

# The efficacy of dynamic irrigation using a commercially available system (RinsEndo®) determined by removal of a collagen 'bio-molecular film' from an *ex vivo* model

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## Abstract

**McGill S, Gulabivala K, Mordan N, Ng Y-L.** The efficacy of dynamic irrigation using a commercially available system (RinsEndo®) determined by removal of a collagen 'bio-molecular film' from an *ex vivo* model. *International Endodontic Journal*.

**Aim** To compare the efficacy of three irrigation protocols using an established *ex vivo* bio-molecular film model.

**Methodology** Thirty human teeth with single straight canals were randomly allocated to three groups [static, manual-dynamic, automated-dynamic (RinsEndo®)]; each with a sub-group ( $n = 5$ ) for needle position at 4 or 10 mm short of the working length (WL). The root canals were prepared to apical size 40, taper 0.08. The teeth were split longitudinally into two halves and a standard coat of stained-collagen was applied to the canal surfaces. The re-assembled teeth were irrigated using one of the protocols with the irrigation needle at one of two positions. Digital images of the canal surfaces, before and after irrigation with 18 mL of 2.5% NaOCl, were used to score surface

coverage with stained-collagen using image-analyses (ipWin4®). The data were analysed using linear regression models.

**Results** The canal area covered with stained-collagen was significantly ( $P < 0.001$ ) less after dynamic irrigation (manual/automated) compared with static irrigation; but automated-dynamic irrigation was significantly ( $P = 0.037$ ) less effective than manual-dynamic irrigation. The 'orientation of needle port', 'corono-apical level of canal' and 'apical extent of needle placement' were significant ( $P < 0.001$ ) factors influencing efficacy of irrigation. Residual collagen was most evident in the coronal third. Deeper penetration of the needle tip resulted in significantly ( $P < 0.001$ ) more effective collagen removal.

**Conclusions** Automated-dynamic irrigation was significantly more effective (16%) than static irrigation but significantly less effective (5%) than manual-dynamic irrigation. Irrigation was more effective (7%) when the needle was placed closer to WL.

**Keywords:** bio-molecular film, irrigation, RinsEndo, root canal.

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## Introduction

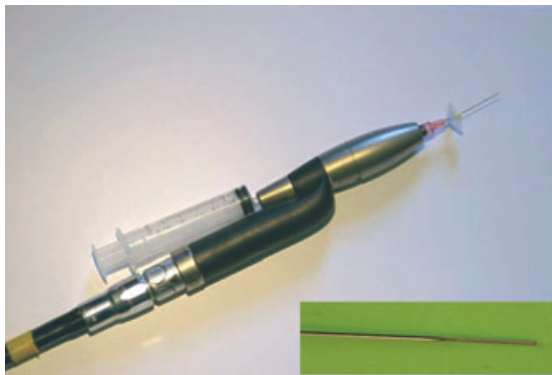
One of the principal roles of root canal irrigation is to assist in the removal of the bacterial biofilm from uninstrumented surfaces (30–50% of the root canal

wall) (Peters *et al.* 2001, Gulabivala *et al.* 2005). The variable nature of such bacterial biofilms (Nair 1987, Richardson *et al.* 2005, Rojekar *et al.* 2006) and even those generated artificially in extracted teeth (Patel *et al.* 2007), renders such models ineffective for the purposes of evaluating the factors influencing irrigation efficacy without prohibitive sample sizes. For this reason, a predictable *ex vivo* bio-molecular film model was proposed as a standardized and simple screening tool, its purpose was to mimic the hydrodynamic

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behaviour of a bacterial biofilm (Huang *et al.* 2007). This model was able to reveal a significant relationship between apical canal enlargement, canal taper and irrigant agitation. In the previous study, the irrigant was manually agitated with a well-fitting gutta-percha cone (manual-dynamic irrigation); the effect of number of cycles (per unit volume of irrigant) was investigated. The maximum number of strokes was 100; some clinicians balk at the laborious nature of such a procedure, whilst others applaud its utter simplicity and cost-effectiveness. The former group may therefore wish to adopt commercially available devices for automating agitation of the irrigant.

A number of automated systems specifically designed for agitation of the irrigant in the root canal system are available on the market and include: sonic (Sabins *et al.* 2003); ultrasonic (Cunningham *et al.* 1982a,b, Sabins *et al.* 2003) or pressure alternation systems. Examples of the latter group include RinsEndo<sup>®</sup> (Braun *et al.* 2005, Muselmani *et al.* 2005), Endo-Vac<sup>®</sup> or like (Fukumoto *et al.* 2006, Nielsen & Baumgartner 2007) and other variations (Lussi *et al.* 1993, Walters *et al.* 2002). Sonically or ultrasonically activated smooth or fluted stainless steel instruments bear the potential risks of instrument separation, ledge formation or irrigant extrusion beyond the apical foramen. The RinsEndo<sup>®</sup> system (Dürr Dental GmbH & Co. KG, Bietigheim-Bissingen, Germany) (Fig. 1) uses pressure-suction technology to deliver the irrigant solution ( $6.2 \text{ mL min}^{-1}$ ) and activates (1.6 Hz) it automatically (Dürr Dental). It has been shown in an extracted tooth model to be superior to conventional static irrigation in removal of pulp tissue (Braun *et al.* 2005) and killing of *Enterococcus faecalis* (Muselmani *et al.* 2005). Its efficacy has not been compared with manual dynamic irrigation (Huang *et al.* 2007). The aim of this study



**Figure 1** RinsEndo<sup>®</sup> system.

was to evaluate the efficacy of three irrigation protocols (static, manual-dynamic, automated-dynamic using RinsEndo<sup>®</sup>) for removal of a standard stained-collagen film coating the root canal walls of extracted teeth (Huang *et al.* 2007).

## Materials and methods

### Collection and preparation of extracted teeth

Thirty extracted human permanent teeth (maxillary or mandibular anterior teeth, mandibular premolars) with single canals, straight mature roots and with no caries or resorption, were collected and stored in 4% formal saline. They were randomly assigned to three experimental groups (group A for static irrigation, group B for manual-dynamic irrigation, group C for automated-dynamic irrigation). The 10 teeth in each group were randomly divided into two further equal subgroups (Table 1).

After accessing, the tooth length was determined by placing a size 10 stainless-steel K flex file (Kerr UK Ltd, Peterborough, UK) in the canal and extending it until visible at the apical foramen. All the teeth were decoronated to give a standardized length of 18.0 mm; the working length was set at 18.0 mm; at the apical terminus.

The canals were prepared using SystemGT<sup>®</sup> instruments (Dentsply Maillefer, Ballaigues, Switzerland) in a 70 : 1 controlled-torque, low speed rotary handpiece at 300 rpm, to apical size 40 with a 0.08 taper following the manufacturer's protocol (Dentsply Maillefer). During instrumentation, each tooth was irrigated with 50 mL of 2.5% sodium hypochlorite (NaOCl) (Teepol<sup>®</sup> bleach; Teepol products, Egham, UK), delivered with a 3 mL Monoject<sup>®</sup> Luer lock syringe with a 27 gauge needle (Sherwood Medical, St Louis, MO, USA).

**Table 1** Allocation of tooth samples

Groups (mode of irrigation)	Subgroups	No. of teeth	Level of irrigation needle tip penetration (mm from apical foramen)
A (static irrigation)	A1	5	4
	A2	5	10
B (manual-dynamic irrigation)	B1	5	4
	B2	5	10
C (automated-dynamic irrigation)	C1	5	4
	C2	5	10

Each tooth was partly embedded in silicone putty (President Putty Coltène®, Altstätten, Switzerland) to create a set matrix which allowed reassembly of the tooth for irrigation tests after splitting. The tooth was then grooved on the buccal and palatal surfaces along its entire length using a diamond disc (Abrasive Technology Inc., Westerville, OH, USA) and cushioned on another silicone matrix to split into two halves using an osteotome and mallet. The two halves of the canal were randomly assigned as side facing (F) or opposing (O).

Four layers of collagen solution (type I rat tail collagen in 0.6% acetic acid solution, First Link Ltd., Birmingham, UK) mixed with Chinese calligraphic ink (Kai-Ming, Tainan, Taiwan), in a ratio of 5 : 1, were applied to the canal surfaces using a small brush and the solvent allowed to evaporate from the acid solution at room temperature for 48 h to enable the collagen to form a gel.

Each split half of the root was equally divided into coronal, middle and apical sections and marked with a sharp pencil. The split tooth was reassembled for irrigation tests and disassembled for examination. Digital images were taken before and after irrigation with 18 mL of NaOCl using a digital camera (Cool- SNAP-Procf, MediaCybernetics®, Silver Springs, MD, USA) (Huang *et al.* 2007).

### Irrigation experiments

The irrigation protocols for canals in group A (static irrigation) and B (manual-dynamic irrigation) were adopted from Huang *et al.* (2007) whilst the protocol for canals in group C (automated-dynamic irrigation) was adopted from the manufacturer's instructions for RinsEndo (Dürr Dental).

The reassembled canals in group A (subgroups 1 and 2) were irrigated with 2.5% NaOCl, delivered with a Monoject® endodontic 3 mL syringe through a Luer-lock 30 gauge Max-I-Probe™ needle (Maillefer, Ballaigues, Switzerland) at a rate of 6 mL min<sup>-1</sup>. The irrigating needle was inserted to 4 mm (subgroup A1) or 10 mm (subgroup A2) short of the working length and a total of 18 mL of solution was delivered in six 3 mL boli. The open side-port of the needle always faced the canal side designated 'A', in a fixed orientation.

For the canals in group B (subgroups B1 and B2), the protocol for irrigation was the same as for group A with the addition of intra-canal push-pull manipulation of a size 40/0.08 taper gutta-percha point (SybronEndo,

Orange, CA, USA). One hundred push-pull strokes (each with a 5 mm amplitude and reaching the working length) were performed *after* introducing the *first* 3 mL of the irrigant, followed by delivery of the next 6 mL in two 3 mL boli. Therefore, 100 push-pull strokes were used for each 9 mL of irrigation. This pattern was repeated twice until the entire 18 mL was delivered with a total of 200 push-pull strokes.

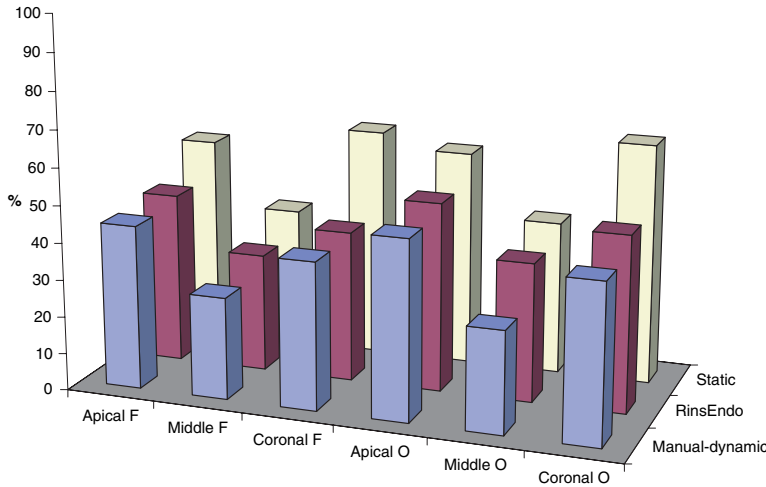
For the canals in group C (subgroups C1 and C2), the protocol for irrigation was similar to that for group A. The irrigant was delivered and agitated by activation of the RinsEndo® handpiece (Dürr Dental) using the needle provided. The supplied 6-mL syringe was filled with 3 mL of NaOCl and delivered by the handpiece at a manufacturer's set rate of 6.2 mL min<sup>-1</sup>. Six cycles with 3 mL each were completed, with a 30-s pause between, until 18 mL of irrigant had been delivered. The compressed air pressure supplying the hand-piece was adjusted to 4 bars to ensure it was within the recommended range (2.3–4.2 bar).

### Image and statistical analyses

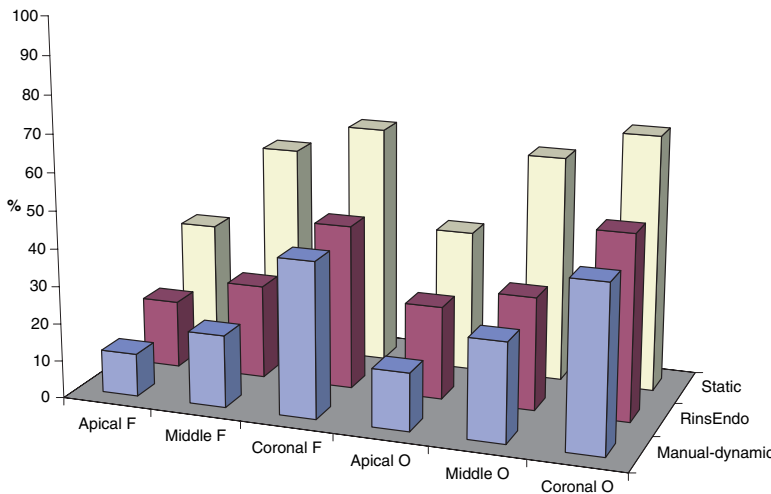
The digital images were analysed using IPWIN4® (MediaCybernetics®, Silver Spring, MD, USA) software to quantify residual canal coverage by the stained collagen. In total, 360 images (12 images per tooth) were taken and digitized. The means and standard deviations of the percentage of canal surface coverage with *residual* stained collagen after irrigation were calculated for each coronal-apical section, on each side of the canal. The generalized estimating equation (GEE) approach (STATA 9; STATA Corporation: College Station, TX, USA, 2005) was used to investigate the influence of the mode of irrigation as well as other predictive factors (coronal-apical level of canal, apical extent of needle placement, orientation of irrigation needle port) on the efficacy of irrigation using 'percentage of canal coverage with residual collagen' as the independent variable. The 'clustering' effect of the measurements taken from different levels of the same canal surface was accounted for in the GEE linear regression model.

### Results

The mean percentages of canal surface coverage with residual stained collagen after static, manual-dynamic or automated-dynamic irrigation are summarized in Figs 2 and 3; some obvious trends were evident. There was less canal coverage with residual stained collagen



**Figure 2** Mean percentage of canal surface coverage with residual collagen by different levels (apical, middle, coronal) after irrigation with the needle placed at 10 mm from the apical terminus (F: surface facing the open side-port of needle; O: surface opposing the open side-port) for groups A2 – C2.



**Figure 3** Mean percentage of canal surface coverage with residual collagen by different levels (apical, middle, coronal) after irrigation with the needle placed at 4 mm from the apical terminus (F: surface facing the open side-port of needle; O: surface opposing the open side-port) for groups A1 – C1.

after manual- or automated-dynamic irrigation compared with static irrigation. The canal surface facing the open side-port of the needle had less residual collagen after irrigation than the opposing surface. The level of the canal had an influence on residual collagen. The least residual collagen was found in the ‘apical levels’ for subgroups A1–C1 (needle at 4 mm) and ‘middle levels’ for subgroups A2–C2 (needle at 10 mm).

The efficacy of irrigation measured as ‘percentage of canal coverage with residual stained collagen’ was significantly ( $P < 0.05$ ) influenced by all four explanatory variables. In descending order of effect (according to the absolute Z-values in Table 2), the relative ranking was: orientation of port of needle, mode of irrigation, apical extent of needle placement and coronal level of the canal (Table 2). Both automated-dynamic irrigation (RinsEndo®) (16%) and manual-dynamic irrigation (21%) were significantly ( $P < 0.001$ ) more

effective in removing stained collagen from the root canal than static irrigation. RinsEndo® was significantly ( $P = 0.037$ ) less effective (21–16 = 5%) than manual-dynamic irrigation. Irrigation was significantly ( $P < 0.001$ ) more effective when the needle was placed closer to the apical foramen (7%) and on the canal surface facing the port of the needle (4%) than the contrary. After irrigation, the coronal third had significantly ( $P < 0.001$ ) more area (14%) covered with residual stained collagen than the apical third, whilst there was no significant ( $P = 0.289$ ) difference between the apical and middle thirds.

**Discussion**

The test model adopted (Huang et al. 2007) allowed quantification and comparison of the efficacy of removal of a bio-molecular film from the root canal

**Table 2** Linear regression model incorporating three significant explanatory variables simultaneously using 'percentage of surface coverage with residual stained collagen as the dependent variable

Explanatory variable	Coefficient	95% CI for coefficient	P-value	Z-value
Constant	43.1	39.4, 46.9	<0.001	
Mode of irrigation				
Static irrigation	1	–	–	
Manual-dynamic irrigation	–20.7	–24.2, –17.2	<0.001	–11.5
Automated-dynamic irrigation	–16.0	–19.3, –12.7	<0.001	–9.6
Corono-apical level of canal ( <i>apical third</i> )				
Apical	1	–	–	
Middle third	–1.8	–7.6, 4.0	0.289	–0.6
Coronal third	13.8	7.9, 19.7	<0.001	4.6
Level of needle penetration				
Needle tip at 4 mm from apical foramen	1	–	–	
Needle tip at 10 mm from apical foramen	7.4	4.9, 10.0	<0.001	5.7
Canal surface				
Port of needle facing side	1	–	–	
Port of needle opposing side	4.4	3.7, 5.1	<0.001	11.9

walls by different irrigation regimens. The model is, however, limited to single-rooted teeth with simple straight canals as teeth with curved canals are less predictably split. The thickness of four layers of stained-collagen applied by the author (SM) ranged between 9–37  $\mu\text{m}$ , which was greater than in the previous study (Huang *et al.* 2007), which reported a range of 5–15  $\mu\text{m}$ . Both these ranges were nevertheless near the reported range of thickness of bacterial biofilms at 21–30  $\mu\text{m}$  (Distel 2002). Previous pilot studies had confirmed that the stained collagen could neither be decolourized by 2.5% sodium hypochlorite solution even after soaking for 12 h nor be flushed out by irrigation with water alone, but it could be gradually flushed out by sodium hypochlorite solution (Huang *et al.* 2007).

All the canal preparations were standardized to the larger dimensions (apical size 40/taper 0.08) previously tested (Huang *et al.* 2007) to facilitate the optimal efficacy of both static and dynamic irrigation. Most other irrigation parameters, such as position of placement of the irrigation needle, rate of irrigant delivery and orientation of port of needle, were adopted from the previous work (Huang *et al.* 2007) to facilitate comparison of results. In the present study, an extra irrigation parameter, apical extent of needle placement was tested by including appropriate sub-groups. The reason for introducing this variable, given that its effect in static irrigation was already known (Sedgley *et al.* 2005), is that the RinsEndo<sup>®</sup> manufacturer's instructions (Dürr Dental) suggest that the apical third of the canal may be effectively rinsed with the cannula restricted to the coronal third of the root canal because

of the pulsating nature of the fluid flow. The RinsEndo<sup>®</sup> handpiece had a pre-set irrigant delivery rate of 6.2  $\text{mL min}^{-1}$  which was slightly higher than 6  $\text{mL min}^{-1}$  used for static and manual-dynamic irrigation groups but the difference was negligible.

The results of this study showed that all four explanatory variables: orientation of port of needle, mode of irrigation, corono-apical level of the canal and apical extent of needle placement had significant influence on the efficacy of irrigation. The former three significant influencing factors were consistent with the previous report (Huang *et al.* 2007). In contrast to their findings, the present study found no significant difference in the efficacy of stained collagen removal from the apical and the middle thirds. This difference may be attributable to the two different levels of needle placement (4 or 10 mm from apical terminus) used in this study in contrast to the 4 mm position adopted by Huang *et al.* (2007). The corono-apical level of canal with the least area covered with residual stained collagen corresponded to the apical extent of needle placement and site of deposition of the irrigant bolus (Figs 2 and 3). This finding would appear to contradict the suggestion by the manufacturer that needle position need not influence apical irrigation with RinsEndo<sup>®</sup>.

This study found that both approaches of dynamic (manual and automated) irrigation resulted in significantly less residual stained collagen than with static irrigation, consistent with previous findings (Huang *et al.* 2007) and of those who agitated the irrigant with hand files, irrigation needles (Cecic *et al.* 1984, Druttman & Stock 1989), or ultrasonic activation

(Druttman & Stock 1989, Lee et al. 2004a,b, van der Sluis et al. 2005a,b). Although manual-dynamic irrigation may be deemed laborious by some clinicians, it was significantly ( $P = 0.037$ ) more effective (5%) than automated-dynamic irrigation using RinsEndo<sup>®</sup>. This difference may be explained in several ways: (i) the push-pull motion of a well-fitting gutta-percha point in the canal may generate higher intra-canal pressure changes in the way that a drain plunger does during pushing movements, leading to more effective delivery of irrigant to the 'untouched' canal surfaces; (ii) the frequency of push-pull motion of the gutta-percha point (33 Hz – 100 strokes per 30 s) is higher than the frequency (1.6 Hz) of positive-negative hydrodynamic pressure generated by RinsEndo<sup>®</sup>, possibly generating more turbulence in the canal; and (iii) the push-pull motion of the gutta-percha point probably acts by physical stretching, folding and cutting of fluid laminas in the viscously dominated environment of the root canal system (Wiggins & Ottino 2004). The latter, probably allows better mixing of the canal fluids; fresh unreacted solution with the spent reacted molecules of the active NaOCl irrigant.

Based on the present study and within any limitations imposed by the model, the removal of a surface adherent bio-molecular film from the root canal surface may be facilitated by placing the irrigation needle close to the apical foramen as well as movement of the needle during delivery, a needle design with multiple ports (Nielsen & Baumgartner 2007), agitation of irrigant with a well-fitting gutta-percha point or an automated device such as RinsEndo<sup>®</sup>. Further research would be helpful to optimize the working parameters of the RinsEndo<sup>®</sup> system.

## Conclusions

Automated-dynamic irrigation using RinsEndo<sup>®</sup> was significantly more effective (16%) in removing stained collagen from root canal walls than static irrigation. However, it was significantly less effective (5%) than manual-dynamic irrigation. Irrigation was significantly more effective when the needle was placed closer to the apex and on the canal surface facing the needle side port.

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