



RESEARCH ARTICLE

A COMPARISON OF APICAL BACTERIAL EXTRUSION IN MANUAL, MTWO AND V TAPER ROTARY INSTRUMENTATION TECHNIQUES- AN IN -VITRO STUDY

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ABSTRACT

Apical extrusion of irrigants and debris is an inherent limitation associated with cleaning and shaping of root canals and has been studied extensively because of its clinical relevance as a cause of flare-ups. Many factors affect the amount of extruded intracanal materials. The purpose of this study was to assess the bacterial extrusion by using manual, Mtwo NiTi rotary system and V(variable) taper rotary instrumentation techniques. Thirty six human mandibular premolars were inoculated with *Enterococcus faecalis*. The teeth were divided into 3 experimental groups (n=10) and 1 control group (n=6). The root canals of experimental groups were instrumented according to the manufacturers' instructions by using manual technique, Mtwo NiTi rotary system and V (variable) taper rotary instruments. Sterilized saline was used as an irrigant and bacterial extrusion was quantified as colony-forming units/milliliter. Results obtained were statistically analysed with suitable statistical test.

INTRODUCTION

The inter-appointment flare up is a complication characterized by Pain and Swelling, which commences within a few hours or days after root canal procedures (Siqueira, 2002; Sjögren, 1990; Seltze, 1985). Causative factors of inter-appointment flare comprise of mechanical, chemical and microbial factors. Mechanical irritation is mainly by overinstrumentation (Haapasalo, 2005 and Schilder, 1974). Chemical irritation by irrigants, intracanal medicaments and over extended filling materials. Microbial irritation caused by microorganisms and their products in root canal system to the periradicular tissues. Extruded bacteria and their products can generate acute inflammatory response (Weine, 1975). All preparation techniques and instrumentation are reported with extrusion of infected debris. More recently root canal preparation with engine driven Ni-Ti instruments has become popular, thus this study to evaluate number of bacteria extruded (Young, 2007

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and Haapasalo, 2014), from extracted teeth ex vivo after root canal instrumentation using a manual technique and two engine driven rotary file systems.(Mtwo and Vtaper)

MATERIALS AND METHODS

Thirty six freshly extracted, single-rooted human mandibular pre-molars with mature apices and curvatures between 0° and 10° and apical diameter confirming to #15 K-file were selected. Presence of a single canal was confirmed with radiographs. Teeth with calcified canals, canals with large apical foramina, and more than 1 canal were excluded. The teeth were divided into 3 experimental groups (n = 10) and 1 control group (n = 6). The teeth were cleaned of debris and soft tissue and stored in saline solution. Endodontic access cavities were prepared with no. 2 round bur, pulp remnants were extirpated with a fine barbed broach, and working length of the canals was established at 1 mm short of the file penetration length, when the tip of the file was just visible at the apex. Double coat of nail varnish is applied to seal any lateral or accessory canals from which the extrusion of bacteria may occur. Glass vials were taken, holes were created in centre of rubber stoppers, fixed at level of CEJ, vented with 23 gauge needle and

sterilized. Broth preparation: 3 grams of broth powder (Tryptone Soya Broth) was weighed on weighing balance and added into 100ml of distilled water in the flask. The mouth of the flask was closed with a sterile cotton plug, then covered with aluminum foil followed by wrapping with blotting paper and then tying it with a thread (to prevent contamination). Then it was kept on the hot plate and gently shaken till the media powder got dissolved homogeneously and then the broth was autoclaved at 121°C at 15psi for 15 min. Preparation of culture: *Enterococcus faecalis* MTCC code 4399 equivalent to ATCC29212, stains was obtained from IMTECH, Chandigarh was used in this study. It was grown on Tryptone Soya Agar (TSA) for 72 hours. The culture was suspended in 10ml of Trptone soya broth and incubated at 37°C. The turbidity was adjusted to 0.5 McFarland standards.

Standardization of samples: McFarland standards are used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range to standardize microbial testing. Original McFarland standards were made by mixing specified amounts of barium chloride and sulphuric acid together. Mixing the two compounds forms a barium sulfate precipitate, which causes turbidity in the solution. A 0.5 McFarland standard is prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$), with 9.95 mL of 1% sulfuric acid (H_2SO_4).

Contamination of the specimens: 50 micro liters of inoculum containing *E. faecalis* was added to the samples. The samples were placed in incubator at 37°C for 21 days. Canals were replenished with fresh bacterial suspension every 48 hrs. The root canals of experimental groups were instrumented according to the manufacturer's instructions by using manual technique, Mtwo NiTi rotary system and V (variable) taper rotary instruments in LAF. Laminar air flow utilizes HEPA (High Efficiency Particulate Air) Filters for removing 99.97% microbes from air. Mtwo is a four file rotary system operated at 250 rpm, sequence used and torque in Ncm are, 10/.04 at 1.2, 15/.05 at 1.3, 20/.06 at 2.1 and 25/.06 at 2.3 using single length technique. V taper file System is a 3 file system operated at 250 rpm with torque of 2.4. The file sequence used was 17/.04, 20/.06 and 25/.06. Manual group was prepared coronally with Gates Glidden Drill No 3, 2, 1 and then apical preparation was completed till 25 no file. Sterilized saline was used as an irrigant, and bacterial extrusion was quantified as colony-forming units/milliliter. 0.01ml of NaCl from test apparatus plated on brain heart agar for 24 hrs Colony forming units were calculated.

Counting of colony forming units: Colony Forming Unit (CFU or cfu) is a measure of viable bacterial or fungal cells. In direct microscopic counts (cell counting using haemocytometer) where all cells, dead and living, are counted, but CFU measures only viable cells. For convenience the results are given as CFU/mL (colony-forming units per milliliter) for liquids, and CFU/g (colony-forming units per gram) for solids. CFU can be calculated using Miles and Misra method, it is useful to determine the microbiological load and magnitude of infection in blood and other samples.

The CFU/ml can be calculated using the formula:

$$\text{cfu/ml} = (\text{no. of colonies} \times \text{dilution factor}) / \text{volume of culture plate.}$$

Statistical Analysis

- Colony forming units were expressed as mean \pm standard deviation. Statistical analysis was performed using SPSS for Windows, Version 12.0.1
- Colony forming units were analyzed using one way ANOVA. Multiple comparisons were performed using Tukey's post hoc test, with a significance level of < 0.05 .

RESULTS

Maximum extrusion is seen with the manual group and is significant to other groups. No significant difference was found in number of CFU among the engine driven techniques.





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

GROUPS	TOTAL(N)	MEAN (CFU ml ⁻¹)	SD
Manual	10	53.8	16.67
Mtwo	10	20.34	1.47
V taper	10	13.69	5.69
Control	6	5.4	1.04

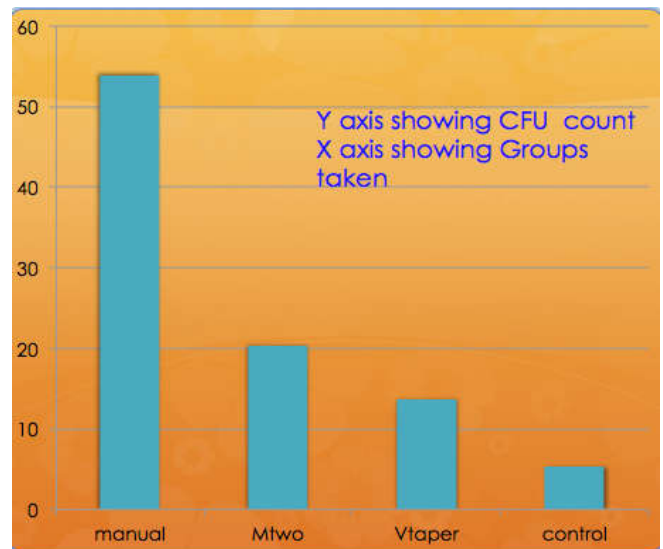
Table 2. Analysis of variance

ANOVA					
CFU	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13213.483	3	4404.494	47.103	.000
Within Groups	2992.273	32	93.509		
Total	16205.756	35			

Table 3. Post hoc test

Post Hoc Tests						
Multiple Comparisons						
Dependent Variable: CFU						
Tukey HSD						
(i) Group	(j) Group	Mean Difference (i-j)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
manual	mtwo	33.46000*	4.32455	.000	21.7432	45.1768
	v-taper	40.17000*	4.32455	.000	28.4532	51.8868
	control	52.66000*	4.99356	.000	39.1306	66.1894
	mtwo	-33.46000*	4.32455	.000	-45.1768	-21.7432
mtwo	v-taper	6.71000	4.32455	.420	-5.0068	18.4268
	control	19.20000*	4.99356	.003	5.6706	32.7294
	v-taper	-40.17000*	4.32455	.000	-51.8868	-28.4532
v-taper	mtwo	-6.71000	4.32455	.420	-18.4268	5.0068
	control	12.49000	4.99356	.079	-1.0394	26.0194
	manual	-52.66000*	4.99356	.000	-66.1894	-39.1306
control	mtwo	-19.20000*	4.99356	.003	-32.7294	-5.6706
	v-taper	-12.49000	4.99356	.079	-26.0194	1.0394

*. The mean difference is significant at the 0.05 level.



DISCUSSION

Apical extrusion is one of the common problem encountered during cleaning and shaping of root canals (Van der Sluis, 2006; Gu, 2009; Seltzer, 1985). This extrusion may have deleterious effect on the prognosis of root canal treatment as it may lead to flare up. A true complication characterized by the development of pain, swelling or both that commences within few hours or days after root canal treatment (Charara, 2016; Mozo, 2012). The aim of study was to access the apical extrusion of intracanal bacteria as result of root canal shaping by 4 different instrumentation techniques. Common to all techniques were the amount and type of irrigant .To compare the amount of apically extruded bacteria by standardized tooth type, curvature and size of apical foramen was selected. Enterococci faecalis was used as bacteriological marker as it has been implicated in persistant root canal infections and commonly found in biofilm. In present study 0.9% saline solution was used for irrigation because it has no antibacterial effect. In this way elimination and extrusion of bacteria is dependent on the mechanical action of the instruments (Van der Sluis, 2007; Haapasalo, 2010 and Gu, 2009). Maximum extrusion is seen with the manual group and is significant to other groups. In the manual group linear filing motion, files may act as pistons pushing irrigating solutions and debris toward the apex and taper being less there remains no space for the coronal movement of the debris and the pitch is close, so accumulation and subsequent cleaning of the debris is impaired(Siqueira, 2003; Van der Sluis, 2006). Rotation during instrumentation with engine-driven techniques results in collection of debris into the flutes of the instrument and its evacuation out of the root canal in a coronal direction and an increased taper facilitates the outward movement of the debris (Adorno, 2015 and Boutsoukias, 2007). Al-Omari and Dummer (AlOmari, 1995), verified that techniques involving a linear filing motion created a greater mass of debris than those involving some sort of rotational action. Both engine-driven systems used in this study work in continuous rotation, and a file with continuous rotation is considered to act like a screw conveyor improving transportation of dentin chips and debris coronally.

Conclusion

All instrument 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 CFU among the engine driven techniques.

Conflicting Interest : None.

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