International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 5S, 2025
https://www.theaspd.com/ijes.php

In vitro study to assess apical extrusion of intracanal bacteria using NiTi file instruments

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Abstract

Background:
Apical extrusion of bacteria during root canal instrumentation is a major concern, as it can lead to postoperative pain and flare-ups. Nickel–Titanium (NiTi) rotary and reciprocating instruments are commonly used in endodontics due to their efficiency and safety. However, their role in bacterial extrusion remains debated.

Materials and Methods:
An in vitro study was conducted on 45 extracted single-rooted human mandibular premolars. The canals were contaminated with *Enterococcus faecalis* and divided into three groups (n = 15 each) based on the instrumentation system: Group A - ProTaper Universal (rotary), Group B - WaveOne Gold (reciprocating), and Group C - TruNatomy (rotary). Each canal was instrumented according to the manufacturer's protocol. Bacterial extrusion was collected in vials containing sterile saline and incubated. Quantification was performed by measuring colony-forming units (CFUs) using standard microbiological techniques.

Results:
All systems caused some degree of bacterial extrusion. Group B (WaveOne Gold) showed the highest mean CFU count ($4.1 \times 10^4 \pm 1.3 \times 10^3$), followed by Group A (ProTaper Universal) ($2.8 \times 10^4 \pm 1.1 \times 10^3$), and Group C (TruNatomy) demonstrated the least extrusion ($1.5 \times 10^4 \pm 9.5 \times 10^2$). The differences were statistically significant ($p < 0.05$).

Conclusion:
Although bacterial extrusion was evident with all tested NiTi systems, reciprocating instrumentation resulted in greater bacterial extrusion compared to rotary systems. TruNatomy files exhibited the least apical extrusion, suggesting better control and potential clinical advantage.

Keywords:
Apical extrusion, NiTi instruments, *Enterococcus faecalis*, root canal therapy, WaveOne Gold, ProTaper, TruNatomy, bacterial contamination.

INTRODUCTION

Effective root canal treatment involves thorough debridement and disinfection of the root canal system while preventing contamination of periapical tissues. One of the recognized procedural complications during endodontic instrumentation is the extrusion of debris and microorganisms beyond the apical foramen, which can provoke periapical inflammation and postoperative discomfort (1). Apical extrusion is particularly concerning when it includes viable bacteria, such as *Enterococcus faecalis*, a common pathogen implicated in persistent endodontic infections (2,3).

The advent of Nickel–Titanium (NiTi) rotary and reciprocating file systems has significantly enhanced canal shaping efficiency and reduced procedural errors due to their superelastic properties and improved flexibility (4). Nevertheless, these systems are not immune to causing apical extrusion of debris and microorganisms (5). Several in vitro studies have attempted to compare the extent of microbial extrusion between different file designs and kinematics, with varying outcomes depending on instrument taper, tip design, cross-sectional geometry, and motion dynamics (6-8).

Rotary systems like ProTaper Universal have been widely used due to their progressive taper and active cutting efficiency, but they are known to extrude debris to some extent (9). On the other hand, reciprocating systems such as WaveOne Gold claim to minimize extrusion by mimicking balanced force technique with a single-file approach (10). Recently introduced systems like TruNatomy incorporate heat-treated NiTi and an innovative slim design that purportedly preserves pericervical dentin and reduces intracanal stress, which may influence the extent of apical extrusion (11).

Since apical extrusion cannot be completely avoided, understanding how different NiTi systems influence bacterial extrusion is crucial for improving clinical outcomes and minimizing post-treatment complications. This study aims to evaluate and compare the amount of apically extruded bacteria using ProTaper Universal, WaveOne Gold, and TruNatomy file systems under standardized in vitro conditions.

MATERIALS AND METHODS

This in vitro study was conducted using 45 freshly extracted human mandibular premolars with single straight canals and mature apices. Teeth with fractures, calcifications, or resorptive defects were excluded. The teeth were cleaned of soft tissue and calculus and stored in 0.1% thymol solution until use.

Sample Preparation

All teeth were decoronated to standardize the root length to 16 mm. The working length was determined by inserting a #10 K-file until it was visible at the apical foramen and subtracting 1 mm. The canals were instrumented manually up to #15 K-file to maintain patency and ensure standardization before mechanical preparation.

Microbial Contamination

The roots were autoclaved and then contaminated with a standard suspension of *Enterococcus faecalis* (ATCC 29212), prepared in brain heart infusion (BHI) broth to a concentration of approximately 1.5×10^8 CFU/mL (McFarland standard 0.5). Each canal was inoculated with the bacterial suspension and incubated at 37°C for 24 hours to allow adherence.

Experimental Groups

The specimens were randomly divided into three groups (n = 15 each) according to the file system used for instrumentation:

- **Group A:** ProTaper Universal (Dentsply Maillefer, Ballaigues, Switzerland) – Rotary
- **Group B:** WaveOne Gold (Dentsply Sirona) – Reciprocating
- **Group C:** TruNatomy (Dentsply Sirona) – Rotary

Each group was prepared following the respective manufacturer's protocol using a torque-controlled endodontic motor. Irrigation was performed using 2.5 mL of sterile saline with a 30-gauge side-vented needle after each file use. Total irrigation volume per canal was standardized to 10 mL.

Collection of Apically Extruded Bacteria

The experimental setup for collecting extruded bacteria was adapted from the method proposed by Myers and Montgomery. Each root was inserted into a pre-sterilized glass vial filled with 1 mL of sterile saline, with the apex suspended freely to avoid external contamination. A 27-gauge needle was used in each lid to equalize air pressure.

After instrumentation, 100 µL of the solution containing apical extrudates from each vial was serially diluted and cultured on BHI agar plates. The plates were incubated aerobically at 37°C for 48 hours, after which colony-forming units (CFUs) were counted manually using a digital colony counter.

Statistical Analysis

The data were recorded as mean CFUs ± standard deviation (SD) for each group. One-way ANOVA was applied to compare differences between groups, followed by Tukey's post hoc test for pairwise comparisons. A p-value < 0.05 was considered statistically significant.

RESULTS

All three file systems tested resulted in varying degrees of apical extrusion of *Enterococcus faecalis*. The number of colony-forming units (CFUs) extruded from the apical foramen differed significantly among the groups. Group B (WaveOne Gold) exhibited the highest bacterial extrusion, whereas Group C (TruNatomy) showed the lowest.

Table 1 summarizes the mean CFU counts and standard deviations for each group. The mean number of CFUs extruded in Group B was significantly higher compared to Group A and Group C ($p < 0.05$). A statistically significant difference was observed between the groups based on one-way ANOVA, and pairwise comparisons confirmed that Group C had significantly less extrusion than the other groups.

Table 1: Mean and Standard Deviation of Apically Extruded *E. faecalis* (CFU/mL) Among Study Groups

Group	Instrumentation System	Mean CFUs ($\times 10^4$)	Standard Deviation (±SD)
Group A	ProTaper Universal	2.80	1.10
Group B	WaveOne Gold	4.10	1.30
Group C	TruNatomy	1.50	0.95

Statistical significance: $p < 0.05$ (ANOVA)

As shown in **Table 1**, all systems caused some degree of bacterial extrusion, with the reciprocating system (Group B) resulting in the highest mean CFUs. Rotary systems (Group A and Group C) showed comparatively lower values, with TruNatomy being the most effective in minimizing extrusion.

DISCUSSION

Apical extrusion of intracanal bacteria remains a significant concern in endodontic practice, as it is often associated with postoperative pain, flare-ups, and delayed periapical healing (1,2). The present study evaluated and compared the extent of bacterial extrusion using three different NiTi instrumentation systems—ProTaper Universal, WaveOne Gold, and TruNatomy—and demonstrated that all systems caused some degree of extrusion, with statistically significant differences among them.

The findings of this study align with earlier literature reporting that reciprocating file systems tend to extrude more debris and microorganisms than continuous rotary systems (3,4). WaveOne Gold, a reciprocating single-file system, showed the highest level of *E. faecalis* extrusion in our study. This can be attributed to its larger taper and aggressive cutting action during apical progression, which may lead to increased apical pressure and debris expulsion (5,6).

ProTaper Universal, a full-sequence rotary system, exhibited moderate bacterial extrusion. Its progressive taper and multiple shaping files may contribute to better coronal flaring and debris removal but may still direct microorganisms toward the apex, especially in curved or narrow canals (7,8). In contrast, TruNatomy demonstrated the lowest extrusion among the systems tested. This could be due to its conservative design, reduced core diameter, and enhanced flexibility, which allow for improved debris transportation coronally and reduced periapical pressure during shaping (9,10).

The bacterial species used in this study, *Enterococcus faecalis*, is frequently isolated from failed root canal cases due to its resistance to antimicrobial agents and ability to penetrate dentinal tubules (11). Thus, its use in extrusion models provides a clinically relevant assessment of microbial contamination risk during instrumentation.

It is essential to acknowledge that complete prevention of apical extrusion is practically impossible, as all instrumentation techniques will inherently push some material beyond the apex (12). However, choosing systems that minimize this risk is crucial for patient comfort and treatment success.

Our findings support those of Bürklein and Schäfer (13), who reported greater debris extrusion with reciprocating files compared to rotary ones. Similarly, De-Deus et al. suggested that kinematics and cross-sectional design significantly influence debris transport and extrusion behavior (14). The reduced extrusion observed with TruNatomy may indicate its potential advantage in minimizing postoperative complications, although further clinical validation is needed (15,16).

One limitation of this in vitro study is the lack of simulation of periapical resistance offered by vital tissues in vivo. Additionally, variations in operator technique, canal anatomy, and irrigation methods can influence the degree of extrusion. Nonetheless, the standardized experimental design used here reduces bias and allows for reliable inter-group comparison.

CONCLUSION

In conclusion, while no system could entirely eliminate bacterial extrusion, the results suggest that file design, taper, and motion kinematics play crucial roles in minimizing apical contamination. Among the systems tested, TruNatomy appears to be most conservative in this regard, highlighting the importance of minimally invasive endodontic concepts in contemporary practice.

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