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Apical extrusion of intracanal bacteria following use of various instrumentation techniques

A. Kuştarcı[1], K. E. Akpınar[1], Z. Sümer[2], K. Er[3] & B. Bek[4]

[1]Department of Endodontics, School of Dentistry; [2]Department of Microbiology, School of Medicine, Cumhuriyet University, Sivas; [3]Department of Endodontics, School of Dentistry, Karadeniz Technical University, Trabzon; and [4]Department of Prosthodontics, School of Dentistry, Cumhuriyet University, Sivas, Turkey

Abstract

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Aim To evaluate the number of bacteria extruded apically from extracted teeth *ex vivo* after canal instrumentation using a manual technique and three engine-driven techniques utilizing nickel–titanium instruments (K3, RaCe, and FlexMaster).

Methodology Seventy extracted human mandibular premolar teeth with similar dimensions were used. Access cavities were prepared and root canals were then contaminated with a suspension of *Enterococcus faecalis* and then dried. The contaminated roots were divided into four experimental groups of 15 teeth each and one control group of 10 teeth. *G1*. RaCe group: the root canals were instrumented using RaCe instruments. *G2*. K3 group: the root canals were instrumented using K3 instruments. *G3*. FlexMaster group: the root canals were instrumented using FlexMaster instruments. *G4*. Manual technique group: the root canals were instrumented using K-type stainless steel instruments. *G5*. Control group: no instrumentation was attempted. Bacteria extruded from the apical foramen during instrumentation were collected into vials. The resultant microbiological samples were removed from the vials and then incubated in culture media for 24 h. The number of colony-forming units (CFU) was determined for each sample. The data obtained were analysed using the Kruskal–Wallis one-way analysis of variance and Mann–Whitney *U*-tests, with $\alpha = 0.05$ as the level for statistical significance.

Results There was a significant difference between experimental-control and engine-driven-manual technique groups ($P < 0.05$). The manual technique was associated with the greatest extrusion of microorganism.

Conclusions All instrumentation techniques extruded intracanal bacteria apically. No significant difference was found in the number of CFU among the engine-driven techniques; manual techniques extruded significantly more microorganisms.

Keywords: apical extrusion, bacteria, engine-driven techniques.

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Introduction

The inter-appointment flare-up is a complication characterized by the development of pain, swelling or both, which commences within a few hours or days after root canal procedures. A true flare-up is of sufficient severity to require an unscheduled visit for emergency treatment (Siqueira 2003). Studies have reported varying frequencies of flare-ups, ranging from 1.4% to 16% (Morse *et al.* 1986, Torabinejad *et al.* 1988, Barnett & Tronstad 1989, Walton & Fouad 1992, Siqueira *et al.* 2002). The causative factors of inter-appointment flare-ups comprise mechanical, chemical and/or microbial injury to the pulp or periradicular tissues (Seltzer & Naidorf 1985, Torabinejad *et al.* 1988). Mechanical and chemical injuries are often associated with iatrogenic factors. Examples of mechanical irritation

Correspondence: Dr Kürşat Er, Department of Endodontics, School of Dentistry, Karadeniz Technical University, Trabzon 61080, Turkey (Tel.: +90 462 3774735; fax: +90 462 3253017; e-mail: kursater@ktu.edu.tr).

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causing periradicular inflammation include instrumentation (mainly overinstrumentation) and overextended filling materials. Examples of chemical irritation include irrigants, intracanal medicaments and overextended filling materials. However, microbial injury caused by microrganisms and their products that egress from the root canal system to the periradicular tissues is conceivably the major and the most common cause of inter-appointment flare-ups (Bartels *et al.* 1968, Seltzer & Naidorf 1985).

All preparation techniques and instruments have been reported to be associated with extrusion of infected debris, even when preparation is maintained short of the apical terminus (Vande Visse & Brilliant 1975, Martin & Cunningham 1982, Al-Omari & Dummer 1995, Reddy & Hicks 1998, Ferraz *et al.* 2001, Er *et al.* 2005, Tinaz *et al.* 2005). Vande Visse & Brilliant (1975) first quantified the amount of debris apically extruded during instrumentation. They found that instrumentation with irrigants produced extrusion, whereas instrumentation without irrigants produced no collectible debris. Martin & Cunningham (1982) reported that less debris was extruded when the intracanal preparation was accomplished with an ultrasonic instrument. Al-Omari & Dummer (1995) verified that techniques involving a linear filing motion, such as the stepback techniques, created a greater mass of debris than those involving some sort of rotational action. Reddy & Hicks (1998) were the first to compare apical debris extrusion between manual instrumentation and engine-driven techniques. When comparing the mean weights of apically extruded debris, they noted that the stepback technique produced significantly more debris than the engine-driven techniques and the balanced force technique.

The purpose of this study was to evaluate the number of bacteria extruded apically from extracted teeth *ex vivo* after canal instrumentation using a manual technique and three engine-driven techniques utilizing nickel–titanium instruments (K3, RaCe, and FlexMaster).

Materials and methods

Selection and prepration of teeth

Seventy freshly extracted human single-rooted mandibular premolar teeth with mature apices and curvatures between 0 and 10 degrees were selected. Buccal and proximal radiographs were taken (Schick Tech. Inc., Long Island City, NY, USA) to ensure that the teeth had single canals. Calcified canals and canals with large apical foramina were excluded. The teeth were cleaned of debris and soft tissue remnants and were stored in physiological saline solution at +4 °C until required. Endodontic access cavities were prepared (Endo Access Bur; Dentsply Maillefer, Ballaigues, Switzerland) in a high-speed handpiece. The pulp chambers were accessed, and any missing coronal tooth structure was replaced with acid-etched composite resin (Charisma; Heraeus Kulzer, Dormagen, Germany) to create a reservoir for contamination of the root canals with a suspension of *Enterococcus faecalis.* Pulp remnants were extirpated with a fine barbed broach, with care taken not to push the broach through the apical foramen.

Test apparatus

A previously described method was used (Er *et al.* 2005; Fig. 1). Briefly, vials with rubber stoppers were adjusted for use by using a heated instrument to create a hole through the in centre. The tooth was inserted under pressure into the rubber stopper, which was fixed to the cementoenamel junction by means of cyanoacrylate cement (Quickstar; Furkan Inc., Istanbul, Turkey). Two coats of nail varnish were applied to the external surface of all roots to prevent bacterial microleakage through lateral canals. The rubber stopper with the tooth was then fitted into the opening of the vial. The apical part of the root was suspended within the vial, which acted as a collecting container for apical material extruded through the foramen of the root. The vial was vented with a 27-gauge needle alongside the rubber stopper during insertion to equalize the air pressure inside and outside the vial and used to be an electrode for the electronic working length determination during canal instrumentation.

The entire model system was sterilized in ethylene oxide gas for a 12-h cycle using the anprolene and 74 °C gas sterilizer (Andersen Products Inc., Haw River, NC, USA).

Contamination with *E. faecalis*

A pure culture of *E. faecalis* (ATCC 29212) was used to contaminate root canals. A suspension was prepared by adding 1 mL of a pure culture of *E. faecalis* grown in brain–heart infusion broth (Difco, Detroit, MI, USA) for 24 h, to fresh brain–heart infusion broth. Then, McFarland standard number 0.5 was used to evaluate the broth to ensure that the number of bacteria was

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Figure 1 The experimental model system.

1.5×10^8 colony-forming units (CFU) mL^{-1}. Before contamination of root canals, a sterile size 15 K-file was placed 1 mm beyond the foramen to create a hole in the nail varnish that covered the apical foramen. In this way, a standard size of foramen and apical patency was achieved. After this procedure, each root canal was filled completely with the *E. faecalis* suspension using sterile pipettes using a size 10 K-file to carry the bacteria down the length of the canals. The contaminated root canals were then dried in an incubator at 37 °C for 24 h.

Glass vials were entirely filled with 0.9% NaCl solution. The tooth-rubber stopper-needle unit was fitted into the mouth of the vial. The contaminated roots were divided into four experimental groups of 15 teeth each, and one control group of 10 teeth.

Root canal preparation

One operator, using aseptic techniques, carried out the canal preparation and sampling procedures on each specimen under a Class I laminar airflow cabinet to prevent airborne bacterial contamination.

Working length determinaton in all teeth was achieved using an Endomaster (EMS, SA, Switzerland) endodontic handpiece with the electronic apex locator mode switched on. The lip clip was attached to the needle. K-files were attached to the file holder cord and placed into the root canals and advanced apically root canal until the LED read 1 mm. Engine-driven instruments were prepared with Endomaster endodontic handpiece at low-speed (300 rpm) and using the automatic reverse function mode.

A total volume of 7 mL 0.9% NaCl solution was used for each root canal as an irrigant because of the different numbers of the files in groups. The irrigant was delivered by disposible plastic syringes with a 27-gauge stainless steel needle that had been placed passively down the canal, up to 3 mm from the apical foramen without binding.

The instrumentation sequences used were:

Group 1 (RaCe group).
RaCe instruments (FGK, La Chaux-de-Fonds, Switzerland) were used in a crowndown manner according to the manufacturer's instructions using a gentle in-and-out motion. Instruments were withdrawn when resistance was felt and changed for the next instrument. File sequences used were: size 25, 0.06 taper was used half of the working length, size 25, 0.04 taper was used between half and two-thirds of the working length, instruments of size 20, 0.02 taper, 25, 0.02 taper, 30, 0.02 taper were used to the working length.

Group 2 (K3 group).
K3 instruments (SybronEndo, West Collins, CA, USA) were used in a crowndown manner according to the manufacturer's instructions using a gentle in-and-out motion. Instruments were withdrawn when resistance was felt and changed for the next instrument. File sequences were: size 25, 0.06 taper was used half of the working length, size 20, 0.06 taper was used between half and two-thirds of the working length, instruments

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of size 20, 0.04 taper, 25, 0.04 taper, and 30, 0.04 taper were used to the working length.

Group 3 (FlexMaster group).
FlexMaster instruments (VDW, Munich, Germany) were used in a crowndown manner according to the manufacturer's instructions using a gentle in-and-out motion. Instruments were withdrawn when resistance was felt and changed for the next instrument. file sequences used were: size 20, 0.06 taper was used half of the working length, size 30, 0.04 taper was used two thirds of the working length, size 25, 0.04 taper was used three quarters of the working length, size 20, 0.04 taper was used between three quarters of the working length and working length, instruments of size 20, 0.02 taper, 25, 0.02 taper, 30, 0.02 taper were used to the working length.

Group 4 (Manual technique group).
K-file instruments (Dentsply Maillefer) were used in a stepback manner (Walton & Rivera 2002) and preparation was performed with rotational forces (Walton & Rivera 2002). K-files were used first with a quarter clockwise rotation followed by a pull-back motion and used repeatedly until they reached the working length. Apical preparation was continued up to size 30 and the stepback technique was used with a reduction of 1 mm for each file until size 45.

Group 5 (Control group).
No instrumentation.

Prior to and at the end of canal preparation, 0.01 mL NaCl solution was taken from the experimental vials to count the bacteria; the suspension was plated on brain-heart agar at 37 °C for 24 h. Colonies of bacteria were counted using a classical bacterial counting technique (Collins *et al.* 1995) and the results were given as number of CFU.

Statistical analysis

Statistical tests were performed using SPSS (Version 9.0; SPSS Inc., Chigago, IL, USA). Data were analysed statistically using Kruskal–Wallis one-way analysis of variance and Mann–Whitney *U*-tests. The level of statistical significance was set at $P = 0.05$.

Results

No growth was observed when checking the sterilization of the whole apparatus. The mean numbers of extruded bacteria for the groups are presented in Table 1. The data was not normally distributed.

Table 1 The mean number of extruded bacteria for each instrumentation technique

Groups	Total (*n*)	Mean (CFU mL^{-1})	SD
RaCe	15	20.73	10.71
K3	15	16.73	8.08
Flexmaster	15	27.47	16.54
Maual Technique	15	41.87	23.74
Control	15	0.50	0.85

KW: 39.706; $P < 0.05$; SD, standard deviation.

Most apical bacteria were extruded when K-type stainless steel instruments were used with a stepback technique. There were statistically significant differences between RaCe-control and RaCe-hand, K3-hand, K3-control, FlexMaster-control, and hand-control group ($P < 0.05$). The difference between other groups was not statistically significant ($P > 0.05$).

Discussion

The aim of this study was to assess the apical extrusion of intracanal bacteria as a result of root canal shaping by four different instrumentation techniques. Common to all techniques were the amount and type of irrigant and the operator. To increase the probability that the amount of apically extruded bacteria was a result of instrumentation, a standardized tooth model was used to decrease the number of variables. The teeth were selected according to tooth type, canal size at the working length, and canal curvature. This ensured that the number of apically extruded bacteria was exactly to be due to the instrumentation technique and not to tooth morphology.

Enterococci faecalis was used as bacteriological marker. *Enterococci* are normally found in the human intestine but may be found also in the oral cavity. In plaque, saliva, on mucosal surfaces, and gingiva (Haapasalo *et al.* 1983, Smith *et al.* 1987, McCrary *et al.* 1989, Rams *et al.* 1992). *E. faecalis* has been implicated in persistent root canal infections and more recently has been identified as the species most commonly recovered from root canals of teeth with post-treatment disease (Sundqvist *et al.* 1998, Siqueira & Rocas 2004).

Martin & Cunningham (1982) demonstrated greater debris extrusion when canals were instrumented at a length where the file was observed to just protrude through the apical foramen versus 1 mm short of the

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apical foramen. Myers & Montgomery (1991) clearly showed that a working length 1 mm short of canal length contributed to significantly less debris extrusion. Beeson *et al.* (1998) reported that, when the instrumentation was performed to the apical foramen, significantly more debris was forced apically than when instrumentation was 1 mm short. In this study, canal working length was 1 mm short of the apical foramen and working length measurements were completed with on Endomaster electronic apex locating handpiece with 'autoreverse function mode'.

In the present study, 0.9% saline solution was used for irrigation because it has no antibacterial effect; in this way elimination and extrusion of bacteria depended on the mechanical action of the instruments. Previous studies (Byström & Sundqvist 1981, Ørstavik *et al.* 1991, Siqueira *et al.* 1999), in which no antibacterial irrigants were used, have reported that the mechanical action of instrumentation and irrigation was effective in reducing the number of bacterial cells in the root canal. However, total elimination of bacteria was not achieved in most of the treatments. Siqueira *et al.* (1999) demonstrated that the instrumentation by hand or rotary instruments and irrigation by the flushing action of saline solution are remove more than 90% of bacteria cells from the root canal.

The extrusion produced by the various techniques was expected, because it is considered as a side effect of all canal instrumentation techniques (Vande Visse & Brilliant 1975). The results of this study demonstrated that all the instrumentation techniques tested created apically extruded bacteria *ex vivo*. However, all engine-driven instruments using the crowndown technique extruded less intracanal bacteria than manual instruments using the stepback technique. Early flaring of the coronal part of the preparation may improve instrument control during preparation of the apical third of the canal (Goerig *et al.* 1982). Also a rotatory motion tends to direct debris towards the orifice, avoiding its compactation in the root canal (Beeson *et al.* 1998). Reddy & Hicks (1998) suggested that rotation during instrumentation, in both the engine-driven techniques and the balanced force technique, tended to pack dentinal debris into the flutes of the instruments and directed them towards the orifice. Er *et al.* (2005) first reported the amount of intracanal bacteria extruded apically during instrumentation using a new experimental model. Tinaz *et al.* (2005) reported that manual instrumentation with K-files and engine-driven instrumentation with the ProFile 0.04 taper system (Dentsply Maillefer) perform similar with respect to apical extrusion in teeth with varying extent of apical patency. They also found a tendency for increased apical extrusion with both techniques as the diameter of the apical foramen increased. In the normal stepback technique, the reason for more apical extrusion of bacteria may be that the file in the apical one third acts as a piston, that tends to push debris through the foramen and less space is available to flush out debris coronally.

In a previous study, Er *et al.* (2005) evaluated the number of bacteria extruded apically after root canal instrumentation using two engine-driven instruments. They reported that the difference between the two instrument groups was not statistically significant. The present results achieved a similar trend and the difference between the three engine-driven instrument groups was not significant.

Conclusions

All instrumentation techniques extruded intracanal bacteria apically. However, engine-driven nickel titanium instruments extruded less bacteria than a manual technique. No significant difference was found in number of CPU among the engine-driven techniques.

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