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Efficiency evaluation of different activation methods on dentinal tubule penetration by a bioactive glass-based sealer using confocal laser scanning microscopy: An *in vitro* study

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Abstract:
Dentinal tubule penetration of bioactive glass-based sealers is influenced by the activation technique used during obturation. Therefore, it is of interest to compare the ultrasonic, sonic, lentulospiral and manual activation methods for dentinal tubule penetration of a bioactive glass-based sealer using confocal laser scanning microscopy. Sixty extracted mandibular premolars were prepared, obturated after sealer activation (n=15/group) and evaluated at coronal, middle and apical thirds using CLSM. Ultrasonic activation produced the greatest penetration at all root levels (322.58±4.34, 295.74±4.37 and 197.42±4.22 µm), followed by sonic (291.02 µm ,250.55 µm and 179.69 µm), lentulospiral (265.75 µm ,213.77 µm and 145.21 µm) and manual (155.47 µm, 114.03 µm and 95.70 µm) activation, with significant intergroup differences (P<0.001). Thus, data shows that ultrasonic activation significantly enhances dentinal tubule penetration of bioactive glass-based sealer and may improve obturation quality compared with conventional techniques.

Keywords: Bioactive glass–based sealer; dentinal tubule penetration; ultrasonic activation; sonic activation; lentulospiral; confocal laser scanning microscopy (CLSM)

Background:
Endodontic treatment aims to preserve the natural tooth by eliminating microorganisms from the root canal system and preventing reinfection through an effective three-dimensional seal **[1].** Despite significant advancements in rotary instrumentation, irrigation protocols and obturation techniques, failure of root canal treatment continues to be reported, often attributable to inadequate sealing of the complex root canal anatomy. Microleakage resulting from poor adaptation of obturating materials may permit bacterial ingress, leading to persistent periapical pathology and eventual treatment failure. Gutta-percha's biocompatibility, dimensional stability and ease of handling make it the gold standard for core obturating materials. Nevertheless, gutta-percha cannot seal abnormalities in the canal on its own and does not adhere to dentin **[2].** As a result, endodontic sealers are essential to obturation because they improve the adaptability between the obturating material and the canal walls by filling voids, lateral canals, isthmuses and dentinal tubules. Penetration of sealers into dentinal tubules enhances micromechanical interlocking, reduces interfacial gaps and contributes to the entombment of residual microorganisms that may survive Chemomechanical preparation. Many endodontic sealers have been created over time, such as resin-based, calcium hydroxide-based, zinc oxide-eugenol-based and bioceramic sealers. Because of their capacity to interact with dentin and adjacent tissues, bioactive glass-based sealers have recently attracted more attention **[3].** These sealers are capable of releasing calcium ions, maintaining an alkaline environment and inducing hydroxyapatite formation at the sealer–dentin interface **[4]**. Such bioactivity may enhance the biological seal, promote mineralization and contribute to long-term obturation stability. Despite these advantages, bioactive glass–based sealers contain a silicone-based carrier matrix that imparts relatively high viscosity and limited inherent flow. From a clinical perspective, this rheological behavior may restrict penetration into dentinal tubules, particularly in the apical third where tubule density and diameter are reduced **[5].** Therefore, reliance on passive sealer placement techniques may compromise the potential benefits offered by these bioactive materials. To overcome these limitations, various sealer placement and activation techniques have been proposed. Manual placement using hand files remains the most commonly employed technique due to its simplicity and familiarity. Lentulospiral placement utilizes centrifugal force to distribute sealer along canal walls and has been widely advocated for improved sealer distribution **[6]**. Sonic and ultrasonic activation techniques introduce energy into the canal system, generating hydrodynamic agitation that may improve sealer flow, penetration and adaptation. Ultrasonic activation, in particular, produces acoustic microstreaming and cavitation, which have been shown to enhance the penetration of irrigants and sealers into dentinal tubules **[7].** Evaluation of sealer penetration into dentinal tubules requires reliable and accurate imaging techniques. Confocal laser scanning microscopy (CLSM) has emerged as a preferred method for assessing sealer penetration, as it allows visualization of fluorescent-labeled materials under hydrated conditions without inducing dehydration artifacts **[8].** Therefore, it is of interest to evaluate and compare the effect of ultrasonic, sonic, lentulospiral and manual activation techniques on the dentinal tubule penetration of a bioactive glass–based sealer using confocal laser scanning microscopy.

Materials and Methods:
Study design and ethical considerations:
This *in vitro* experimental study was conducted following approval from the institutional ethics committee. Extracted human teeth were obtained with informed consent and stored according to institutional guidelines.

Sample selection and standardization:

A total of sixty extracted mandibular premolars with straight canals and completely formed apices were chosen. Teeth with numerous canals, resorption, calcifications, caries, restorations, or cracks were not included. To standardize the root length to 12 mm, all specimens were decoronated using diamond disc with water cooling.

Root canal preparation:
Using a size 10 K-file, the working length was determined by deducting 1 mm from the length of the apical foramen. ProTaper Gold rotary instruments up to size F3 were used in a crown-down method for biomechanical preparation. During instrumentation, 5.25% sodium hypochlorite was used for irrigation. 17% EDTA was used for one minute to remove the smear layer and then saline was used as a final rinse.

Sealer preparation and group allocation:
Following the manufacturer's recommendations, a bioactive glass-based sealer (GuttaFlow Bioseal, Coltene, Switzerland) was combined with 0.1% Rhodamine B dye (1 mg/mL) for fluorescence visibility. Based on the sealer activation method, samples were split into four groups (n = 15) at random:
[1] Group A: Ultrasonic activation
[2] Group B: Sonic activation
[3] Group C: Lentulospiral activation
[4] Group D: Manual activation

Sealer placement, activation and obturation:
After drying the canals with paper points, the labelled sealer was applied to canal walls. In Group A, activation was performed using a K15 ultrasonic tip at 25–30 kHz (power 3) placed 1–2 mm short of working length for 20 seconds, inducing acoustic microstreaming and cavitation. In Group B, a size 30 sonic activator tip was used 2 mm short of working length at 1–6 kHz for 20 seconds to generate hydrodynamic agitation. In Group C, a size 30 lentulospiral in a slow-speed handpiece (300 rpm) was used for 20 seconds, utilizing centrifugal force for sealer distribution. In Group D, manual activation was performed using a master gutta-percha cone with push–pull strokes (approximately 100 strokes/min) for 20 seconds. All canals were obturated using warm vertical compaction with gutta-percha and obturation quality was radiographically verified. Specimens were stored at 37°C and 100% humidity for 48 hours to allow sealer setting. Samples were sectioned longitudinally under water cooling and analyzed at coronal, middle and apical thirds using confocal laser scanning microscopy at 10 × magnifications to evaluate sealer penetration depth.

Statistical analysis:
One-way ANOVA and Tukey's post-hoc test were used to examine the data, with significance set at $p < 0.05$.

Results:
Confocal laser scanning microscopy (CLSM) analysis showed statistically significant differences in dentinal tubule penetration among all experimental groups at the coronal, middle and apical thirds of the root canal ($p < 0.05$). Mean penetration depth values for all groups are presented in **Table 1,** while intergroup pairwise comparisons are shown in **Table 2**. Among all activation methods, ultrasonic activation (Group A) showed the greatest sealer penetration at every root level **(Table 1; Figures 1–3)**. The mean penetration depth in this group was 322.58 ± 4.34 µm at the coronal third, 295.74 ± 4.37 µm at the middle third and 197.42 ± 4.22 µm at the apical third. Sonic activation (Group B) showed the second-highest penetration values, with mean depths of 291.02 ± 5.30 µm, 250.55 ± 5.52 µm and 179.69 ± 5.29 µm at the coronal, middle and apical thirds respectively. Lentulospiral activation (Group C) showed moderate penetration values, 265.75 ± 3.11 at coronal third, 213.77 ± 3.01 at middle third and 145.21 ± 3.05 at apical third whereas manual activation (Group D) consistently exhibited the lowest penetration depths 155.47 ± 2.75 at coronal third, 114.03 ± 2.53 at middle third and 95.70 ± 2.48 at apical third **(Table 1)**. A progressive reduction in penetration depth from coronal to apical thirds was observed in all groups **(Figures 1–3).** The coronal third showed maximum sealer penetration, followed by the middle third, while the apical third showed the least penetration. This trend was consistent irrespective of the activation technique used. One-way ANOVA revealed highly significant intergroup differences at all three root levels ($p < 0.001$). Tukey's post-hoc analysis further showed that ultrasonic activation produced significantly greater penetration than sonic, lentulospiral and manual activation techniques at all thirds of the root canal **(Table 2)**.

Table 1: Comparison of sealer penetration depth (µm) among experimental groups at coronal, middle and apical thirds (Mean ± SE) by Anova

Group	Coronal third (µm)	Middle third (µm)	Apical third (µm)
Group A	322.58 ± 4.34	295.74 ± 4.37	197.42 ± 4.22
Group B	291.02 ± 5.30	250.55 ± 5.52	179.69 ± 5.29
Group C	265.75 ± 3.11	213.77 ± 3.01	145.21 ± 3.05
Group D	155.47 ± 2.75	114.03 ± 2.53	95.70 ± 2.48

Table 2: Multiple pairwise comparisons of sealer penetration depth among groups at coronal, middle and apical thirds (Tukey post-hoc test)

Comparison	Coronal third – Mean difference (95% CI)	P value	Middle third – Mean difference (95% CI)	P value	Apical third – Mean difference (95% CI)	P value
Group A vs Group B	31.56 (16.56 to 46.56)	< 0.001	45.19 (30.09 to 60.30)	< 0.001	17.73 (3.06 to 32.40)	< 0.05
Group A vs Group C	56.83 (41.83 to 71.82)	< 0.001	81.97 (66.86 to 97.07)	< 0.001	52.21 (37.54 to 66.88)	< 0.001
Group A vs Group	167.11 (152.10 to 182.10)	< 0.001	181.71 (166.60 to 196.80)	< 0.001	101.72 (87.05 to 116.40)	< 0.001

D						
Group B vs Group C	25.27 (10.27 to 40.26)	< 0.001	36.77 (21.67 to 51.88)	< 0.001	34.48 (19.81 to 49.15)	< 0.001
Group B vs Group D	135.55 (120.50 to 150.50)	< 0.001	136.51 (121.40 to 151.60)	< 0.001	83.99 (69.32 to 98.66)	< 0.001
Group C vs Group D	110.28 (95.28 to 125.30)	< 0.001	99.74 (84.64 to 114.80)	< 0.001	49.51 (34.84 to 64.18)	< 0.001

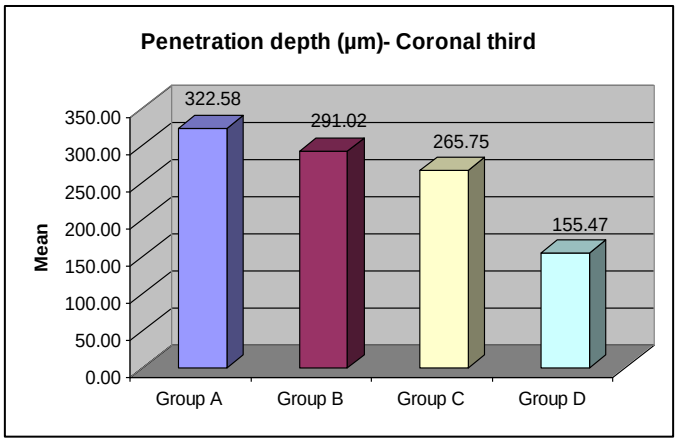

Figure 1: Comparisons of difference in mean penetration depth between four groups at coronal third

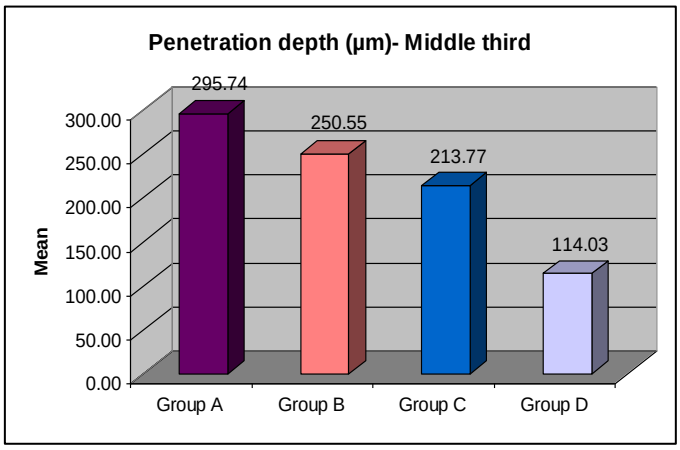

Figure 2: Mean penetration depth of four groups at middle third

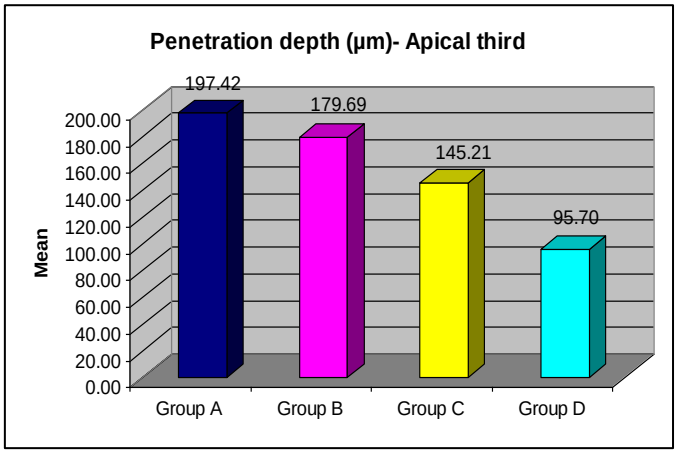

Figure 3: Mean penetration depth of four groups at apical third.

Discussion:

The quality of root canal obturation is a critical determinant of long-term endodontic success. Effective obturation prevents coronal and apical microleakage and entombs residual microorganisms within dentinal tubules that may persist despite thorough chemo mechanical preparation. Dentinal tubule penetration of sealers is therefore an essential parameter, as it enhances micromechanical retention, improves sealer–dentin interface adaptation and contributes to overall sealing efficacy. In the present study, dentinal tubule penetration of a bioactive glass–based sealer was evaluated using four activation techniques. Bioactive glass–based sealers represent a newer class of materials with favourable biological properties, including calcium ion release, alkaline pH and hydroxyapatite formation at the sealer–dentin interface, which may enhance periapical healing and long-term stability. However, their silicone-based carrier matrix, composed of polydimethylsiloxane with long hydrophobic chains and high molecular weight, increases viscosity and reduces flow. This inherent property limits penetration when passive placement techniques are used due to increased internal resistance **[9]**. Ultrasonic activation showed the highest mean sealer penetration at all root levels—coronal (322.58 µm), middle (295.74 µm) and apical (197.42 µm). Greater penetration in the coronal third can be attributed to wider tubules and higher permeability, which is consistent with findings by Nikhil *et al.* (2025) **[10]**. These results are in agreement with Tomar *et al.* (2022), who reported superior sealer penetration with ultrasonic activation compared to sonic and lentulospiral techniques **[11]**. The enhanced performance is primarily due to acoustic microstreaming and cavitation. Van der Sluis *et al.* showed that acoustic microstreaming and cavitation generated during ultrasonic activation create hydrodynamic forces capable of enhancing sealer penetration into dentinal tubules **[12]**. Although penetration decreased toward the apical third due to reduced tubule diameter, sclerosis and limited permeability, ultrasonic activation still achieved the highest values in this region. This may be attributed to sustained microstreaming and localized temperature rise, which reduces sealer viscosity and improves flow. Prasad *et al.* reported that increased temperature lowers sealer viscosity and enhances penetration and noted improved sealer adaptation due to ultrasonically induced heat **[13]**. Sonic activation demonstrated mean penetration values of 291.02 µm (coronal), 250.55 µm (middle) and 179.69 µm (apical), which were significantly lower than ultrasonic activation ($P < 0.001$) but higher than lentulospiral and manual techniques. This reduced performance is attributed to the lower operating frequency of sonic devices (1–6 kHz) compared to ultrasonics (25–30 kHz), resulting in less

intense hydrodynamic effects. Tan *et al.* reported that sonic activation produces gentle hydrodynamic agitation that improves sealer distribution but lacks the shear intensity of ultrasonics **[14]**. Despite the absence of cavitation and significant heat generation, sonic activation achieved notable penetration. Pérez Alfayate *et al.* highlighted that oscillatory motion of flexible sonic tips helps displace air voids and enhances penetration compared to passive techniques **[15]**. Plotino *et al.* further emphasized that flexible sonic tips can better navigate canal curvatures and transmit agitation uniformly, supporting improved apical penetration **[16]**. Lentulospiral activation showed moderate penetration coronal (265.75 µm), middle (213.77 µm) and apical (145.21 µm) ranking below ultrasonic and sonic techniques. Its mechanism is purely mechanical, relying on rotational movement to generate centrifugal force that distributes sealer along canal walls. Nikhil and Singh reported that this centrifugal thrust effectively coats canal walls, explaining its improved performance over manual placement **[17]**. However, it does not facilitate deep penetration due to the absence of hydrodynamic effects such as microstreaming or cavitation. Dash *et al.* emphasized that lentulospirals lack dynamic agitation and cannot effectively propel sealer into dentinal tubules **[18]**. Penetration decreased from coronal to apical thirds, reflecting anatomical narrowing and reduced mechanical efficiency. The relatively low apical penetration (145.21 µm) highlights the limitations of this technique in sclerotic regions. Guinesi *et al.* noted that rotary sealer placement may lead to coronal pooling or backflow, thereby reducing apical penetration **[19].** Manual activation exhibited the lowest penetration - coronal (155.47 µm), middle (114.03 µm) and apical (95.70 µm). This technique lacks both mechanical and hydrodynamic energy, relying solely on push–pull motion. The apical third showed the least penetration due to limited adaptation and higher risk of void formation. Keles *et al.* emphasized that passive placement is associated with voids and poor adaptation **[20]**. Additionally, push–pull motion may generate hydraulic back-pressure, restricting sealer flow and limiting tubule infiltration. Across all groups, penetration decreased from coronal to apical thirds due to reduced tubule diameter, increased sclerosis and decreased dentin permeability. This was also supported by Barbhai *et al.* who reported that bioceramic and bioactive glass-based sealers achieved superior dentinal tubule penetration compared with resin-based and ZOE-based sealers, with bioactive glass exhibiting the greatest percentage of tubule penetration **[21]**. Kashaf *et al.* also highlighted that dentinal tubule penetration is influenced by several interacting factors, including sealer flow characteristics, particle size, irrigation procedures and dentinal tubule density. The present investigation extends these observations by showing that, even when the same bioactive sealer is used, activation technique significantly influences penetration depth throughout the root canal, with ultrasonic activation providing the greatest benefit **[22].** Confocal laser scanning microscopy enabled accurate evaluation under hydrated conditions, minimizing artifacts. However, the *in vitro* design remains a limitation, as clinical variables such as canal complexity and intraoral conditions could not be replicated. Clinically, the findings suggest that activation techniques, particularly ultrasonic activation, significantly enhance dentinal tubule penetration of bioactive glass–based sealers. Incorporating such energy-driven techniques into routine endodontic practice may improve obturation quality and long-term treatment outcomes.

Conclusion:

Ultrasonic activation was the most effective method for enhancing dentinal tubule penetration of a bioactive glass–based sealer, followed by sonic and lentulospiral activation. Manual activation resulted in the least penetration. The use of energy-driven activation techniques may improve the sealing efficacy of bioactive sealers and contribute to improved clinical outcomes in endodontic practice.

Advancement to knowledge:

The major advancement of this study lies in its comparative evaluation of modern activation techniques specifically for a bioactive glass–based sealer (GuttaFlow Bioseal), a material with promising bioactive properties but limited flow due to its high viscosity. Unlike previous studies that mainly focused on conventional or bioceramic sealers, this study demonstrated that energy-driven activation methods.

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