability to break disulfide bonds involved in the generation of extra polysaccharides (EPS). Its antioxidant properties may indirectly affect the metabolism and EPS synthesis of the bacterial cells.

Quah *et al*. concluded that NAC demonstrated bactericidal activity against *E. faecalis*.[10] Choi *et al*. revealed NAC has greater biofilm disruption and antimicrobial efficacy than calcium hydroxide or chlorhexidine.[11] In the present study, NAC in powder form with propylene glycol as a vehicle was used.[12]

Along with the use of intracanal medicaments, its complete removal is also essential. Residual medicament may interfere with sealer penetration into dentinal tubules. Several irrigant activation techniques are studied for the effective removal of intracanal medicament. The efficacy of irrigant activation techniques in the removal of NAC was measured by maximum penetration of GuttaFlow bioseal sealer into dentinal tubules.

GuttaFlow bioseal, a silicon-based, bioceramic sealer has shown outstanding biocompatibility as it resembles hydroxyapatite. It comprises a blend of zirconium dioxide, microsilver, poly-dimethylsiloxane, gutta-percha powder (particle size <30 µm), and platinum catalyst.[13] Single-cone obturation technique was used owing to biomineralization properties of the sealer and due to uniformity achieved by this technique.[14] Despite superior properties of bioceramic sealer, prior to obturation, residual intracanal medicaments need to be completely removed as they might affect the quality of obturation increasing microleakage.

In the present study, we compared diode laser activation and passive ultrasonic irrigation in effectively removing NAC medicament. The results showed diode laser activation showed highest mean depth of penetration in coronal, middle, and apical thirds of the canal suggesting better removal of NAC in all the three regions of the root canal. These results are in accordance with a previous study by Marchesan *et al*., where 980 nm diode laser showed ultramorphological changes which ranged from smear layer removal to fusion of dentinal tubules.[15] Lasers can also

Table 1: Dentinal tubule penetration of sealer in the three groups and three regions of the root canal

Factor	Level of factor	*n*	Mean	SD	95% CI for mean		*F*	*P*
					Lower	Upper		
Groups	I - diode laser activation	81	728.52[a]	152.58	694.79	762.26	107.0705	0.0001*
	II - passive ultrasonic agitation	81	687.97[b]	108.23	664.03	711.90		
	III - no agitation (positive control)	81	489.49[c]	146.72	457.05	521.93		
Regions	Coronal	81	738.85[a]	140.92	707.69	770.01	64.2930	0.0001*
	Middle	81	625.84[b]	170.77	588.08	663.60		
	Apical	81	541.29[c]	144.00	509.45	573.13		

All values expressed as mean and SD. Different lower case letters (a, b, c) indicate a significant difference between the groups. The statistical test used: One-way ANOVA followed by Tukey's multiple *post hoc* test. The level of significance: * $P \leq 0.05$ is considered a statistically significant association. SD: Standard deviation, CI: Confidence interval

Table 2: Region-wise mean depth of penetration of the three irrigant activation techniques

Level of factor	*n*	Mean	SD	95% CI for mean		*P*
				Lower	Upper	
Diode laser activation with coronal	27	824.21[a]	149.75	764.97	883.45	0.0001*
Diode laser activation with middle	27	729.27[b]	109.57	685.93	772.62	
Diode laser activation with apical	27	632.09[c]	134.29	578.97	685.21	
Passive ultrasonic agitation with coronal	27	787.53[a]	69.44	760.06	815.00	
Passive ultrasonic agitation with middle	27	691.36[b]	72.07	662.85	719.87	
Passive ultrasonic agitation with apical	27	585.01[c]	68.64	557.86	612.17	
No agitation (positive control) with coronal	27	604.81[c]	71.89	576.37	633.25	
No agitation (positive control) with middle	27	456.89[d]	165.31	391.50	522.28	
No agitation (positive control) with apical	27	406.77[d]	108.25	363.95	449.59	

All values expressed as mean and SD. Different lower case letters (a, b, c, d) indicate a significant difference between the groups. The statistical test used: Tukey's multiple *post hoc* followed by one-way ANOVA test. The level of significance: * $P \leq 0.05$ is considered a statistically significant association. SD: Standard deviation, CI: Confidence interval

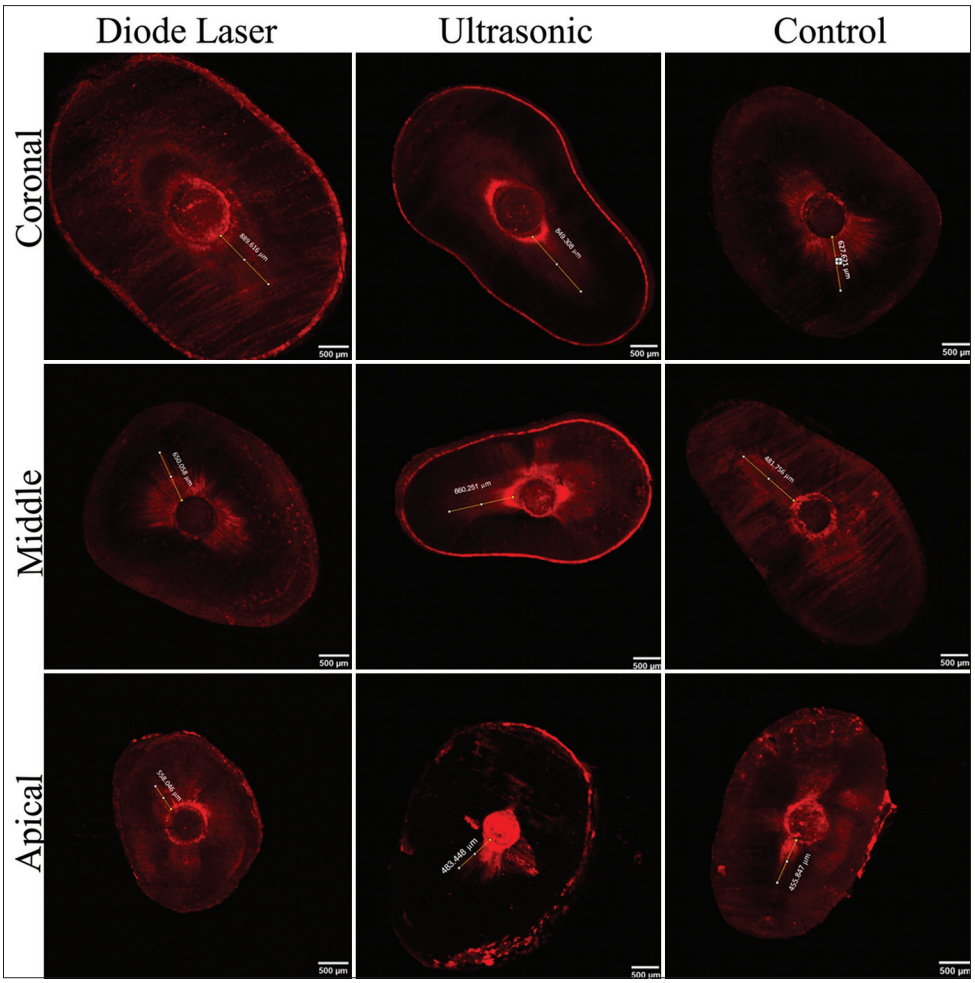

Figure 1: CLSM Images depicting penetration of GuttaFlow Bioseal sealer

eliminate highly resistant *E. faecalis* species in addition to cleaning and sterilizing the root canal dentin.[16] Saraswathi *et al*. concluded significantly better smear layer removal when 940 nm diode laser irradiation was combined with NaOCl and EDTA irrigation with minimum additional loss of mineral content.[17]

Laser energy or laser radiation leads to temporary cavitation in the irrigant through optical breakdown. It intensifies fluid exchange and thus the removal of debris by producing vapor bubbles with secondary cavitation effects. Owing to these properties, laser activation is clinically effective in removing biofilm, smear layer, and dentin debris from the root canal system.[16]

A flexible 200 µm fiber-optic tip with high power density at the tip contributes to better sealer penetration in the apical third.[18] Diode lasers have shown better dentinal tubules penetration at an output power range of 0.5 to 7 W.[17] Based on the previous literature and the present findings, laser activation is effective in removing NAC medicament at lower power settings, in lesser time with more advantages.

Another method studied in great detail is passive ultrasonic irrigation. In our study, passive ultrasonic agitation showed similar efficacy as diode laser activation in all the three regions of the canal. By cavitation and acoustic streaming, passive ultrasonic agitation could enhance cleaning in intricate canal anatomic locations. Two parts of flow pattern are produced, the steady part, acoustic streaming,[19] causes two jets of irrigating solution to flow outward continuously from the instrument along the direction of the oscillating tip. The rapid component, acoustic cavitation, causes pressure variations in the irrigant which causes bubbles to form and burst generating large shear stress on the surface of canals, improving cleaning.[20] In clinical use, ultrasonic agitation is undoubtedly the most common method for irrigant activation. Many studies have found 1 min of ultrasonic agitation resulted in significantly cleaner canals and isthmi.[16] LAI has recently gained popularity due to its increased awareness and efficacy.

In previous studies, by de Groot SD *et al*., laser-activated irrigation showed no significant difference when compared to passive ultrasonic irrigation, whereas in several studies, lasers have proven to be more effective.[21]

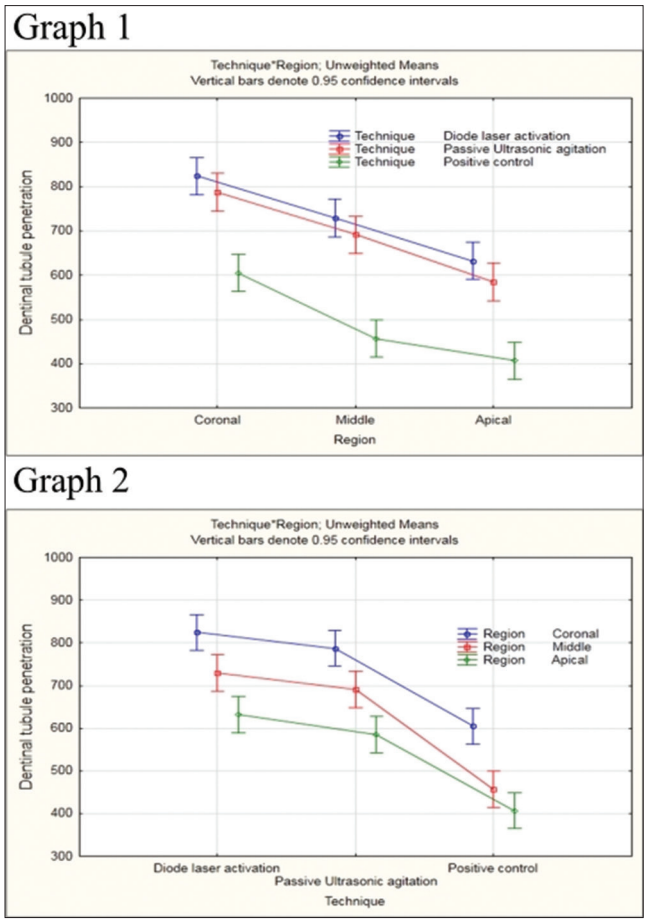

Figure 2: Comparison of sealer penetration among Graph 1- Group I, II and III and Graph 2- coronal, middle and apical third region

In agreement with existing literature, no agitation (Group III) showed least mean depth of penetration. Syringe irrigation has a weak flushing activity that depends on the diameter of the needle, its depth of placement, and structure of root canal system. To guarantee fluid exchange, irrigating needles should be positioned no more than 1 mm from working length.[22]

The results are in accordance to a systematic review on calcium hydroxide removal, which stated ultrasonic irrigation was superior to syringe needle irrigation and apical negative pressure irrigation.[23]

The study has few limitations, an increased power setting of diode laser more than 2 watt might significantly affect the results, although higher power has also demonstrated deleterious effects on radicular dentin. Stereomicroscopic analysis could be done to assess tubule penetration but has some drawbacks due to the laborious gold-sputtering and vacuum phases.

Within the limitations, the present results explain that laser-activated irrigation and ultrasonic agitation have a positive influence on the removal of NAC medicament from the root canals.

The study also demonstrated that, across all groups, sealer penetrated in coronal third the highest, followed by middle third, and then apical third. The tubule density of dentin and its diameter is highest in the coronal region and falls with depth; the lowest density is seen apically.[24] The apical one-third of the tubules also exhibit obliteration from sclerosis as a result of aging or continuous functional loading.[25] Despite the intricacies, diode laser irrigation performed better in the apical third of the root canal.

CONCLUSIONS

Diode laser activation was most effective in the removal of NAC from all three regions, coronal, middle, and apical thirds of the root canal. Passive ultrasonic agitation had comparable results to diode laser activation, thus can be considered as effective when NAC is used as intracanal medicament.

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Conflicts of interest
There are no conflicts of interest.

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