Comparative evaluation of apical bacterial extrusion following root canal instrumentation using different endodontic file systems: An *in vitro* study

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**Abstract**

**Aim:** This study aims to evaluate the amount of apical extrusion of bacteria during root canal instrumentation using K3XF, Protaper Gold, Edge taper platinum, and Hyflex CM Rotary systems.

**Materials and Methods:** Sixty freshly extracted maxillary incisors teeth collected in saline. Access cavity prepared and canals were made free of bacterial and pulp. The teeth were mounted on the bacteria collecting apparatus. Root canals were contaminated with the Fusobacterium Nucleatum (ATCC25586) and dried at 37°C for 24 h. In Group 1 (Control group): No instrumentation was done and biomechanical preparation done in all other groups with Group 2: Hand K-files, Group 3: Protaper gold, Group 4: K3XF, Group 5: Edge taper platinum, and Group 6: Hyflex CM rotary file systems. Then, the extrude was collected, and it is incubated in Mueller-Hinton agar for 24 h and the number of colony forming units were counted and statistical comparison was done using Kruskal-Wallis test and Mann-Whitney U test.

**Results:** Hand K-files extruded more bacteria when compared to other four rotary systems, K3XF file system extruded least number of bacteria.

**Conclusion:** All instrumentation techniques extruded intracanal bacteria apically. However, engine-driven nickel-titanium instruments extruded less bacteria than the manual technique. The K3XF rotary file system comparatively extruded less bacteria than other rotary file systems.

**Keywords:** Apical extrusion; edge taper platinum; fusobacterium nucleatum; Hyflex CM; K3XF

**INTRODUCTION**

The endodontic instrumentation mainly aims at disinfection and thorough debridement of root canal. Debridement of the pulp canal chemomechanically is necessary for successful endodontic treatment. In reality, the root canal anatomy is unpredictable with curvatures, ramifications and location of foramen, hence to clean and shape these canals successfully requires appropriately designed endodontic equipment along with different types of irrigation systems to thoroughly remove the debris. Debris such as dentinal filings, necrotic pulp tissue, bacteria, and their products may be extruded from apical foramen into the periradicular region which has been referred to as “worm of necrotic debris,” which may lead to periapical inflammation and postoperative flare-ups. The major cause of root canal treatment failure is due to the bacteria that extruded...
from the root canal, which are mainly Gram-positive, Gram-negative, and obligate anaerobes. *Enterococcus faecalis*, *Fusobacterium nucleatum*, *Propionibacterium alactolyticus*, *Propionibacterium propionicum* are the commonly isolated species of microbes.\[^3\]

Currently, all preparation techniques and instruments are associated with extrusion of bacteria, even when the preparation is maintained short of the apical terminus and manual instrumentation happens to produce greater extrusion when compared to engine driven rotary preparation. The studies so far have proven that none of the various techniques and instruments can clean and shape the root canal system without producing some apically extruded bacteria.\[^4\]

Crown-down techniques have demonstrated to extrude lesser number of bacteria than step-back technique, in various previous studies. Copious and frequent irrigation during biomechanical procedures significantly removes the excised dentin, pulpal debris, and microbial cells from the root canal reducing the risk of procedural accidents, such as blockages and apical extrusion of debris.\[^3,6\] The amount of extruded debris may influence the response of the peri-radicular tissues, hence crown-down techniques using advanced endodontic instruments combined with copious irrigation have the potential to reduce the risk of flare-ups.\[^7\]

This study was done to evaluate which file system (Manual K-file, Protaper gold, Edge taper platinum, Hyflex CM and K3XF) would extrude least amount of apical debris laden with bacteria and therefore reduce the risk of flare-ups. The null-hypothesis tested in this study stated that the amount of apically extruded debris laden with microbes does not differ between the five instrumentation systems.

**MATERIALS AND METHODS**

**Selection and preparation of teeth**
Sixty freshly extracted single-rooted human upper central incisor teeth with mature apices were selected. No data regarding age, sex, or reason for extraction was available. Teeth were stored, disinfected, and handled as per the recommendations and guidelines laid down by the Occupational Safety and Health Administration and Centers for Disease Control and Prevention. Exclusion criteria were multi-rooted teeth, calcified canals and canals with large apical foramina. Access cavities were prepared with high-speed handpiece with Endo access bur (Dentsply Maillefer). The pulp chambers were accessed, to create a reservoir for contamination of the root canals with a suspension of *Fusobacterium Nucleatum* (ATCC 25586) and then the pulp remnants were extirpated carefully with a fine barbed broach. #10 K file was inserted into the canals, to measure the lengths. As the tip of the file was visible at the apex, working length considered was 1 mm short of the file penetration length.

**Test apparatus preparation**
The test apparatus used to evaluate the bacterial extrusion was a previously described method by Er et al. 2005.\[^8\] Glass vials were used in which holes were made in the center of the rubber stoppers. The study tooth was fixed at the cemento-enamel junction level into the center of the rubber stopper. Nail varnish of two coats was applied onto the roots external surface for prevention of micro leakage through the lateral canals or other cemental breaches. The rubber stopper containing the tooth was then fitted into the glass vial mouth. The vial acted as a collecting container for the apical material extruded through the foramen of the root, in which the apical root portion of the tooth was suspended. The rubber stopper of the vial was vented with a 23-guage needle for equalizing the air pressure outside and inside [Figure 1]. The entire model system was then sterilized in ethylene oxide gas for a 12-h cycle using the Anprolene AN 74C Gas Sterilizer (Andersen Products Inc., Haw River, NC, USA). To create a hole in the nail varnish, a sterile #10 size K-file was placed 1 mm beyond the apex, to achieve standardized foramen and patency.

**Contamination with F. Nucleatum**
The root canals were then contaminated by a pure culture of *F. Nucleatum* (ATCC 25586, Microbiologics, U.S.A). A suspension was prepared by adding 1 ml of fresh pure culture of *F. Nucleatum* (ATCC 25586, Microbiologics, U.S.A) grown on brain-heart infusion broth for 24 h, anaerobically using AnaeroGas Pack (HIMEDIA, U.S.A). Contamination of the root canals was done using 10 µL of the above prepared suspension in a sterile micropipette of class I laminar airflow cabinet, to prevent any airborne contamination, and then a size #10 K file was used to carry the bacterial suspension down the length of the root canals carefully 1 mm short

![Figure 1: Experimental model used in the study](image-url)
of apex. An incubator at 37°C for 24 h was used to dry the contaminated roots. Glass vials were then filled entirely with 0.9% NaCl solution. The contaminated roots were then randomly divided into six experimental groups of 10 teeth each. A single operator, using aseptic techniques, carried out the preparation, and sampling procedures on each specimen covered by a rubber dam sheet to obscure the root apex during preparation.

**Preparation of root canal**

Root canal preparation was carried out under aseptic conditions. A constant total volume of 10 ml of 0.9% NaCl solution was used for irrigation in all specimens, subsequently root canal preparation was carried out with different file systems in different groups, corresponding to the sequence of instrumentation. The instrumentation sequences used were as follows:

- **Group I** – Control group: No instrumentation was done for the root canals of the control group
- **Group II** – Manual technique group: K-files (Dentsply Maillefer) were used in a step back manner. Apical preparation was performed up to #25 K-file and the step back technique was used with a reduction of 1 mm for each file until #45 K-file. Recapitulation was done with #10 K-file before progressing to the next larger instrument and frequent irrigation was done
- **Group III** – ProTaper Gold system group: ProTaper Gold instruments were used according to the manufacturer’s instructions in a crown down manner using a gentle in and out motion, after establishing glide path with K-files #10 or #15 stainless steel files to the working length. File sequences used were: Shaping file S1 was moved apically to 2 mm short of working length, SX files were then used until there was resistance (4 to 5 mm from W/L), which was then followed by S1 and S2 taken to the full working length. At the apical one-third finishing files F1, F2 were moved to the working length
- **Group IV** – K3XF G Pack system group: Instrumentation with the K3XF Rotary Ni–Ti Technique according to the manufacturer’s instructions, canals were prepared with #25/.12 K3XF instrument to the resistance followed by 0.10 taper and 0.08 taper instruments. Initial glide path was achieved using #10, #15, and #20 K-files. Canals were further prepared with 0.06 taper with #25 K3XF instruments to the resistance from largest instrument to smallest reaching the working length. After middle third scouting with #10 K-files
- **Group V** – Edge taper platinum system group: Edge taper platinum instruments were used according to the manufacturer’s instructions in a crown down manner using a gentle in and out motion, after establishing glide path with K-files #10 or #15 stainless steel files to the working length. Shaping and Finishing files S1, SX, S2, F1, F2 were used in sequence to complete the biomechanical preparation similar to that of Protaper Gold rotary files system
- **Group VI** – Hyflex CM system group: Instrumentation with the Hyflex CM Rotary Technique according to the manufacturer’s instructions, canals were prepared with #25/.08 Hyflex CM instrument to the resistance followed by initial glide path was achieved using #10, #15, and #20 K-files. Canals were further sequentially prepared with #20/.04, #25/.04, #25/.06 Hyflex CM instruments reaching the working length.

All the canals were overall irrigated with 10 ml of saline. At the end of root canal preparation, 100 µl of NaCl solution was taken from the experimental vials for counting the bacteria; and the suspension was inoculated on Mueller–Hinton agar and incubated at 37°C for 24 h. Classic bacterial counting method was used to count the colonies of bacteria and the results were given as the number of colony forming units (CFU). Statistical analysis was done by using SPSS software version 21 (IBM Statistics IBM Corp., NY, USA). The data were analyzed using Kruskal–Wallis one-way analysis of variance [Table 1] and Mann–Whitney U-tests [Table 2]. Statistically significant value was set at *P* < 0.05.

**RESULTS**

Data regarding the number of bacteria extruded are presented in Table 1. Bacterial growth was observed in all the experimental groups. Most apical bacteria extrusion was seen when K-type stainless steel hand instruments were used with the step back technique. There were statistically significant differences between, Manual-control, K3XF with ProTaper gold, Edge taper platinum, Manual and control groups, and also Hyflex CM with Protaper gold, Edge taper platinum, Manual and control groups (*P* < 0.05). The differences between Manual-ProTaper gold groups, Manual-Edge taper platinum, and Protaper gold-Edge taper platinum groups were not statistically significant (*P* > 0.05).

**DISCUSSION**

The main purpose of this study was to evaluate the apical extrusion of intracanal bacteria as a result of shaping of the root canal by Step-back and Crown-down technique. A standardized tooth model was used to decrease the number of variables. Single rooted maxillary central incisors were selected with mature apices and least possible

| Groups                  | Total (n) | Mean (CFU/mL) | SD    |
|-------------------------|-----------|---------------|-------|
| Control                 | 10        | 9.30          | 10.08 |
| K-File Manual           | 10        | 206.60        | 91.04 |
| ProTaper Gold           | 10        | 203.90        | 91.43 |
| K3XF                    | 10        | 124.10        | 55.98 |
| Edge Taper Platinum     | 10        | 202.4         | 87.16 |
| Hyflex CM               | 10        | 133.00        | 63.75 |

SD: Standard deviation
Table 2: Mann-Whitney U-test for intergroup comparison

| Groups         | K-File Manual | ProTaper gold | K3XF  | Edge Taper platinum | Hyflex CM |
|----------------|---------------|---------------|-------|---------------------|-----------|
| K-File Manual  | -             | 0.96          | 0.02  | 0.96                | 0.04      |
| ProTaper Gold  | 0.96          | -             | 0.02  | 0.96                | 0.04      |
| K3XF           | 0.02          | 0.02          | -     | 0.03                | 0.70      |
| Edge Taper Platinum | 0.96          | 0.96          | 0.03  | -                   | 0.04      |
| Hyflex CM      | 0.04          | 0.04          | 0.70  | 0.04                | -         |

Table: Mann-Whitney U-test for intergroup comparison

P<0.05 significant

variations to minimize the effect of tooth morphology on the extrusion of the debris during instrumentation.[10] Access cavities were done in all teeth and the length of the canal was measured and working length was kept 1 mm short of the apical foramen with #10 file visible at the apex. If the working length is taken at the apex or instrumentation done beyond the apex would lead to extrusion of more debris.[11]

Fusobacterium nucleatum was used in this study as there was no literature on its usage. It is one of the several Gram-negative bacteria seen along with Prevotella intermedia, Porphyromonas gingivalis, and Peptostreptococcus micros in endodontic flare-ups. These organisms, in synergy, are able to worsen a periapical inflammatory lesion.[12-15] In the present study, bacterial extrusion model as described by Er et al.[16] was used unlike other studies where they measure the quantitative amount of debris extrusion which have shortcomings like inability to ensure that the collecting devices are not contaminated, and the material extruded is extremely low, usually in fractions of milligrams or micrograms making the collection of that fractions difficult.[16,17]

The cleaning and shaping were performed in each group according to the manufacturer’s instructions for all the file systems. The apical preparation was standardized at ISO size #25 irrespective of the type of preparation whether step back or crown down to avoid any variations in the amount of bacterial extrusion due to apical enlargement.[18] To preserve the bacteria in the canal and in the apically extruded debris, 10 ml of 0.9% saline solution was used for irrigation in all groups as it is not antimicrobial. Hence, the extrusion of the bacteria depended only on the mechanical action of cleaning and shaping of the instruments.

The results of this study demonstrated that all the instrumentation techniques whether manual step back technique or engine driven crown down technique, resulted in more bacterial extrusion when compared to control group with no instrumentation. Statistically significant differences where seen between the groups (P < 0.05).

All the file systems used in this study extruded the apical microbial debris thus null hypothesis is rejected. Although the method of use (i.e., rotational motion) is similar for these types of instruments, but due to variable cutting action of each instrument there may be differences in the amount of apically extruded bacteria. The engine driven systems, i.e., Protaper gold, Edge taper platinum, Hyflex CM and K3XF systems, uses the crown-down technique. According to Shovelton 1964, the greatest number of microorganisms in the root canal are found in the coronal third, hence initial preparation of the coronal section of the root canal helps in reducing the number of microorganisms which may be pushed apically.[19] In addition, pre-flaring of the coronal part might improve the instrument control during the preparation of the apical third of the canal, moreover the rotary motion tends to direct debris toward the orifice, which does the packing of the dentinal debris into the flutes of the rotary instruments, hence avoiding their compaction into the root canal.[1,20]

Many previous studies have proven that manual cleaning and shaping technique, using the hand k-files, in a step back method with linear filing motion creates a greater mass of debris.[14] The main reason for more apical extrusion of bacteria and debris might be, the file in the apical one third acts as a piston, which tends to push debris out of the foramen into the periapical area and also to flush out the debris coronally from the apical third very little space is available.[11,21,22]

K3 XF showed less extruded debris when compared with Protaper Gold and Edge taper platinum as the files have variable pitch which ensures the debris is driven more coronally. The files have better centering ability and stability due to the third radial land. K3 XF has better cyclic fatigue resistance and has a positive rake angle which increases the cutting efficiency. Their increasing variable helical flute angle from tip to handle helps to dislodge the dentin chips from the working area, coronally to the orifice. [23] These observations are in accordance with previous studies done by Ghogre et al., and Zan et al.[24,25]

Hyflex CM showed less extrusion similar to K3XF which is in accordance with the study conducted by Capar et al.[26] and Labbaf et al.[27] Unwinding the spirals of Hyflex, may lead to decrease in the cutting ability and cleaning efficiency of instrument, decreasing the production of dentinal chips, debris, and therefore less apical extrusion. ProTaper Gold files extruded apical debris similar to hand k-files. ProTaper gold and Protaper universal have a convex triangular cross-sectional design and a flute design that combines multiple tapers within the shaft, they cut the dentine more effectively with increased risk of debris and irrigants extrusion, the long pitch design of the ProTaper instruments can cause greater amount of debris and irrigants to be
extruded. The results of this study are in accordance with Mohammed et al.[28] and Alani and Al-Huwaizi.[29]

There are no studies done on apical extrusion of debris using the Edge taper platinum files hence was selected for the study. The files were introduced in the market with the claim that it is manufactured using heat-treatment technology and has a design similar to that of ProTaper Gold. So that could be the reason of similar amounts of extrusion like that in ProTaper Gold group.

Limitations of study

This is an in-vitro study done on extracted teeth with single root and straight canal. Multirooted teeth, curved canals, calcifications and other variations are not tested. Irrigating solutions required for proper disinfection of canal such as sodium hypochlorite, chlorhexidine, and ethylenediaminetetraacetic acid were not used which might have reduced the number of bacteria extruding through the apex. Only one strain of bacteria was tested. Rotary files of different blade designs and at different speeds were used which may influence the amount of debris extruded.

CONCLUSION

Within the limitations of this study, it is inferred that all instrumentation techniques extruded some amount bacteria through the apical foramen. Compared to Crown-down engine-driven groups, Step-back hand instrumentation technique extruded more bacteria, with significant difference in CFU. Although there was no significant difference in the number of CFU among the engine-driven techniques, K3XF and Hyflex CM extruded less bacteria when compared to Protaper Gold and Edge taper Platinum rotary file systems. However, several factors should be considered for better prognosis of the endodontic therapy, such as instrumentation technique, instrument type, size, preparation end points, and irrigation solutions used, which affect the amount of debris extruded from the root canal system.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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