



## **Evaluation of Apical Extrusion of E-Faecalis during Root Canal Instrumentation with Self Adjusting file, Twisted file Adaptive & Wave One Gold**

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### **Abstract**

#### **Introduction**

All preparation techniques and instrumentation have been reported to be associated with extrusion of infected debris, irrespective of the preparation being maintained short or up to the apical terminus. Extrusion of even a small amount of debris can provoke postoperative inflammation, pain and delay the healing process

### **Aim**

To compare the apical extrusion of E. Faecalis using Self Adjusting File System, Twisted file Adaptive, and WaveOne Gold file system.

### **Methods and Materials**

A total of 120 extracted human single rooted tooth with intact cementum were selected for this study, the prepared samples were then randomly divided into 4

groups with sample size of 30 in each group namely- Self Adjusting File, Twisted File Adaptive, WaveOne Gold and Control group.

Test apparatus to determine apical extrusion of intracanal bacteria. A pure culture of *E. Faecalis* (ATCC29212) was used to contaminate the root canal.

All three groups were instrumented as per the manufacture instruction except the control group.

### **Statistical Analysis**

Comparison of mean and SD between all groups was done by using one way ANOVA test, after which Post Hoc Turkey's HSD test was carried out to assess whether the mean difference between a pair of group is significant or not.

### **Results**

Mean colony count for Self adjusting file group is  $423 \pm 35.19$  CFU/ml. Mean colony count for Twisted File Adaptive group is  $557.73 \pm 23.28$  CFU/ml. Mean colony count for WaveOne Gold group is  $642.23 \pm 13.86$  CFU/ml.

Mean colony count for control group is 0.

### **Conclusion**

It means the colony count of *E. Faecalis* formed in Self Adjusting File system is less as compared to Twisted File Adaptive and WaveOne Gold.

### **Keywords**

Self Adjusting file, twisted file Adaptive, WaveOne Gold & *E. faecalis*

### **Introduction**

All preparation techniques and instrumentation have been reported to be associated with extrusion of infected debris, irrespective of the preparation being maintained short or up to the apical terminus.[1-5] Extrusion of even a small amount of debris can provoke postoperative inflammation, pain and delay the

healing process.[6] Its complication may include pain, swelling or both, the combination is called flare-up.[7] The flare-up incidence during endodontic treatment ranged between 1.4% upto 16% results in an antigen-antibody complex formation leading to severe inflammatory response and post-operative flare-up.[8] However main microbial species causing the failure of root canal treatment include

*Enterococcus Faecalis*, *Propioni bacterium lactolyticus*, and *Propioni bacterium propionicum*. [9] In particular, *E. Faecalis* is the most commonly isolated species for the treatment of diseases.[10] *E. Faecalis* is gram-positive cocci that singly in pairs or short chains can survive harsh environments include in g extreme alkaline pH, salt concentrations. It resists bile salts, detergents, heavy metals, ethanol and a zide desiccation. It can survive a temperature of  $60^{\circ}\text{C}$ . The prevalence of *E. Faecalis* in primary endodontic infection is 40% and in persistent endodontic infection 24% to 77%. [11,12] A recent review reported that inflammatory reaction due to debris extrusion is influenced by the type of movement and instrument design. However, there have been contradictory results on motion kinematics and debris extrusion.[8] Thus this study was done to compare the apical extrusion of *E. Faecalis* using Self Adjusting File System, Twisted File Adaptive, and WaveOne Gold and to evaluate their efficiency in minimizing the amount of apical debris extrusion during the use.

### **Materials and Methods**

The present study was designed as a prospective cross-sectional study. 120 freshly extracted human single-rooted teeth with complete root formation were extracted from patients above 50 years of age requiring extractions due to periodontal reasons. Teeth with canal curvature  $0-10^{\circ}$  and apical diameter

confirming to 10 No. K file (DENTSPLY Maillefer, Ballaigues, Switzerland) were selected for the study. These freshly extracted teeth with intact cementum were cleaned for stains, tissue debris, calculus and were stored in 0.2% thymol after that the teeth were immersed in 5% sodium hypochlorite (NaOCl, Vishal Dento Care Pvt. Ltd. Ahmedabad, India) for 30 min to remove organic tissues.

### **Test Apparatus**

Test apparatus to determine apical extrusion of intra canal bacteria. The tooth was forced through the rubber stopper of a vial after endodontic access cavity preparation. Two coats of nail varnish were applied to

the external surface of the root and then the tooth with the rubber stopper fitted into the mouth of the vial. A 23-gauge needle was inserted into the vial through the rubber stopper to equalize the air pressure. The entire apparatus was sterilized in an autoclave. Before the experiment, the vial was filled with normal saline solution. The hole was created in nail varnish that covered the apical foramen using a 10 No. K-file. In this way, the standard size foramen and apical patency were achieved. The tooth with a rubber stopper was placed into the mouth of the vial. The same procedure was repeated to all experimental teeth.



**Figure 1:** Test Apparatus

### **Preparation of Enterococcus Faecalis**

A pure culture of *E. faecalis* (ATCC29212) was used to contaminate the root canal, the suspension was prepared by adding 1 ml of pure culture of freshly prepared *E. faecalis* grown in the brain–heart infusion broth for 24 h (BHI; Himedia Laboratories Pvt. Ltd; Mumbai, India). The McFarland standard number 0.5

was used to evaluate the broth to ensure that number of bacteria was  $1.5 \times 10^8$  colony forming units (CFU) ml/l. The root canal was filled with the *E. faecalis* suspension. During incubation, canals were hand instrumented with 10 No. K-file to carry the bacteria down the length of the canal. The contaminated root

canal was dried at 37°C for 24h. Single operator, using aseptic techniques, carried out the preparation and sampling procedures on each specimen under a class I

laminar air flow cabinet to prevent airborne bacterial contamination.



**Figure 2: Broths Medium Sample Distribution**

The prepared samples were then randomly divided into 4 groups with a sample size of 30 in each group

Samples were equally divided into four groups for instrumentation with different techniques:

**Sample size**

**Instrumentation**

**Sample Distribution**

The prepared samples were then randomly divided into 4 groups with sample size of 30 in each group

GROUP No.	FILES	SAMPLE SIZE
1	The Self-Adjusting File (SAF)	30
2	The Twisted File Adaptive (TFA)	30
3	The WaveOne Gold (WOG)	30
4	Control	30

**Group1:** The Self-Adjusting File (SAF)

The Self-Adjusting File (SAF; ReDent-Nova, Ra'anana, Israel) is a single file system, which is a hollow compressible design. The SAF is used with vibrating movement accompanied by continuous irrigation with any desired solution. Thirty teeth were prepared with SAF as per the manufacturer's instructions.

**Group2:** The Twisted File Adaptive (TFA)

The Twisted File Adaptive (TFA; Sybron Endo, Orange, CA, USA) system's unique motion features a combination of continuous and reciprocating movement. The system first rotates the file clockwise (CW), and when the TFA instrument is subjected to no or very light stress, the system works with intermittent rotation with 600° CW and stops. On the other hand, with increased instrumentation stress, the TFA instrument adapts to a reciprocating motion. Thirty teeth were prepared with TAF as per the manufacturer's instructions.

**Group3:** The WaveOne Gold (WOG)

The WaveOne Gold (WOG; DENTSPLY Maillefer, Ballaigues, Switzerland) is a novel file system manufactured using a thermal process that enhances the cyclic fatigue resistance and flexibility of the instruments. This single file reciprocating system has four tip sizes: Small (20/0.07), Primary (25/0.07), Medium (35/0.06), and Large (45/0.05). The files have a parallelogram-shaped off-centre cross-section with 85° cutting edge in contact with the canal with a variable and reducing taper. Thirty teeth were prepared with WOG as per the manufacturer's instructions.

**Group4: No instrumentation (Control)**

No instrumentation was performed. Scouting was done with a 10 No. K file after each instrument to ensure canal patency. A volume of 7 ml saline was used as an irritant during the instrumentation of each sample. The

entire procedure was performed under a class II laminar air flow cabinet to prevent air-borne bacterial contamination. Subsequently, after root canal preparation 0.1 ml of saline was taken from the experimental vial to count the bacteria and incubated in BHI agar (Himedia Laboratories Pvt. Ltd; Mumbai, India) at 37°C for 24 h. Colonies of bacteria were counted using a colony counter (Yarco colony counter) following a classical bacterial counting technique as described. Scouting was done in all four groups SAF, TFA, WOG, Control with 10 No. K File.

**Statistical Analysis and Methods**

Data was collected by using a structure proforma. Data entered in MS Excel sheet and analysed by using SPSS 23.0 version IBM USA. Quantitative data were expressed in terms of Mean and Standard deviation. Comparison of mean and SD between two groups was done by using an unpaired test to assess, whether the mean difference between groups is significant or not. Descriptive statistics of each variable was presented in terms of Mean, standard deviation, standard errors of the mean.

Comparison of mean and S.D. between all groups was done by using one-way ANOVA test. If ANOVA comes significant, then Post Hoc Turkey's HSD test was carried out to assess whether the mean difference between a pair of the group is significant or not. A p-value of <0.05 was considered as statistically significant whereas a p-value <0.001 was considered as highly significant.

**Result**

**Table (1):** Comparison of colony count (CFU/ml) between all files systems

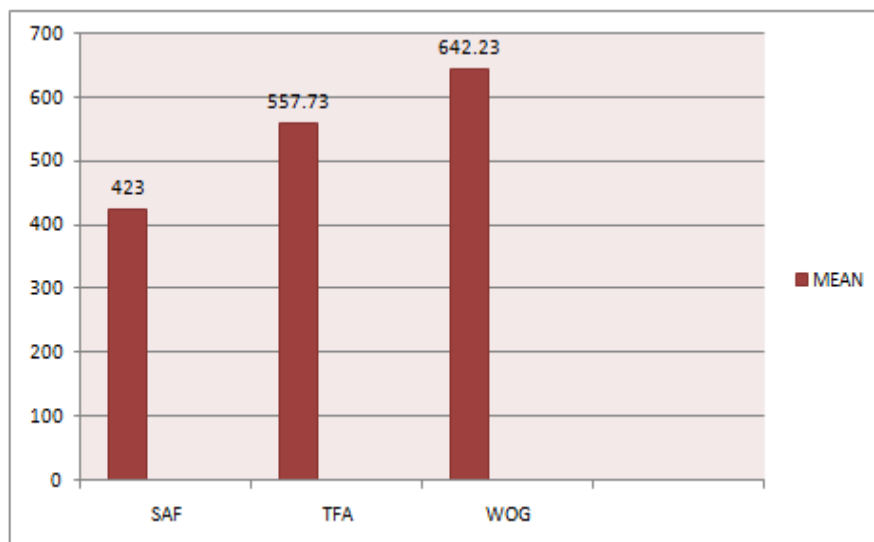
	GROUP	N	MEAN	STD DEVIATION	F	P	INFERENCE
Colony count (CFU/ml)	SAF	30	423.00	35.19	4948.07	0.00001 (<0.001)	Highly significant
	TFA	30	557.73	23.28			
	WOG	30	642.23	13.86			
	CONTROL	30	.00	.00			
	TOTAL	120	405.74	248.96			

The mean colony count of the SAF group is 423±35.19CFU/ml. The mean colony count of the TFA group is 557.73±23.28 CFU/ml. The mean colony count of the WOG group is 642.23±13.86 CFU/ml. The mean colony count of the control group is 0.

To know whether the mean difference between individual groups is significant or not we applied Post Hoc Turkey’s HSD test. The results are as follows:

**Table (2):** Indicates that the difference in the mean is significant at 0.01 level

	TFA	WOG	CONTROLS
SAF	-134.33**	-219.23**	423**
TFA		-84.5**	557.73**
WOG			642.23**





**Graph (1):** Comparison of colony count (CFU/ml) between all files systems

This test shows that the mean difference in colony count between SAF and TFA, between SAF and WOG, between TFA and WOG, is statistically highly significant ( $p < 0.001$ ). Also when the three file systems are compared with the control group, the resulting difference between the mean colony counts is significant. ( $p < 0.05$ ) It means the colony count formed in the SAF file system is less as compared to TFA and WOG.

### **Discussion**

*E. Faecalis* has been used as a biological marker in a great part of endodontic studies and its importance for endodontic research is well documented.[13-18] *E. Faecalis* is a Gram-positive coccus, facultative anaerobe, present in human gastrointestinal infections and common in secondary apical periodontitis. The microbial flora of teeth with persistent apical periodontitis presents mainly simple species of Gram-positive organisms, *E. Faecalis* represents the species most commonly recovered, and the overall healing rate of re-treatment was 74%.[19] Adhesion to the dentin surface is an essential step that determines the pathogenic potential of *E. Faecalis*. Kristich, et al, used independent experimental approaches to characterize bio film formation by *E. Faecalis*, which forms robust bio films and its development is modulated by the prevailing environmental conditions. [20]

The endodontic flare-up is a true complication characterized by the development of pain, swelling, and discomfort which commences within a few hours or days after root canal procedures and requires an emergency treatment.[21] The major reason cited for such distressing occurrence is extrusion of debris

present within and created during instrumentation of root canal system into periapical tissues resulting in persistent periapical inflammation. [22] Shovelton DS, Seltzer, Naidorf and Siqueria have reported that along with debris, the bacteria are also extruded through the apical foramen. [23,24] The number of bacteria extruded apically has a direct correlation with the weight of debris (quantitative factor), type, and virulence of bacteria (qualitative factor). Naidorf studied the immunological aspects of flare-up and stated that various mediators released during flare-up will cause damage to the cell membrane resulting in prostaglandin release, bone resorption, amplification of the kinin system and ultimately pain for the patient.[25] Furthermore, Perrini and Fonzi have found numerous mast cells in human periapical lesions discharging vasoactive amines into the periapical tissues and initiating an inflammatory response. [25,26] The present study aimed to assess the extrusion of intra canal bacteria as a result of canal shaping by different instrumentation techniques.

The methodology employed in this study was similar to that described by Er et al. The amount and type of irrigant and operator are common to all the techniques, if *E. Faecalis* was chosen as the bacteriological marker because it can survive alone without symbiotic support from other bacteria.

Since Vande Visse and Brilliant conducted the first study concerning the apical extrusion of debris in 1975, up to date variable factors that may affect apical debris extrusions such as irrigation, preparation technique, and nickel-titanium (NiTi) file systems used for preparation have been studied.[27-29]

In this study, a generally accepted experimental model was used to collect apically extruded debris,[30] it is important to emphasize that the current results

cannot be directly extrapolated to the clinical situation because of the absence of any periapical tissue simulation that may inhibit debris extrusion. Although the periapical tissues are not mimicked, this technique allows a comparison of the file systems. The literature presents controversial results, especially when comparing reciprocating single file and continuous rotation multi file systems in terms of apical extrusion. [31]

Concerning movement kinematics, some authors have stated that reciprocal motion may act as a mechanical piston that appears to increase the transportation of debris toward the apex, while continuous rotation provides the coronal transportation of dentin.[32,33] However, other authors have suggested that reciprocating motion imitates the balanced force technique that causes less debris extrusion.[34,35]

The present study compared three different instrumentation protocols, a vibratory file (SAF), a continuous and reciprocating file (TFA) and a reciprocating file (WOG), a comparison yet to be reported in the literature. The results obtained from the current study may be explained by differences in the instrument design, movement kinematics between the SAF, TFA and WOG systems. SAF a single-file system, devoid of a central metal core and any cutting edge or flutes, instead has an abrasive surface.[34] The SAF is operated with a transline in-and-out vibratory motion and associated with continuous simultaneous irrigation. This continuous flow of the irrigant does not build up any pressure in the canal as the metal meshwork allows the free escape of the irrigant. In the narrowest apical part of a canal prepared up to 20 No. K-file, the SAF is effective; leaving more than 38% of the canal cross-section free for back flow of fluid and dentinal debris.[36,37] deMelo Ribeiro et al stated that in the apical third, the SAF system created cleaner inner

canal walls when compared to the rotary system.[38] In our result, the mean colony count of the SAF group is  $423 \pm 35.19$  CFU/ml. The mean colony count of the TFA group is  $557.73 \pm 23.28$  CFU/ml. When we compared the mean colony count between two files, it was found to be statistically highly significant ( $p < 0.001$ ). (**Table 1, Graph 1**)

In our result, the mean colony count of the SAF group is  $423 \pm 35.19$  CFU/ml. The mean colony count of the WOG group is  $642.23 \pm 13.86$  CFU/ml. When we compared the mean colony count between two files, it was found to be statistically highly significant ( $p < 0.001$ ). (**Table 2**) The Twisted File Adaptive (TFA; Sybron Endo, Orange, CA, USA) system's unique motion features a combination of continuous and reciprocating movement. The system first rotates the file clockwise and when the TFA instrument is subjected to no or very light stress, the system works within term it tent rotation with  $600^\circ$  clockwise and stops.

On the other hand, with increased instrumentation stress, the TFA instrument adapts to a reciprocating motion. SM1(20/0.05), SM2(25/0.06), and SM3(35/0.04) files are available for narrow canals.

In our study, the mean colony count of the TFA group is  $557.73 \pm 23.28$  CFU/ml. The mean colony count of the WOG group is  $642.23 \pm 13.86$  CFU/ml. When we compared the mean colony count between two files, it was found to be statistically highly significant ( $p < 0.001$ ). (**Table 2**) The WOG file was associated with the maximum debris extrusion apically in the present study, which is similar with other studies.[39-41] The WOG files are characterized by a modified triangular cross-section, which results in decreased cutting efficacy and smaller chip space resulting in extrusion of the for med debris after instrumentation,



periapically.[38,39] The WOG files also exhibit a larger taper of 0.08 at the apical 3mm, which can be attributed to excessive debris formation periapically, and extrusion periapically.[38]

In our study, the mean colony count of the SAF group is  $423 \pm 35.19$  CFU/ml. The mean colony count of the TFA group is  $557.73 \pm 23.28$  CFU/ml. The mean colony count of the WOG group is  $642.23 \pm 13.86$  CFU/ml. The mean colony count of the control group is 0. (Table 2)

This test shows that the mean difference in colony count between SAF and TFA, between SAF and WOG, between TFA and WOG, is statistically highly significant ( $p < 0.001$ ). Also when the three file systems are compared with the control group, the resulting difference between the mean colony counts is significant. ( $p < 0.050$ ) It means the colony count formed in the SAF file system is less as compared to TFA and WOG. According to the results obtained from the current study, the result of debris from instrumentation of root canals was extruded periapically regardless of the file design and different kinematic motions used. The reciprocating file WOG resulted in maximum debris extrusion ( $P < 0.01$ ), whereas the vibratory file SAF resulted in the least debris extrusion in the three groups tested ( $P < 0.01$ ). Thus, the null hypothesis was rejected.

TFA motion depends on the stress loaded on the file, so reciprocating angles may vary along with a wide range, while there might only be reciprocating or continuous rotation during the entire root canal preparation.[39]

Another factor that may affect debris extrusion is the design of the file, for example, the cross-section, rake angle, helical angles, distance between flutes, taper, tip design, flexibility, alloy, and several files.[40]

In a study by Dietrich et al [41] comparing the reciprocal movement to the SAF, reciprocal instrumentation resulted in more debris in the apical part of the root canal than the SAF or a rotary system. The amount of debris formed in the apical third may also cause its extrusion periapically. Every effort should be made to limit the periapical extrusion of intracanal material during treatment that has the potential to bring about serious systemic diseases such as endocarditis, brain abscess, and septicemia, particularly in compromised patients. Further in vivo research in this direction could provide more insight into the biologic factors associated with correlations and consequences of apically extruded debris and may focus on bacterial species that essentially play a major role in post instrumentation flare-ups.

### **Summary & Conclusion**

In the present in vivo study, the extrusion of debris associated with three different file processing designs that used different kinematic motions was assessed. Within its limitations, it can be concluded that three file systems used for instrumentation resulted in extrusion of debris even though the working length was maintained 1mm short of the apex. The SAF that used a vibratory motion with continuous irrigation resulted in significantly less debris extrusion when compared to TFA and WOG file systems. The results of the current study are favorable to the SAF file system, but further studies clinically evaluating the incidence of post instrumentation pain with these instrumentation systems can provide a better understanding of these file systems.

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