Journal of Applied Dental and Medical Sciences
NLM ID: 101671413 ISSN:2454-2288
Volume 9 Issue 3 July-Sept 2023

Original Research

https://www.ncbi.nlm.nih.gov/nlmcatalog/101671413
www.joadms.org

To compare and evaluate the apical extrusion of E. Faecalis from infected root canal system by using rotary and reciprocating file systems- An in vitro study

Uma Mahajan [1], Sonal Deole [2], Sadashiv Daokar [3], Jyoti Magare [4], Swapnil jadhav [5], Kalpana Pawar [5]

[1] Professor, Department of Conservative dentistry and Endodontics, C.S.M.S.S Dental college and Hospital, Aurangabad, Maharashtra, India
[2] Postgraduate student, Department of Conservative dentistry and Endodontics, C.S.M.S.S Dental college and Hospital, Aurangabad, Maharashtra, India
[3] Professor and H.O.D, Department of Conservative dentistry and Endodontics, C.S.M.S.S Dental college and Hospital, Aurangabad, Maharashtra, India
[4] Professor, Department of Microbiology and Pathology, C.S.M.S.S Dental college and Hospital, Aurangabad, Maharashtra, India
[5] Senior Lecturer, Department of Oral And Maxillofacial Surgery, M.I.D.S.R Dental college and Hospital, Latur , Maharashtra, India
[6] Professor, Department of Conservative dentistry and Endodontics, C.S.M.S.S Dental college and Hospital, Aurangabad, Maharashtra, India

ARTICLE INFO

Keywords:
Protaper,rotary,file system,

ABSTRACT

All available biomechanical preparation techniques and instruments are associated with some amount of apical extrusion of bacterial and pulpal content, which is considered as the major cause of root canal treatment failure. Hence the present study aims to evaluate the amount of apical extrusion with different file systems.

Aim: To compare and evaluate apical extrusion of E. faecalis from infected root canal system using rotary and reciprocating file systems.

Methods and materials: Sixty mandibular premolars teeth with single canals were collected. Access cavities prepared and WL measured by keeping 1 mm short of file penetration length. The teeth mounted on the bacteria collecting apparatus and contaminated with E. faecalis. The contaminated samples were then divided into three experimental groups and prepared according to manufacturers instructions. Group 1: ProTaper gold rotary file group; Group 2: Mtwo rotary file group; Group 3: RECIPROC blue reciprocating file group. The extrude was collected and incubated on brain–heart infusion agar Colonies counted in CFU number. Obtained data were statistically analysed and compared with ONE WAY ANOVA Test -using IBM SPSS-20 software.

Results: RECIPROC blue reciprocating file system extruded more bacterias when compared to ProTaper gold and Mtwo rotary file systems, Mtwo rotary file system extruded least number of bacteria. Conclusion: All instrumentation techniques extruded intracanal bacteria apically. However, Rotary file systems showed less bacterial extrusion than the Reciprocating file system.

Introduction

The main objective of root canal treatment is the debridement and disinfection of root canal system. The root canal anatomy is unpredictable with number of variations therefore it is important to use appropriately designed endodontic instruments(1).

All the documented root canal instrumentation techniques leads to some or more amount of apical extrusion containing number of intra-radicular bacteria, dentinal chips, necrotic pulp tissue, irrigants etc. Among the products extruded apically, the major cause of root canal treatment failure is intraradicular bacteria. These bacteria are gram positive, gram negative and obligate anaerobes; Enterococcus faecalis, Fusobacterium nucleatum, Propionibacterium alactolyticus, Propionibacterium propionicum are the commonly isolated species of microbes from root canal(1-5,6).

Amongst the diverse bacterial species, Enterococcus faecalis shows highest resistance to chemo-mechanical endodontic procedures; it can survive in nutrient-poor environments and is most commonly associated with persistent apical periodontitis. (4)

Currently available all the preparation techniques such as

Corresponding author: Dr. Sonal, Department of Conservative dentistry and Endodontics, Csmss Dental College, Kanchanwadi Aurangabad – 431011
E-mail address: sonaldeole2013@gmail.com; Phone number: 9922219691

step-back, crown down, crown down pressureless etc; instruments such as conventional, rotary and reciprocating file systems with advanced material, shape, pitch, taper and the motion cycles are associated with some amount of apical extrusion(5).

Studies have shown that significantly higher amount of apical extrusion is seen with push-pull than the rotary motion. Even though Rotary instruments have a tendency to auger dentinal debris into the flutes of the file and directs it coronally; it has facilitated and accelerated the root canal procedures; apical extrusion continues to occur (5-7).

Despite the fact that the Reciprocation motion mimics the kinematics of balanced force technique, which is proven to be a pressure less technique; Reciprocating instruments shows larger amount of dentin cutting in short time period creating forceful movements which results in large apical extrusion(5,8).

Hence the aim of this study is to evaluate which file system and motion (rotary or reciprocating) would show more apical bacterial extrusion and therefore increase the risk of flare-ups.

Method and material

Selection and preparation of teeth- Sixty freshly extracted human mature mandibular premolar teeth underwent digital radiographs to check for a single canal. Access cavities prepared and working length measured with K file which were considered 1 mm short of the file penetration length.

Test apparatus preparation- Glass vials with holes in the center of the rubber stoppers fixed at the cemento-enamel junction in rubber stopper. Nail varnish of two coats applied onto the roots external surface for prevention of micro leakage. The glass vial acts as a collecting container for the apical material extrusion. The rubber stopper vented with a 23-guage needle for equalizing the air pressure outside and inside. The entire model system then sterilized in an Autoclave. (Fig 1)

Contamination with E. Faecalis - A pure culture of E. Faecalis used to contaminate root canals. A sample prepared by adding 1 ml of a pure culture of E. Faecalis, grown in brain– heart infusion broth for 24 h, to fresh brain–heart infusion broth. Each root canal were completely filled with the E. Faecalis sample using insulin syringe and carried to working length with 10 K-file. The contaminated root canals were then placed in incubator at 37o C for 24 h.

Before the experiment, the vials filled with Normal saline solution. A hole created in the nail varnish that covered the apical foramen using a 10 K-file. During this procedure, only 1–2 mm of instrument extruded. The contaminated roots then divided into three experimental groups of 20 teeth each. Group 1, PROTAPER GOLD rotary file group; (fig 2) Group 2, Mtwo rotary file group; (fig 3) Group 3, RECIPROC blue reciprocating file group. (fig 4)

Before the beginning of and after the end of laboratory tests, 0.1 mL Normal Saline solution taken from the experimental vials in order to count the bacteria; the suspension incubated in brain–heart infusion agar at 37oC for 24 h. Colonies of bacteria were then counted and the results given as number of CFU. (fig 5a and 5b)

Root canal preparation- Root canal preparation carried out under aseptic conditions. Glide path prepared with 10k file and Canals enlarged upto 25 number k file.

Irrigation- Between instrumentation each canal irrigated with 2 ml of 3% sodium hypochlorite using a syringe and a 30-gauge needle that were placed 1 mm short of the working length. Final irrigation were carried out with 5 ml of normal saline.

Subsequently root canal preparation carried out with different file systems in different groups, corresponding to the sequence of instrumentation.

Group 1, ProTaper gold rotary file group. ProTaper Gold file used in a crown down manner according to the manufacturer's instructions using a gentle in and out motion. The instrumentation sequence were SX instrument at two-thirds of working length, S1 and S2 at 1 mm less than working length, and then F1, F2 upto working length.

Group 2, Mtwo rotary file group. Mtwo instruments were also be used in a crown down manner according to the manufacturer's instructions using a single length technique with a gentle in-and-out motion. Therefore, all files of the instrumentation sequence used to the full Working length of the root canal.

Group 3, RECIPROC blue reciprocating file group. According to the manufacturer's instructions a single file used for complete root canal preparation. The instrument advanced apically using an in -and - out pecking motion. Gentle apical pressure applied with a brushing action against the lateral walls. This procedure repeated until the instrument reach working length.

Result:

All the values obtained from the study were tabulated and subjected to the statistical analysis using ANOVA test and Post- hoc Tukey's test using IBM SPSS-20 software, at the significance level of 0.05 ($P \leq 0.05$=Significant).

Data regarding the number of bacteria extruded are presented in Table 1,2 and graph 1 and 2. Bacterial growth was observed in all the experimental groups. Most apical bacteria extrusion was seen with RECIPROC blue

reciprocating file system. There were statistically significant differences between ProTaper gold, Mtwo rotary file group and RECIPROC blue reciprocating file group ($P < 0.05$). The differences between Mtwo rotary file and ProTaper gold rotary file groups were not statistically significant ($P > 0.05$).

Discussion

Debris gets extruded into the periradicular tissue during root canal instrumentation (9,10) which can cause inflammation, delay periapical healing and lead to postoperative pain and flare-up in both vital and necrotic pulp cases.(4,11-13) Seltzer, et al.(12) indicated that a quiescent chronic inflammatory periapical lesion may give a violent inflammatory reaction after the endodontic instrumentation. (14,15)

Post treatment flareup is one of the common problems that the practitioner encounters following endodontic treatment which is an undesirable and distressing situation. (4,16-17) Hence practitioner should adopt clinical procedures that have the potential to prevent or at least reduce the incidence of Flare-ups (18,19) one of this procedure is selection of instrumentation techniques that extrude less amount of debris apically. All instrumentation techniques extrude some debris apically, but there are differences among them.

Hegde MN, Thatte S (20) and Fairbourn DR, McWalter GM (21) concluded that the Stepback shows more extrusion than Crown down technique. (23) Ruiz-Hubard et al.(24) less extrusion with crown-down pressureless than step-back. (24) Al-Omari and Dummer (25) reported linear filing motion create a greater mass of debris. The filing action of the instrument may act as a piston, pumping the debris through the apex. (9,25, 27-29) Bürklein and Schafer (30) found that rotary motion had lower apical than Reciprocating motion.

ROTARY MOTION: Rotation convey debris in coronal direction; provides a more constant 360° engagement of the file tip in the canal that forces it to follow the canal and results in better control for maintaining the central axis of the canal, reducing the incidence of ledging or perforation. (31,32)

RECIPROCATING MOTION: In 1985, Roane et al (33) introduced the balanced force instrumentation. Numerous reports indicated good results that were obtained with this technique for preparation of curved canals. (33, 19, 31) In 2008, a new concept of reciprocation was proposed by Dr. Ghassan Yared(34) using only one NiTi instrument. Advantages of reciprocating motion are binding into the root canal dentin is less frequent, reducing torsional stress; decreased risk of instrument fracture; lower cyclic fatigue. (35) Studies by You et al. (36) and Franco et al. (37) found minimal differences in cleaning and shaping ability of rotary and reciprocating motions.

ProTaper Gold ROTARY FILE (Dentsply Maillefer, Ballaigues, Switzerland): Works in crown down manner using a gentle in and out motion with instrumentation sequence SX, S1, S2, F1 and F2 according to manufacturers instructions. (38,39)

Mtwo ROTARY FILE (VDW dental): Works in a crown down manner with light pressure in an apical direction, using a single length technique with a gentle in-and-out motion. (40,41)

RECIPROC blue RECIPROCATING FILE (VDW dental): A single file R25 Red (0.25-8%) having a size 25 at the tip and a taper of 0.08 over the first 3 mm with 0.5 N.cm torque used for complete root canal preparation. (41)

Many factors affect the amount of extruded intra-canal material. These factors include the instrumentation method / technique, motions, the instrument type and size, the size and length of the canal, the preparation endpoint, and the type and the amount of irrigant used. In the present study, to minimize the effects of the aforementioned factors, standard conditions (other than the instrumentation system used) were created for all of the groups. (3,16, 42)

The teeth were imaged using RVG to ensure that they had single canals and orifices. Single rooted mandibular premolar teeth with straight canals were selected with mature apices and least possible variations to minimize the effect of tooth morphology on the extrusion.(1,2,16,43)

In previous studies, Reddy and Hicks(27)used single-rooted mandibular premolars, Myers and Montgomery(44) used single-rooted maxillary lateral incisors and mandibular premolars, Ferraz et al.(26) used maxillary and mandibular central and lateral incisors.

The working length was kept 1 mm short of the file penetration length. The study by Myers GL, Montgomery S.(44) demonstrated more debris extrusion would have occured if the working length is taken at the apex or beyond the apex. (1,21,44)

A standardized bacterial extrusion model was used to decrease the number of variables as described by Er et al (2)

E. faecalis was used in this study as the bacteriological marker. It is a non fastidious, easy-to-grow aerobic bacterium which has the ability to penetrate dentinal tubules and survive even under unusual environmental stresses and may be extremely resistant to medications used during the endodontic therapy; more associated with asymptomatic cases. (7,20,2,45)

Common to all techniques were the amount and type of irrigant and the operator. (1,25) To simulate the irrigation system with commonly used irrigation systems 5 ml of 3%

Figure 1: Test apparatus

Fig 2 : ProTaper gold rotary file

Fig 3 : Mtwo rotary file

Fig 4 : RECIPROC blue reciprocating file

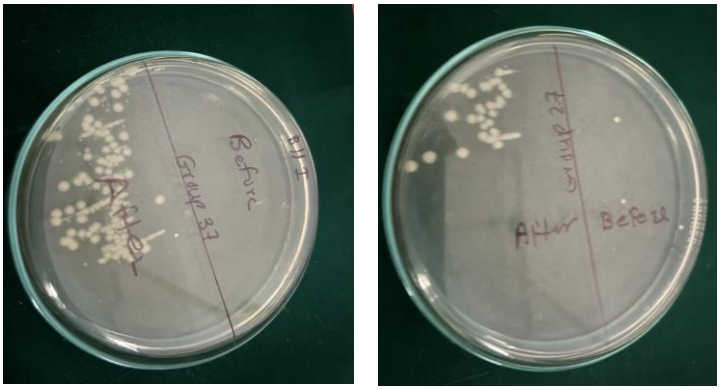


Fig 5a and 5b: Colonies of bacteria grown on BHI Agar plate of sample 37 (RECIPROC blue file group) and 27 (Mtwo file group)

sodium hypochlorite and 10 ml of 0.9% saline solution was used for irrigation in all groups.

The side vented irrigation needle was placed at an established level i.e 1 mm short of the working length and the irrigant was passively injected over 10 seconds to minimize uncontrolled forces on the irrigation syringe. (46)

The apical preparation was standardized at ISO size 25 to avoid any variations in the amount of bacterial extrusion due to apical enlargement (1).

The results of this study demonstrated that all the instrumentation techniques resulted in bacterial extrusion when compared to control group (before instrumentation) with no instrumentation which is in agreement with the

Tabel 1: Comparison of extrusion between ProTaper, Mtwo and RECIPROC blue file systems and Rotary and Reciprocating Motion

	Mean Difference	Std. Deviation	t	df	P value	Result
Protaper	4.75	4.90	4.34	19	0.00	Significant
M2	5.00	3.77	5.93	19	0.00	Significant
Reciprocating Blue	11.80	9.90	5.33	19	0.00	Significant

Table 2 : Multiple Comparison (post Hoc Test)

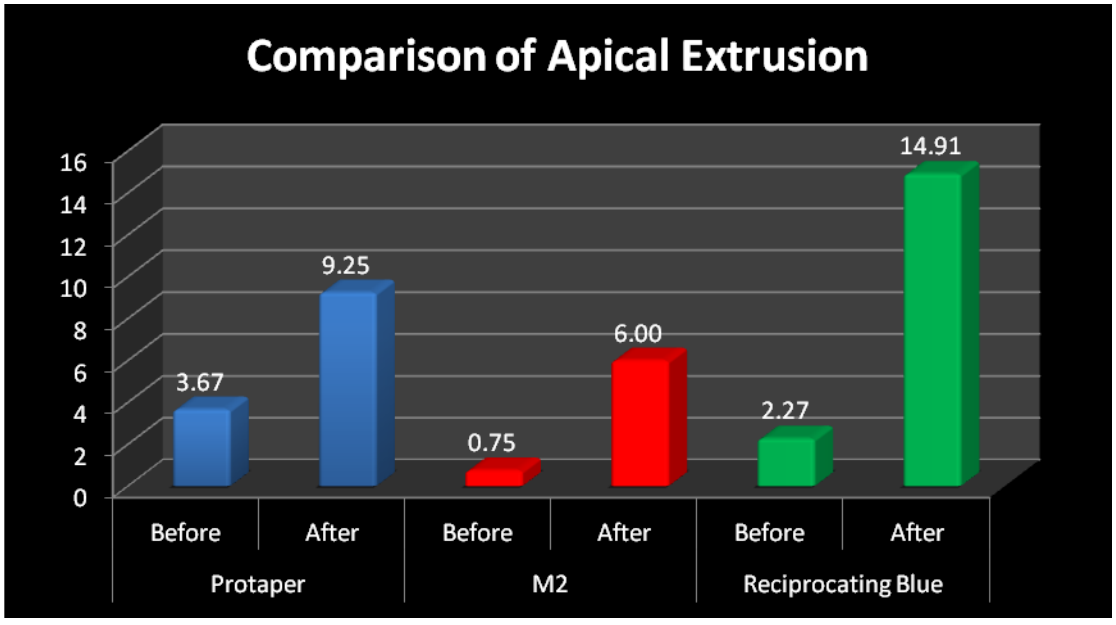
Group I	Group J	Mean Difference (I-J)	Std. Error	Sig.	Result
Protaper	M2	-.250	2.130	.907	Not Significant
	Reciprocating Blue	-7.050*	2.130	.002	Significant
M2	Protaper	.250	2.130	.907	Not Significant
	Reciprocating Blue	-6.800*	2.130	.002	Significant
Reciprocating Blue	Protaper	7.050*	2.130	.002	Significant
	M2	6.800*	2.130	.002	Significant

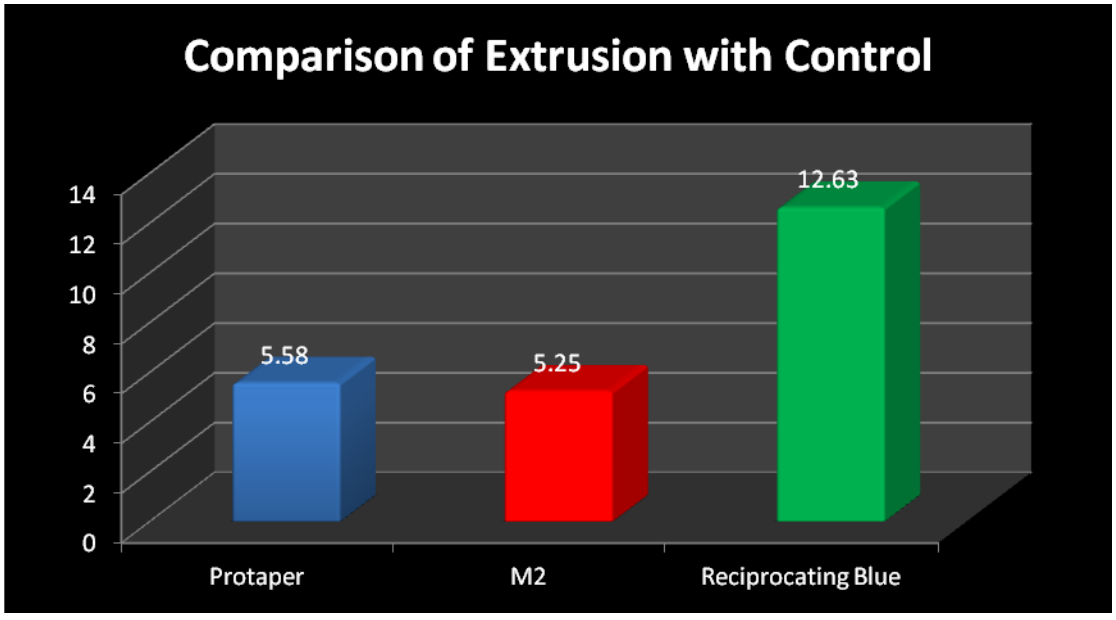
results of previous studies. (3,27,47) These observations are in agreement with previous findings that rotary systems were associated with less debris extrusion than reciprocating systems. The obtained differences between the instruments might have been caused by the i) preparation technique, ii) the different tapers and iii) the cross-sectional design of the instruments. (16)

The reciprocating file RECIPROC blue extruded significantly more debris compared to the multiple-file rotary instrument. These observations are supported by studies from Burklein & Schafer 2012 (30) Kucukyilmaz E, Savas S;(16).

The results of the present study are in contrast with the results of the studies of Kocak S, Kocak MM, Saglam BC et al.(43) and De-Deus G, Neves A, Silva EJ et al; (47)

According to Shovelton 1964, the greatest number of microorganisms are found in the coronal third, hence initial preparation of the coronal section reduces the number of microorganisms. It creates space large enough for debris to be rinsed away in a coronal direction. (4,17,20)





The single-file systems works faster and takes shorter time (16,23), thus they form large amount of debris in short time which creates forceful movement resulting in large apical extrusion. Continuous rotary motion may improve coronal transportation of dentin chips and debris by acting like a screw conveyor which does the packing of the dentinal debris into the flutes of the rotary instruments, hence avoiding their compaction into the root canal (4,20,13) while reciprocal working motion might explain the greater amount of debris extrusion caused by this system.(16)

However, based on the available literature, it was not possible to determine whether it was the reciprocal motion that was responsible for the increased risk of debris extrusion. Further studies using standardized apical tapers are required to answer this question. (16)

Conclusion

All the file system groups showed apical extrusion of E. faecalis from infected root canal systems. Comparison of

mean apical Extrusion within Rotary and Reciprocating File Motion showed significantly more apical extrusion with Reciprocating motion than with Rotary motion. A statistically significant difference in the apical extrusion of ProTaper Gold, Mtwo and RECIPROC blue file systems was seen. Mtwo and ProTaper Gold file systems showed less apical extrusion than RECIPROC blue file system. Highest mean apical extrusion was seen with RECIPROC blue Reciprocating file system. No statistically significant difference in mean apical extrusion of Mtwo and ProTaper Gold Rotary file systems were seen. For the final conclusion further studies should be done to evaluate the efficacy in apical extrusion of Rotary and Reciprocating motion with all the other standardizations.

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