

Evaluation of Apical Sealing Ability Following Different Root Canal Instrumentation Techniques: An In-vitro Study

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Abstract :

Background: The long-term outcome of endodontic therapy is thought to be significantly impacted by apical sealing capacity. Inadequate canal preparation can raise apical microleakage and impair obturation quality, which could result in treatment failure and reinfection. **Materials and Methods:** Seventy-five excised human single-rooted premolars with curved root canals and mature apices were chosen, and they were split into experimental and control groups at random. WaveOne Gold (G1), Reciproc Blue (G2), or manual K-files (G3) were used to instrument root canals. There were also positive and negative control groups. Apical microleakage was evaluated using dye penetration technique after obturation. Shapiro-Wilk normality testing, independent t-tests, Kruskal-Wallis analysis, Mann-Whitney U tests, and Tukey's post hoc comparisons were used for statistical analysis, with a significance level of $p < 0.05$. **Results:** Significant differences in microleakage were observed among the study groups ($p = 0.001$). WaveOne Gold demonstrated the lowest mean microleakage values (1.207 ± 0.030 mm), followed by Reciproc Blue (1.437 ± 0.063 mm), whereas manual K-files showed the highest leakage among the experimental groups (1.833 ± 0.042 mm). Dye penetration analysis also revealed statistically significant differences among groups ($p = 0.001$). Positive control specimens exhibited the highest leakage values, while the negative control group showed no detectable leakage. Post hoc analysis confirmed significant pairwise differences between all experimental groups.

Conclusion: Reciprocating nickel-titanium devices showed better apical sealing ability than manual instrumentation under the constraints of this in vitro study. WaveOne Gold outperformed Reciproc Blue and manual K-files in terms of sealing performance and obturation quality, as evidenced by the lowest microleakage values.

Keywords:

Apical microleakage; Apical sealing ability; WaveOne Gold; Reciproc Blue; Root canal instrumentation; Dye penetration; Endodontic therapy.

1. Introduction

Successful endodontic therapy depends on the complete elimination of microorganisms from the root canal system and the prevention of reinfection through adequate canal preparation, disinfection, and three-dimensional obturation. An effective apical seal is considered one of the most important determinants of long-term treatment success because apical microleakage permits the penetration of bacteria and their by-products into the periapical tissues, leading to persistent inflammation and possible treatment failure (1,9). The apical third of the root canal presents a considerable clinical challenge because of its complex anatomical characteristics, including lateral canals, apical ramifications, fins, and irregular cross-sectional morphology. These anatomical complexities often limit the effectiveness of mechanical instrumentation and irrigation, resulting in residual debris and microorganisms that may compromise the adaptation of obturation materials and reduce sealing quality (14,17). Conventional stainless-steel hand instrumentation has been widely used for root canal preparation for many years. However, manual techniques are associated with several procedural errors, including canal transportation, ledge formation, zipping, and inadequate canal centering, particularly in curved canals. These limitations may adversely affect canal shaping and ultimately increase the risk of apical microleakage (2,18). The introduction of nickel–titanium (NiTi) instrumentation systems has substantially improved the quality of root canal preparation. Reciprocating single-file systems such as WaveOne Gold and Reciproc Blue employ alternating clockwise and counterclockwise movements that reduce torsional stress and cyclic fatigue while maintaining the original canal anatomy. In addition, their thermally treated NiTi alloys provide greater flexibility and fracture resistance, facilitating more conservative canal shaping and enhanced irrigant penetration (3,5,8,12). Several studies have demonstrated that the quality of canal instrumentation directly influences debris removal, smear layer

elimination, canal geometry, and the adaptation of obturation materials. Although reciprocating systems generally exhibit superior shaping ability and cleaning efficiency compared with manual instrumentation, conflicting evidence remains regarding their effect on apical sealing ability and microleakage (7,11,16). Among the various techniques used to evaluate apical sealing ability, dye penetration remains one of the most commonly employed laboratory methods because of its simplicity, sensitivity, reproducibility, and cost-effectiveness. Methylene blue dye has been widely used to compare the sealing ability of different endodontic procedures under standardized experimental conditions, despite the inherent limitations associated with in vitro leakage models (15). Therefore, the present study aimed to evaluate and compare the apical sealing ability of two reciprocating nickel–titanium instrumentation systems (WaveOne Gold and Reciproc Blue) with the conventional manual step-back technique using dye penetration analysis.

2. Materials and Methods

Study Design and Sample Selection

This in vitro experimental study was conducted on seventy-five extracted human single-rooted premolars with mature apices and curved root canals. Teeth extracted for periodontal or orthodontic reasons were selected. Prior to inclusion, all specimens were examined radiographically in both mesiodistal and buccolingual directions and inspected under a stereomicroscope at $\times 20$ magnification to exclude teeth presenting with severe root curvature, internal or external root resorption, root fractures, calcifications, open apices, multiple canals, or root caries. After the removal of calculus and adherent soft tissue remnants, the teeth were immersed in 2.5% sodium hypochlorite for two hours for disinfection and subsequently stored in normal saline at 4°C until use. The crowns were sectioned at the cemento-enamel junction (CEJ) using a high-speed handpiece equipped with a diamond bur under continuous water cooling to obtain a standardized root length of 16 mm. Canal patency was established using a size #15 K-file (Dentsply Maillefer, Switzerland). The working length was determined by advancing the file until its tip became visible at the apical foramen and subtracting 1 mm from this measurement. Teeth with an apical diameter larger than a size #25 K-file were excluded and replaced. The specimens were randomly allocated into five groups ($n = 15$ per group):

Group 1 (WaveOne Gold): Root canals were prepared using WaveOne Gold Primary files (25/.08) operated with a dedicated reciprocating endodontic motor (Dentsply Maillefer, Ballaigues, Switzerland) according to the manufacturer's recommendations using gentle in-and-out pecking motions.

Group 2 (Reciproc Blue): Root canals were prepared using Reciproc Blue R25 files (25/.08) with the VDW Silver Reciproc Endo Motor (VDW, Munich, Germany) following the manufacturer's instructions and employing a reciprocating pecking motion.

Group 3 (Manual K-files): Root canals were instrumented using stainless-steel K-files (Dentsply Maillefer, Switzerland) according to the conventional step-back technique. Apical preparation was completed to size #25, followed by sequential flaring of the canal using progressively larger files up to size #80.

Group 4 (Positive Control): Root canals remained unobturated to permit maximum dye penetration.

Group 5 (Negative Control): Root canals were completely sealed externally to prevent dye penetration.

Irrigation Protocol

During instrumentation, each canal was irrigated with 10 mL of 5.25% sodium hypochlorite. Passive ultrasonic irrigation (PUI) was subsequently performed using an Irrisafe ultrasonic tip size #20/.00 attached to a P5 Newtron XS ultrasonic unit (Acteon, Merignac, France). The ultrasonic tip was positioned 1 mm short of the working length without contacting the canal walls. Irrigation was activated for 20 seconds and repeated three times with fresh irrigant between cycles. Smear layer removal was achieved using 10 mL of 17% EDTA for one minute, followed by the same ultrasonic activation protocol. Finally, the canals were rinsed with distilled water and dried using sterile paper points.

Root Canal Obturation

All canals were obturated using gutta-percha cones in combination with AH Plus epoxy resin sealer (Dentsply DeTrey, Konstanz, Germany). The sealer was introduced into the canal using a size #25 Lentulo spiral operating at low speed to a point 1 mm short of the working length. The master cone was lightly coated with sealer and inserted to the full working length. Root canal obturation was completed using the continuous wave compaction technique with the System B heat source (Kerr Endodontics, Orange, CA, USA). The remaining canal space was backfilled using thermoplasticized gutta-percha delivered with the Obtura III Max system (Obtura Spartan, Fenton, MO, USA), followed by vertical compaction using appropriate pluggers. Excess gutta-percha was removed to the level of the cemento-enamel junction, and the access cavity was restored with glass ionomer cement. The quality of the root canal fillings was verified radiographically in both mesiodistal and buccolingual projections. Specimens were then stored at 37°C and 100% relative humidity for seven days to allow complete setting of the sealer.

Microleakage Evaluation

The root surfaces of all experimental specimens were coated with two layers of nail varnish except for the apical 2 mm. In the positive control group, canals remained unobtured to maximize dye penetration, whereas in the negative control group the apical foramen was sealed with wax and the coronal access cavity was sealed with light-cured glass ionomer cement before complete coating with nail varnish. Following dye immersion, the specimens were sectioned longitudinally, and linear apical dye penetration was measured under a stereomicroscope at $\times 40$ magnification by two independent blinded observers. The average value obtained from both observers was recorded as the final microleakage measurement for each specimen.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 28.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics, including the mean, median, standard deviation (SD), standard error (SE), minimum, maximum, and range, were calculated for each study group. The normality of the data distribution was assessed using the Shapiro–Wilk test. Because the data were not assumed to be normally distributed across all groups, comparisons of microleakage values were performed using the Kruskal–Wallis test.

Pairwise comparisons among the experimental groups were subsequently conducted using Tukey's post hoc multiple comparison test. The coefficient of determination (R^2) was calculated to evaluate the goodness of fit of the statistical model. For apical dye penetration analysis, descriptive statistics (mean $\pm$ SD) were calculated for each group, and differences among groups were assessed at a significance level of $p < 0.05$. Statistical significance was accepted when the p-value was less than 0.05, and highly significant differences were considered at $p \leq 0.001$. The negative control group (G5) demonstrated complete absence of microleakage in all specimens (0.00 mm) and was therefore excluded from the comparative statistical analysis because no variability was present. All statistical tests were two-tailed.

A. Intergroup comparison of microleakage

1. Overall intergroup comparison

Overall analysis demonstrated a progressive increase in microleakage across groups, with G4 showing the highest values, followed by G3, G1, and G2 (Table 1). The differences among all groups were statistically significant ($p = 0.01$), with a high coefficient of determination ($R^2 = 0.9986$). The Kruskal–Wallis test confirmed overall significance ($p = 0.001$), while Figure. 1 illustrated clear separation among groups. G5 served as the negative control group and demonstrated zero microleakage values in all samples; therefore, it was excluded from the statistical analysis

Table 1. Overall descriptive statistics and intergroup comparison of microleakage among all study groups (G1–G4).

Statistics	G1	G2	G3	G4	R ²	p-value
Mean	1.437	1.207	1.833	12.03	0.998 6	0.01
Median	1.440	1.210	1.840	12.00		
SD	0.063 88	0.03086	0.04203	0.3478		
SE	0.016 49	0.00796 8	0.01085	0.08980		
Minimum	1.300	1.150	1.750	11.50		
Maximum	1.540	1.250	1.900	12.50		
Range	0.240 0	0.1000	0.1500	1.000		
Shapiro-Wilk test	0.985 4	0.9477	0.9808	0.9321		
Kruskal-Wallis test						0.001

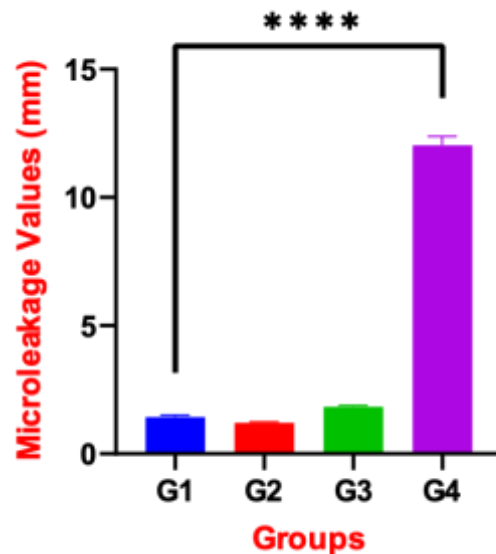


Figure 1. Comparative distribution of microleakage across all study groups (G1–G4).

B. Apical dye penetration analysis

Mean apical dye penetration analysis showed the highest leakage in the Positive Control group (6.95 ± 0.34 mm), whereas the Negative Control group exhibited no leakage (0.00 ± 0.00 mm). Among experimental groups, G3 demonstrated the highest leakage, followed by G2 and G1 (Table 2). Differences among groups were statistically significant ($p = 0.001$), **Table 2. Mean apical dye penetration among study groups.**

Group	Instrumentation Technique	Mean Leakage (mm)	SD	P-value
G1	WaveOne Gold	1.31	0.18	0.001
G2	Reciproc Blue	1.48	0.21	0.001
G3	Manual K-files	1.82	0.27	0.001
G4	Positive Control	6.95	0.34	—
G5	Negative Control	0.00	0.00	—

C. Post hoc multiple comparisons

Tukey's post hoc test confirmed significant pairwise differences among experimental groups (Table3). G1 showed significantly lower leakage than G2 ($p = 0.041$) and G3 ($p = 0.001$), while G2 also differed significantly from G3 ($p = 0.003$), indicating a stepwise increase in leakage from G1 to G3.

Table 3. Tukey's post hoc multiple comparison analysis among experimental groups.

Comparison	Mean Difference	P-value
G1 vs G2	-0.17	0.041
G1 vs G3	-0.51	0.001
G2 vs G3	-0.34	0.003

Discussion

One of the most important elements affecting the long-term success of root canal therapy is still the creation of an efficient apical seal. Microorganisms and their byproducts may be able to enter the periapical tissues through apical microleakage, which could lead to endodontic failure and chronic inflammation. Thus, the current study sought to examine the apical dye penetration and sealing capacity of several instrumentation methods, specifically WaveOne Gold, Reciproc Blue, and manual K-files. (1).

The results of this investigation showed that the examined instrumentation techniques differed significantly from one another. Among the experimental groups, manual K-files had the greatest leakage values, whereas Reciproc Blue and WaveOne Gold had the lowest mean microleakage values. When compared to traditional hand instrumentation, these results imply that reciprocating nickel–titanium systems may offer better canal shape and adaptability, improving the apical seal's quality (4).

Reciprocating motion and its thermally treated nickel-titanium alloy, which increase flexibility and cutting efficiency while preserving canal centralization, may be responsible for Reciproc Blue's exceptional performance. According to earlier research, Reciproc Blue increases canal enlargement and improves shaping ability, which could lead to better irrigant penetration and obturation adaptation. According to Caviedes-Bucheli et al., Reciproc Blue showed a noticeably higher rise in canal volume than WaveOne Gold, suggesting improved shaping efficiency. (4,6). Additionally, better master cone adaptation to canal walls may be linked to the enhanced sealing performance shown with Reciproc Blue. In comparison to WaveOne Gold, Reciproc Blue produced better filling quality and fewer voids in the apical area, according to a CBCT-based study assessing

single-cone adaptation. This could account for the decreased microleakage found in this investigation. (10). Additionally, compared to manual K-files, WaveOne Gold showed considerably lower leakage values. This result supports the idea that reciprocating single-file systems decrease procedural mistakes frequently associated with manual instrumentation and result in more standardized canal preparations. WaveOne Gold's improved metallurgy and design features, such as its parallelogram cross-sectional design and gold heat-treated alloy, may increase obturation quality by increasing flexibility and debris removal effectiveness (4,13). On the other hand, among the experimental groups, manual K-files showed the highest leakage values. The increased operator reliance and decreased shaping uniformity linked to manual instrumentation could account for this. Hand filing methods may result in less consistent canal geometry and insufficient canal wall preparation, which could jeopardize gutta-percha adaption and sealer distribution. Additionally, compared to contemporary reciprocating systems, manual instrumentation has been linked to inconsistent canal preparation and greater debris retention (19). While the negative control group showed no discernible dye penetration, the positive control group had exceptionally high leakage values. These results validate the sensitivity and validity of the experimental model employed in this investigation. While the widespread leaking seen in the positive control group validates the dye penetration method's accuracy in detecting apical leakage, the total lack of leakage in the negative control group shows sufficient exterior sealing of specimens. WaveOne Gold had lower mean dye penetration values than Reciproc Blue, according to the dye penetration investigation, which showed a somewhat different pattern. This discrepancy in microleakage and dye penetration results may be due to variations in evaluation methodology, specimen variability, or sensitivity of the assessment approaches, even though both systems outperformed manual K-files by a large margin. A number of experimental factors, such as dye molecular size, immersion time, and sectioning techniques, are known to affect dye penetration techniques. As a result, in vitro research should anticipate minor differences amongst leakage measurement techniques. Statistically significant pairwise differences between all experimental groups were confirmed by post hoc analysis, which showed a gradual rise in leakage from Reciproc Blue to WaveOne Gold and ultimately to manual K-files. In terms of apical sealing ability, these results confirm that reciprocating nickel-titanium systems are better to traditional manual instrumentation.

When evaluating the results, it is important to take into account the limitations of the current study. First, the study was carried out in vitro, which is unable to fully replicate

the intricate biological environment found in clinical settings. Second, the results could not be applied to more complex canal anatomy because only straight single-rooted premolars were included. Furthermore, dye penetration techniques may not accurately reflect clinical bacterial leakage and offer an indirect evaluation of leakage. Future research utilizing bacterial leakage models or micro-CT analysis is advised to offer a more thorough assessment of sealing quality. Within the constraints of this investigation, manual K-files showed the largest leakage, whereas WaveOne Gold and Reciproc Blue showed the lowest apical microleakage values among the examined instrumentation systems. Therefore, when compared to traditional manual instrumentation methods, reciprocating nickel-titanium systems may offer better obturation quality and superior sealing ability.

Conclusion

Within the limitations of this in vitro study, the instrumentation technique significantly influenced apical sealing ability. WaveOne Gold and Reciproc Blue demonstrated superior sealing performance and significantly lower apical microleakage than manual K-file instrumentation. Manual K-files exhibited the highest leakage values, indicating less effective canal preparation and obturation. These findings suggest that reciprocating nickel–titanium systems provide better apical sealing and may improve the long-term success of endodontic treatment. Nevertheless, further in vivo studies using advanced leakage assessment methods, such as bacterial leakage models and micro-computed tomography (micro-CT), are required to confirm these results.

Conflicts of interest

The authors declare that they have no conflicts of interest related to this work.

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Data availability statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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