

Canal and isthmus debridement efficacies of two irrigant agitation techniques in a closed system

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Abstract

Susin L, Liu Y, Yoon JC, Parente JM, Loushine RJ, Ricucci D, Bryan T, Weller RN, Pashley DH, Tay FR. Canal and isthmus debridement efficacies of two irrigant agitation techniques in a closed system. *International Endodontic Journal*, **43**, 1077–1090, 2010.

Aim To compare canal and isthmus debris debridement efficacies of the manual dynamic irrigation (MDI) and apical negative pressure (ANP) techniques in the mesial root of mandibular first molars with narrow isthmi, using a closed canal design.

Methodology Micro-computed tomography was employed to select 20 teeth, each containing a narrow isthmus. Each root was sealed at the apex with hot glue and embedded in polyvinylsiloxane to simulate a closed canal system. The teeth were submitted to a standardized instrumentation protocol. Final irrigation was performed with either the MDI or the ANP technique using the EndoVac system ($N = 10$). Masson trichrome-stained sections were prepared from completely demineralized roots at 10 canal levels between 1 and

2.8 mm of the anatomical apices. Areas occupied by canals and isthmus of each root and debris in the corresponding regions were digitized by the NIH Image J software and statistically analysed using two-way repeated measures ANOVA.

Results For the instrumented canals, there were no differences between the two groups ($P = 0.131$) in the area occupied by debris at all canal levels ($P = 0.343$). Conversely, for the isthmus, less debris was found in the ANP group ($P < 0.001$) but no differences were seen in each group with respect to the 10 canal levels ($P = 0.352$).

Conclusion Neither technique completely removed debris from the isthmus regions. However, the EndoVac system, which encompasses the ANP concept, removed considerably more debris from narrow isthmi in mandibular mesial roots.

Keywords: apical negative pressure, canal, debris, EndoVac, isthmus, manual dynamic irrigation.

Received 21 December 2009; accepted 6 June 2010

Introduction

Success of root canal treatment is dependent upon the effective removal of bacterial biofilms and their by-products from the root canal system (Chávez de Paz *et al.* 2007, 2008, Estrela *et al.* 2009, Ricucci & Siqueira 2010). Current instrumentation techniques

are inefficient at cleaning all surfaces and irregularities within the root canal space (Siqueira *et al.* 1997, Peters 2004, Burleson *et al.* 2007, Vaudt *et al.* 2009, Williamson *et al.* 2009). Hard and soft tissue debris that remains after mechanical instrumentation can harbour microorganisms and decrease the efficacy of the seal created by root filling materials, possibly leading to treatment failure (Bergenholtz & Spångberg 2004, Nair 2006, Brito *et al.* 2009, Paqué *et al.* 2009). Therefore, irrigant agitation is a necessary adjunct to mechanical instrumentation as a method of chemical debridement to remove debris and bacteria from the

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entire canal system (Nielsen & Baumgartner 2007, Carver *et al.* 2007, de Gregorio *et al.* 2009, Gu *et al.* 2009, Jiang *et al.* 2010).

Apart from the rational selection of irrigants (Zehnder 2006), a sufficient volume of the irrigant, a high flow rate and unrestricted flow of the irrigant along the canal walls are crucial for thorough debridement of the root canal system (Moser & Heuer 1982, Chow 1983, Hsieh *et al.* 2007). As traditional needle irrigation delivers solutions no further than 1 mm beyond the needle tip (Boutsiokis *et al.* 2009, Gao *et al.* 2009), this irrigant delivery technique is relatively ineffective in flushing hard and soft tissue remnants from the apical third of canal walls in the absence of adjunctive irrigant agitation regimes. Gas entrapment prevents optimal irrigant delivery and flow 0–2 mm from the end-point of canals (Tay *et al.* 2010). With the exception of studies performed by Baumgartner and coworkers (Baumgartner & Mader 1987, Albrecht *et al.* 2004, Usman *et al.* 2004, Nielsen & Baumgartner 2007), few *ex vivo* studies have incorporated experimental set ups that truly produce a closed canal system design to simulate the effect of gas entrapment (Fukumoto *et al.* 2006, Tay *et al.* 2010). Other studies that were intended to simulate a closed canal system were nondeliberately flawed as they permitted gas or fluid exchange between the canal and external environment. One example is the procedure of half-embedding demineralized roots in soft silicone whilst allowing the other halves to be covered with a clearing fluid that can move in and out of the roots. Iatrogenic creation of an open canal system may also occur when teeth extracted after meticulously executed *in vivo* irrigation procedures are flushed through unsealed apical foramen to remove blood that accumulates in the root canals during extraction, or by reassembling split roots back into silicone moulds to create a canal that can be easily examined after the irrigation procedures. A plethora of *ex vivo* irrigation studies is available in the literature in which the use of a closed system or an open system design had not been specified. Thus, the reported canal debridement efficacy in studies with ambiguous or unspecified designs on how the issue of gas entrapment was addressed has to be viewed with scepticism.

Successful debridement of posterior teeth is hampered by the difficulty in flushing debris effectively from the isthmi of roots containing multiple canals. Although there is no hard tissue debris associated with these noninstrumented regions, dissolution of soft tissue remnants and eradication of microbes associated

with infected pulpal tissues must rely on the efficacy of irrigants to access these hard-to-reach regions, as well as the generation of a high flow rate that permits a hydraulic force that is strong enough to detach debris that are caught within the predentine collagen network of the noninstrumented isthmus canal walls. Previous *ex vivo* and *in vivo* studies have shown that debridement of the isthmus 1–3 mm from the anatomical apex could be improved with the adjunctive use of ultrasonic agitation (Haidet *et al.* 1989, Walker & del Rio 1991, Archer *et al.* 1992, Evans *et al.* 2001, Gutarts *et al.* 2005, Burleson *et al.* 2007). Nevertheless, the adverse effect of the apical gas entrapment on the efficacy of isthmus debridement has not been appropriately addressed in those *ex vivo* studies. Moreover, a critical issue associated with the aforementioned *in vivo* studies is the failure to adopt a clinically applicable selection criterion to define the mesiodistal isthmus width until the teeth were extracted and prepared for histological examination (Mannocci *et al.* 2005). Perceivably, roots with wider isthmi are more readily accessible to tissue-dissolving irrigants than roots with narrow 'partial isthmi' (Weller *et al.* 1995, Teixeira *et al.* 2003) that are partially obliterated in a three-dimensional manner by calcified or sclerotic dentine (Mannocci *et al.* 2005). Thus, in the absence of an appropriate clinical selection criterion on isthmus width, it is difficult to prevent the unintentional introduction of bias during the execution of an *in vivo* study on isthmus debridement efficacy. Clearly, there is a gap in our current understanding of how the phenomenon apical gas entrapment affects isthmus debridement efficacy in narrow isthmi present in roots with multiple canals.

Different techniques and irrigant delivery devices are available for improving the flow and distribution of irrigating solutions within the root canal system (Gu *et al.* 2009). Manual dynamic irrigation (MDI) has been described as a simple and cost-effective technique for cleaning the walls of instrumented root canals (McGill *et al.* 2008). It involves repeated insertion of a well-fitting gutta-percha cone to working length of a previously shaped canal. The gutta-percha cone is applied in short, gentle strokes to hydrodynamically displace and agitate an irrigant by producing eddy currents. This technique may be useful in circumventing apical gas entrapment because the air bubble located at the 0–2 mm of the apical seat will be completely displaced by repeated gutta-percha insertions. However, the hydrodynamic effect of the MDI technique on isthmus debridement efficacy in a closed canal system is unknown.

The EndoVac system (Discus Dental, Culver City, CA, USA) is an apical negative pressure (ANP) irrigation device that is designed to deliver irrigating solution to the apical end of the canal system and remove debris via a negative pressure mechanism (Schoeffel 2008). The EndoVac has been shown to introduce a higher flow of irrigant and produce better debridement at 1 mm from the working length when compared with needle irrigation (Nielsen & Baumgartner 2007). Additionally, the EndoVac has been shown to extrude less irrigant and, therefore, create less risk of a NaOCl incident (Desai & Himel 2009). Although the ANP suction mechanism could be a means to overcome the obstacle of apical gas entrapment within instrumented canals in anterior teeth, the efficacy of this strategy in removing debris from the apical part of the canal walls in posterior teeth has not been reported except for anecdotal reports of clinical experiences in nonpeer reviewed magazines. In particular, the effectiveness of the EndoVac system in removing debris from narrow isthmi that are associated with the critical apical 0–2 mm of the canal in roots of posterior teeth with multiple canals is unknown. Thus, the objective of the present *ex vivo* study was to compare the canal and isthmus debris debridement efficacies of the MDI technique and the ANP technique at 0–2 mm from the apical stop prepared in the mesial root of mandibular first molars with narrow isthmi, using a closed canal design. The null hypotheses tested were that: (i) there is no difference between the canal debridement efficacy of the two irrigant agitation techniques at different canal levels from the anatomical apex in a simulated closed canal system and (ii) there is no difference between the isthmus debridement efficacy of the two irrigant agitation techniques at different canal levels from the anatomical apex in a simulated closed canal system.

Materials and methods

Recently extracted noncarious human first molars with closed apices that were extracted for periodontal reasons were collected with patient's consent under a protocol approved by the Human Assurance Committee of the Medical College of Georgia. The teeth were stored at 4 °C in 0.9% NaCl solution supplemented with 0.02% sodium azide to prevent bacterial growth until use.

Criterion of tooth selection

As roots with narrow and wide isthmi differ considerably in their ease of debris removal, only those mandibular

first molars with narrow isthmus width between the mesiobuccal and mesiolingual canals were used. The criterion for tooth selection was that the mesiodistal isthmus width of completely patent isthmi or partially obliterated isthmi had to be less than one-quarter of the diameter of the unshaped canals along the canal levels (i.e. 1–2.8 mm from the anatomical apex) from which histological sections would eventually be prepared after cleaning and shaping procedures. As this criterion could not be fulfilled using conventional radiography, micro-computed tomography (micro-CT) was used for non-destructive screening of those mesial roots prior to actual irreversible hemisectioning of the molar teeth. Each tooth was placed apical-coronally inside a custom-made X-ray transparent Styrofoam specimen holder and stabilized with cotton pellets. The specimen holder was attached perpendicularly to the specimen turntable of a micro-CT scanner (SkyScan 1174; SkyScan N.V., Aartselaar, Belgium). Scanning was performed with a spatial resolution of 15 µm using a 0.5-mm-thick aluminium filter at 50 kV and 800 µA, a 0.6° rotation step and 360° rotation. After reconstruction, two-dimensional virtual slices in the axial direction (i.e. apical-coronal axis of the root) were made of the mesial root from 1 to 3 mm coronal to the anatomical root apex using the CT-analyser software (SkyScan) and saved in a 256 grey scale format. Each series consisted of 320–350 virtual sections. Molars with mesial roots that had mesiodistal isthmus widths wider than one-quarter of the diameter of the unshaped canals anywhere along the scanned regions were excluded from the study. Based on the aforementioned criterion, 20 mandibular molars were selected and randomly divided into two groups.

Preparation of the mesial root

Each molar was hemisectioned using a slow-speed Isomet diamond saw (Buehler Ltd, Lake Bluff, IL, USA) under water-cooling to expose the pulp chamber. The coronal portion of each root was flattened with the Isomet saw for accurate and consistent working length measurements. Pulpal contents were gently removed with a spoon excavator. A size 3 cotton pellet was placed inside the pulp chamber together with two size 10 stainless steel hand files that were inserted into the mesiobuccal and mesiolingual canals to prevent clogging of the canal orifices. Reconstruction of the distal wall of the severed mesial root was performed using a dentine adhesive and a resin composite to provide a reservoir for the irrigants (Fig. 1). Working length was determined with the hand files just visible in the apical

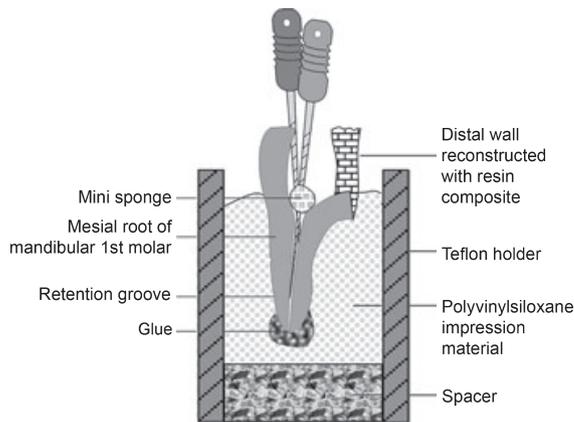


Figure 1 Diagram depicting a sectioned mesial root of a mandibular first molar with its distal wall reconstructed with a dentine adhesive and resin composite prior to inserting it into the polyvinylsiloxane-filled, simulated closed-end fixture.

foramen of each canal. The actual working length was taken to be 1 mm from the anatomical apex. Additionally, shallow horizontal grooves were placed circumferentially around the acellular cementum to provide mechanical retention in the experimental set up. Each root had their apical foramina covered by glue expressed from a hot glue gun. The cementum and set glue were coated with tray adhesive (Dentsply Caulk, Milford, DE, USA).

Experimental design

The roots were divided into two groups of 10 roots each ($N = 10$). Group I consisted of teeth irrigated with the MDI technique and Group II consisted of teeth irrigated with the ANP technique (i.e. the EndoVac system). A custom-fabricated fixture was used to simulate the effect of apical gas entrapment in a closed canal system (Fig. 2). The fixture, in turn, was affixed to the experimental set up (Fig. 3), which enabled consistent canal irrigation and suction of irrigants to be performed simultaneously by a single operator. Briefly, a Teflon holder was assembled on the top of metal parts that enabled the tube to be attached to the experimental set up (Figs 2a and 3). A tight-fitting metal spacer was inserted into the Teflon holder to form the base of the Teflon holder (Figs 1 and 2b) and to prevent extrusion of polyvinylsiloxane (PVS) impression material (Blue Moose; Parkell Inc, Farmington, CT, USA) from the base of the fixture. The Teflon holder with the inserted metal spacer is shown in Fig. 2(c). The Teflon holder also contained an anti-rotation lock on each side to

prevent rotation of the set PVS during cleaning and shaping procedures. After filling the holder with PVS (Fig. 2d), the adhesive-coated root was inserted into the PVS. Excess PVS was removed with a razor blade so that 2 mm of the decoronated surface protruded from the surface of the PVS. A slanted groove was created with a high-speed bur on one side of the exposed root surface (Fig. 2e) of the reconstructed pulp chamber to facilitate adaptation of the master irrigant delivery tip to the canal orifice. This experimental set-up prevented fluid and gases from escaping apically via the apical foramen to the external environment (Baumgartner & Mader 1987, Tay *et al.* 2010).

The experimental set up (Fig. 3) had a vacuum tubing that was connected to the high-vacuum line (Fig. 3 – component 1) for suction of the irrigants. For the ANP group, irrigant was delivered to the canal orifice via the master delivery tube (Fig. 3 – component 2) that was connected to a 20 mL syringe (Fig. 3 – component 3). For the MDI group, this irrigant delivery technique was replaced by direct delivery of the irrigants to 1 mm short of the working length via a 30-G side-vented needle (Maxi-i-Probe; Dentsply-Rinn, Elgin, IL, USA) connected to the 20 mL syringe (not shown). The rate of irrigant from the 20 mL syringe was consistent for both groups (5 mL min^{-1}). A plastic tubing (Fig. 3 – component 4) enclosing the master delivery tube was connected via a ‘T’ connector (Fig. 3 – pointer) to a fluid trap (Fig. 3 – component 5) and the high vacuum line. The fluid trap, in turn, was connected to the ANP suction device (Fig. 3 – component 6). Thus, during irrigation with the ANP technique, irrigant removal was achieved with the apical negative suction, whilst excess irrigant was simultaneously removed via the plastic suction tubing (component 4) over the reconstructed pulp chamber. During MDI, the ANP component of the experimental set up was disabled so that suction of the irrigant was achieved only via the plastic suction tubing placed over the reconstructed pulp chamber.

Experimental groups

Each canal was instrumented to size 35, .04 taper using a crown down approach with Endosequence rotary nickel titanium instruments (Brasseler, Savannah, GA, USA). During all instrumentation, the chamber was flooded with 5.25% NaOCl replenished with 1 mL after each instrument. All post-instrumentation irrigant delivery was performed at an average rate of 5 mL per min, and each group had the same

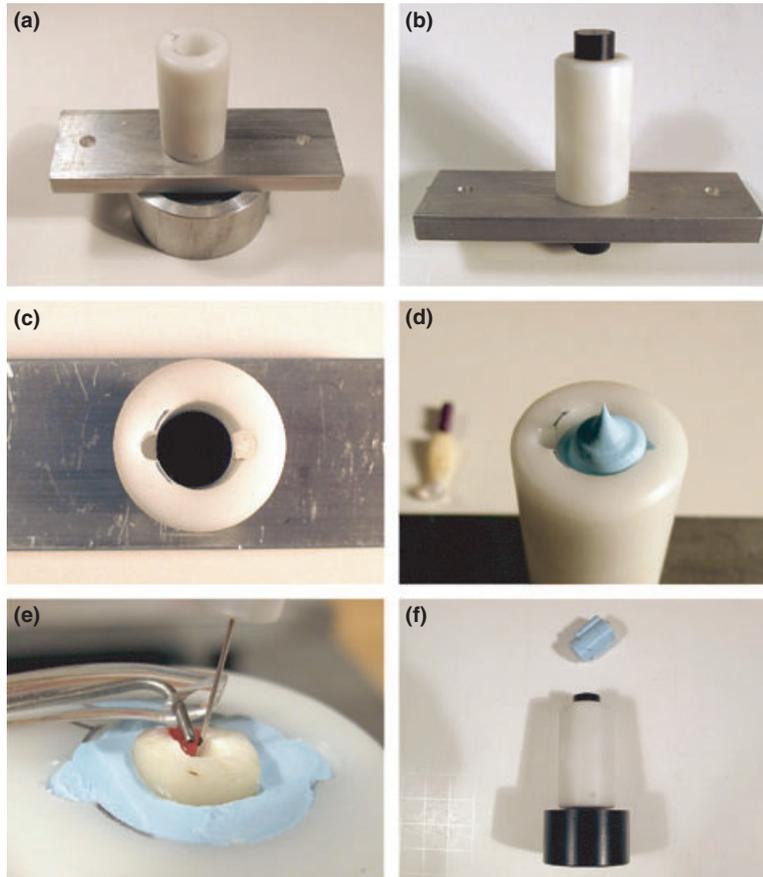


Figure 2 Photographic images of the simulated closed-end fixture employed in this study. (a) The closed canal fixture assembled from its component parts. A Teflon spacer is attached via a metal base to a circular base holder for attachment to the experimental fluid delivery and suction system (see Fig. 3). (b) A (black) spacer is inserted into the Teflon holder. (c) The spacer is pushed to the bottom of the holder to serve as the base to prevent extrusion of the polyvinylsiloxane (PVS) impression material from the bottom of the fixture. Two anti-rotation grooves prevent rotation of the set impression material within the holder during canal cleaning and shaping procedures. (d) The Teflon holder is filled to the top with impression material. The root is inserted into the impression material before it sets. (e) A groove (marked red) is placed on one side of the canal wall dentine to facilitate fitting of the master suction tip. (f) After completion of the canal shaping and irrigation procedures, the PVS impression material is displaced from the Teflon holder with a cap screw for retrieval of the root.

overall total irrigation time of 6 min. The two groups represented the two different agitation techniques that were performed after the use of the last rotary nickel titanium instrument:

Group I

Manual dynamic irrigation that consisted of 5.25% NaOCl for 1 min with short push-pull strokes using a size 35, .04 taper gutta-percha cone followed by 1 min of soaking; 17% EDTA for 1 min with short push-pull strokes using the same gutta-percha cone followed by 1 min of soaking; 5.25% NaOCl for 1 min with short push-pull strokes using the same gutta-percha point

followed by 1 min of soaking. The use of an irrigation protocol that consisted of three irrigation cycles (i.e. initial NaOCl, EDTA, final NaOCl) was based on the work of Yamada *et al.* (1983). The time periods employed for the three irrigation cycles were chosen so that they were similar to those employed for Group II. All push-pull strokes were performed manually at an approximate rate of 100 strokes per min. The selection of a gutta-percha cone corresponding to the canal preparation size and taper ensured that air inside the apical third of the canal was displaced by the gutta-percha cone when the latter was inserted to the working length.

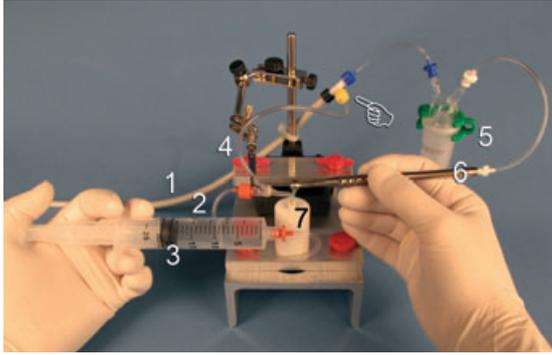


Figure 3 Custom-fabricated experimental set up to facilitate the application of irrigation procedures in a closed canal system design to be performed by a single operator. The different component parts are the following: 1. High vacuum suction line. 2. Connector to a 20 mL syringe. 3. Twenty microlitre syringe. 4. The master suction tip is connected to the high vacuum line via a T-connector (pointer). 5. A separate fluid collection trap is attached to the high vacuum line and to the tubing connecting the macro-cannulus and micro-cannulus of the EndoVac hand piece. This trap measured the volume of irrigant suctioned by the EndoVac system. 6. EndoVac hand piece. 7. Teflon holder as shown in Fig. 2.

Group II

Irrigation with the ANP technique also consisted of three irrigation cycles as reported by Nielsen & Baumgartner (2007). The canals were first irrigated for 30 s with 5.25% NaOCl using the macro-cannulus followed by leaving the canal full of irrigant for 30 s. Three irrigation cycles using the micro-cannulus inserted to full working length followed. The first cycle was 30 s of 5.25% NaOCl followed by 30 s of soaking; the second cycle was 1 min of 17% EDTA followed by 1 min of soaking; the third cycle was 1 min of 5.25% NaOCl followed by 1 min of soaking. The combined, post-instrumental NaOCl irrigation and soaking times prior to the use of EDTA (30 s for macro-cannulus and 30 s for micro-cannulus) employed in Group II were equivalent to the post-instrumental NaOCl irrigation and soaking times employed for Group I. The same volumes of irrigants were also used in Groups I and II.

Upon completion of the respective irrigation protocol, the canals were rinsed with sterile saline and dried with multiple paper points. The access of each canal was temporized with Tempit (Centrix, Shelton, CT, USA). The PVS was removed from the Teflon holder by inserting a cap screw from the bottom of the holder (Fig. 2f). Each instrumented root was removed from the PVS and stored separately in labelled bottles containing

10% formaldehyde as a fixative for any soft tissue debris remaining within the shaped canals as well as the isthmus area.

Preparation for light microscopy

All teeth were completely demineralized in an aqueous solution of 10 wt% formic acid/sodium formate (pH 2.4) for a minimum of 2 weeks at 25 °C under constant stirring. The end-point of demineralization was assessed using digital radiography. The demineralized roots were embedded in paraffin wax for microtomy. Seven-micrometre-thick serial histological sections were prepared from 10 canal levels of each root, beginning at 1.0 mm from the anatomical apex. The 10 canal levels were (from the anatomical apex) the following: 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6 and 2.8 mm. Sixteen serial sections were prepared at each canal level. Glass slides containing the serial sections were stained with Masson's trichrome. All slides derived from the same root were given a random number corresponding to the group to which root belonged and sequentially labelled according to the canal level in which they were sectioned. Only the canal level was known to the evaluator who was assigned to assess the cleanliness of the canal space, who was blind to the group from which the root was derived. Stained sections were examined using an incandescent light microscope (BX 51; Olympus America, Inc., Melville, NY, USA) equipped with a digital camera system (Retiga 4000R OImaging, Burnaby, BC, Canada). Digitized images were taken of the best, technically error-free section as being representative of respective canal level. Images were taken at the highest magnification (20–40×) in which the mesiobuccal and mesiolingual canals and the isthmus region could be included in the same image to facilitate subsequent image analysis of these three components of the canal space under the same scale from a single digitized image.

Image analysis

Digitized images were analysed using the Image J software (National Institute of Health, Bethesda, MD, USA) by a collaborator who was not involved in the canal preparation and irrigation procedures. The outlines of the mesiobuccal and mesiolingual canals and the isthmus between the two canals were traced to delineate the relative surface areas of the respective regions of the canal space. Likewise, areas occupied by

stained debris in the corresponding regions were determined. For each canal level, the percentage area occupied by debris in the shaped canals was calculated as the quotient of the summation of the relative areas occupied by debris in both canals over the summation of the mesiobuccal and mesiolingual canals and multiplied by 100. Likewise, the percentage area occupied by debris within the isthmus region was calculated as the quotient of the relative area occupied by debris in the isthmus region over the relative area of the isthmus multiplied by 100. When a 'partial isthmus' was encountered, debris within the partially exposed isthmus was expressed as the quotient of the partially exposed isthmus. In the case of a highly sclerotic isthmus with multiple, small, partially exposed isthmus loci or an isthmus that extended beyond the shaped canals as canal fins, the sum of areas occupied by debris in those regional areas was divided by the total regional areas to generate an overall debris percentage score. For statistical analysis purpose, a completely blocked isthmus or a nonexistent isthmus as a result of merging of the mesiobuccal and mesiolingual canals was given a null percentage score to achieve a balanced statistical design.

Statistical analysis

Data derived from the canals and those derived from the isthmi were analysed separately by a two-factor repeated measures analysis of variance to examine the effects of the factor 'Experimental Group' and the repeated factor 'Canal Level' and the interaction of these two factors on canal space cleanliness. Pooled data from the 10 canal levels were also analysed to examine if there were differences between the MDI group and the ANP group in terms of cleanliness of the instrumented canals and the noninstrumented isthmus regions. As the normality and homoscedasticity assumptions of the data appeared to be violated, the data were expressed as least square means and the common standard error of the least square means. Least square means are the expected values of group or subgroup means for a balanced design involving the group variables with all covariates at their common mean values. All pair-wise multiple comparisons were performed using the Tukey test. Statistical significance was set at $\alpha = 0.05$.

Results

Figure 4 contains two series of micro-CT scans showing the criterion for tooth selection based on nondestructive

scanning of the root tips of the mesial roots of mandibular first molars. Each series was taken from the 320–350 images reconstructed from the axial direction commencing at 1.0 mm from the anatomical apex of the mesial root and ending at 3.0 mm from the anatomical apex. In the first series (Fig. 4a–d), the root had a narrow isthmus that was narrower than one-quarter of the mesiodistal diameter of the unshaped root canals. However, axially reconstructed images taken from positions more coronal to the anatomical apex showed that the isthmus in this part of the root was wider than the pre-established criterion. Thus, this specimen was considered unacceptable for the study. In the second series (Fig. 4e–f), a hairline-type isthmus is illustrated that was barely visible because of its highly sclerotic nature. As the isthmus was narrower than the pre-established criterion, the specimen was considered acceptable for the study. Although the isthmus was barely visible under micro-CT scanning, it was partially patent (Weller *et al.* 1995) when examined using histological sections. Indeed, in this type of highly sclerotic 'partial isthmus' (an example is shown in Fig. 5), the isthmus is partially obliterated by sclerotic dentine in a three-dimensional manner so that it may appear completely obliterated, partially patent and completely patent at different canal levels. Such an isthmus may also become completely filled with sclerotic dentine at a more coronal canal level after the observation of its complete or partial patency at a more apical level. This makes it very difficult to get irrigants to flow three dimensionally into those patent regions beneath the blocked coronal part of the isthmus.

Figure 5 is a representative example from the MDI group showing fairly clean instrumented canals but incomplete debris removal from the narrow isthmus. The latter extended across the two canals as canal fins along the buccal and lingual aspect of the mesial root. Incomplete debridement of debris from the isthmus region is evident at all canal levels where apical gas entrapment has been shown to be present in a simulated closed canal system (Tay *et al.* 2010). Figure 6 is a representative example from the ANP group showing clean instrumented canals and isthmus at all canal levels. Complete debridement of the very narrow isthmus is evident at 1.0 and 1.2 mm from the anatomic apex with the ANP generated as the micro-cannulus of the EndoVac system was inserted to working length to eliminate the phenomenon of apical gas entrapment.

Table 1 summarizes the percentage area occupied by debris in the instrumented canals (i.e. isthmus

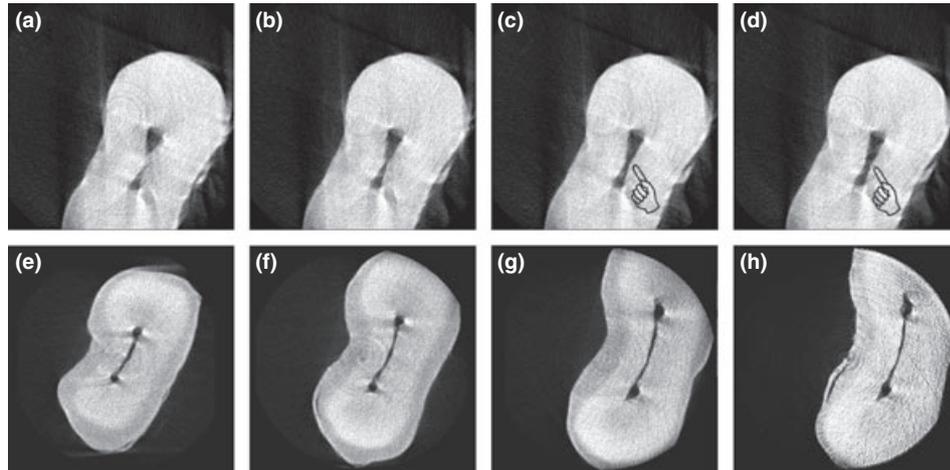


Figure 4 Micro-computed tomography (micro-CT) was used for nondestructive screening of the apical third of the mesial root of mandibular molars (1–3 mm from the anatomic apex). Roots with mesiodistal isthmus widths wider than one-quarter of the diameter of the unshaped canals anywhere along the scanned regions were excluded from the study. (a–d) Representative images from a root that had acceptable narrow isthmus widths at 1.0 mm (a) and 1.6 mm (b) from the anatomical apex but isthmus widths wider than the set selection criterion (pointers) at 2.2 mm (c) and 2.8 mm (d). This root was not included in the study. (e–h) Representative images from a root that had acceptable isthmus widths at all levels [1.0 mm – (e); 1.6 mm – (f); 2.2 mm – (g); 2.8 mm – (h)] of the scanned regions. This root was included in the study. The partial isthmus was barely visible under micro-CT but could be discerned from histological sections.

excluded) of the two experimental groups examined repeatedly at the 10 canal levels. Pooled data from all canal levels are also included. The data were presented as least square means with a common standard error of the least square means. Although there appeared to be more debris at most canal levels for the MDI group, two-way repeated measures analysis of variance revealed no significant difference between the two groups at all canal levels ($P = 0.131$). For each group, there was no difference in the area occupied by debris amongst the 10 canal levels ($P = 0.343$). There was no statistically significant interaction between the factor 'Group' and the repeated factor 'Canal Level' ($P = 0.227$). When the data from the 10 canal levels were pooled together, the difference in debris retention between the MDI group and the ANP group was also statistically insignificant ($P = 0.092$).

Although no statistical comparisons were made between debris retention in the instrumented canals and noninstrumented isthmus regions, there was a general tendency that more debris were retained within the isthmus regions in both groups, with only very few canal levels being completely free of debris in the isthmus regions. Table 2 summarizes the percentage area occupied by debris within the

noninstrumented isthmus regions (i.e. instrumented canals excluded) of the two experimental groups examined repeatedly at the 10 canal levels. Highly significant differences ($P < 0.001$) could be detected between the MDI group and the ANP group for the repeated factor 'Canal Level'. Pair-wise comparisons indicate significant differences ($P < 0.05$) at all canal levels. Conversely, the differences in least square mean values amongst the different 'Canal Level' is not great enough to exclude the possibility that the difference is because of random sampling variability after allowing for the effects of 'Group' differences ($P = 0.352$). Pair-wise comparisons of the canal levels in the MDI group showed that only debris present in the isthmus regions at 1.0 and 2.2 mm were significantly different ($P < 0.05$). There was no difference amongst other canal levels in the MDI group ($P > 0.05$) or amongst all canal levels in the ANP group ($P > 0.05$). The interaction between the factor 'Group' and 'Canal Level' was not statistically significant ($P = 0.559$). When the data from the 10 canal levels were pooled together, a highly significant difference could be detected between the MDI group and the ANP group with respect to isthmus-retained debris ($P < 0.001$).

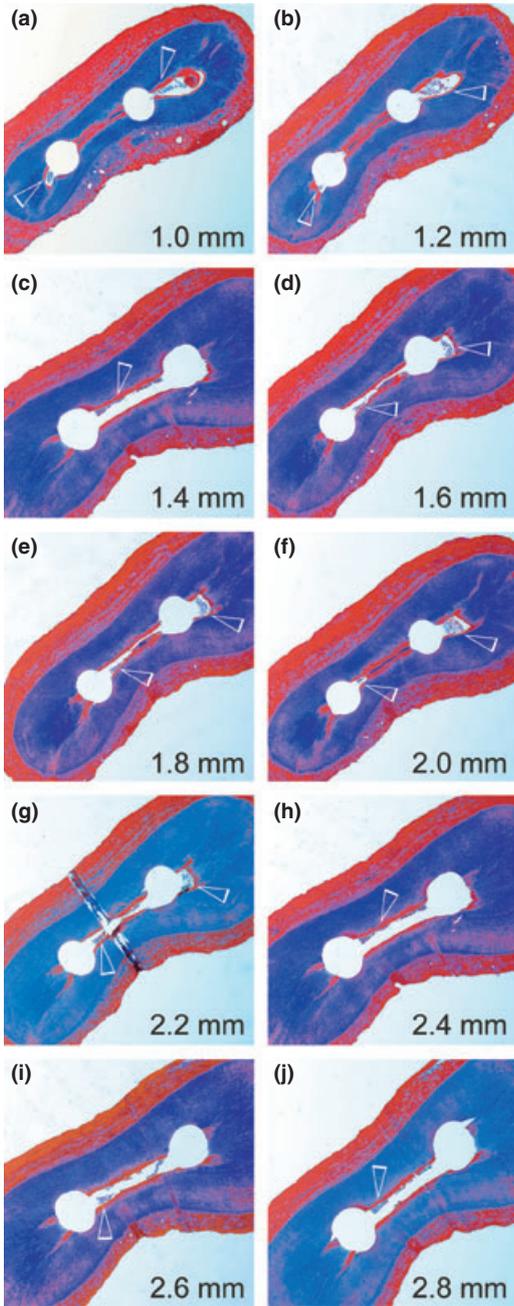


Figure 5 A representative example of a root specimen that was cleaned using the manual dynamic irrigation technique. Figures a–j represent Masson's trichrome-stained sections of the same root taken at the 10 canal levels (original magnification 20–40×). Note that the isthmus between the two canals was sclerotic (stained red) and blocked in (a) (1.0 mm) and (b) (1.2 mm). However, the part of isthmus that extended beyond the canals as canal fins remained patent at these two locations. Debris-containing regions are indicated by open arrows in each section.

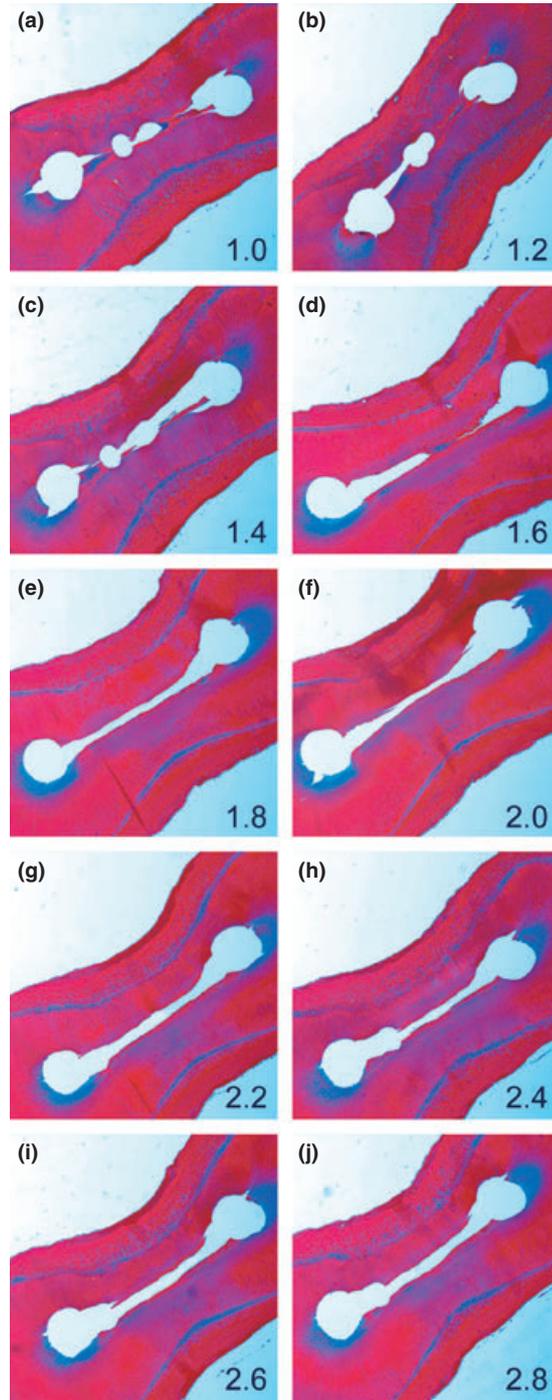


Figure 6 A representative example of a root specimen that was cleaned using the apical negative pressure technique. Figures a–j represent Masson's trichrome-stained sections of the same root taken at the 10 canal levels. No debris could be detected at each canal level. The number at the lower right corner of each image represents canal level in mm (original magnification 20–40×).

Table 1 Percentage of total canal area (mesiobuccal and mesiolingual canals combined) occupied by debris in the manual dynamic irrigation (MDI) and the apical negative pressure (ANP) groups at 10 canal levels

Canal level (from anatomic apex)	MDI (%)*	ANP (%)*
1.0 mm	0.21	0.00
1.2 mm	0.11	0.00
1.4 mm	0.02	0.00
1.6 mm	0.78	0.00
1.8 mm	0.82	0.00
2.0 mm	0.13	0.00
2.2 mm	0.00	0.00
2.4 mm	0.12	0.00
2.6 mm	0.00	0.16
2.8 mm	0.26	0.00
Pooled data from the 10 canal levels**	0.22 ¹	0.02 ¹

*Values are expressed as least square means with the common standard error of the least square means = 0.14.

**When data from the 10 canal levels of each 'Group' are pooled together, the least square mean of the 'MDI' group is 0.22% and that of the 'ANP' group is 0.02%. The common standard error of the least square means is 0.09%. There is no statistical significant difference (same letter superscripts) between the pooled data from the 'MDI' group and the 'ANP' group ($P = 0.092$).

Table 2 Percentage of total isthmus area occupied by debris in the manual dynamic irrigation (MDI) and the apical negative pressure (ANP) groups at 10 canal levels

Canal level (from anatomic apex)	MDI (%)*	ANP (%)*
1.0 mm	15.64 ^{1,B}	0.00 ^{2,a}
1.2 mm	23.59 ^{1,AB}	1.81 ^{2,a}
1.4 mm	24.39 ^{1,AB}	0.00 ^{2,a}
1.6 mm	25.02 ^{1,AB}	0.00 ^{2,a}
1.8 mm	24.76 ^{1,AB}	1.70 ^{2,a}
2.0 mm	25.93 ^{1,AB}	0.70 ^{2,a}
2.2 mm	26.69 ^{1,A}	1.41 ^{2,a}
2.4 mm	21.87 ^{1,AB}	1.84 ^{2,a}
2.6 mm	24.81 ^{1,AB}	2.52 ^{2,a}
2.8 mm	20.81 ^{1,AB}	1.46 ^{2,a}
Pooled data from the 10 canal levels**	23.36 ¹	1.15 ²

*Values are expressed as least square means with the common standard error of the least square means = 0.14. For each column, different letter superscripts denote statistical significance ($P < 0.05$). For each row, different numeric superscripts denote statistical significance ($P < 0.05$).

**When data from the 10 canal levels of each 'Group' are pooled together, the least square mean of the 'MDI' group is 23.36% and that of the 'ANP' group is 1.15%. The common standard error of the least square means is 2.49%. The difference of the pooled data between the 'MDI' group and the 'ANP' group is highly significant ($P < 0.001$).

Discussion

In this study, two irrigation/agitation techniques were selected for comparison of their canal and isthmus debridement efficacy in the mesial root of mandibular molars. These two techniques differ from other irrigant/agitation regimes (Gu *et al.* 2009) in that the agitation/negative suction components are inserted to the working length of the instrumented canals, which is not possible or recommended in other irrigant delivery or agitation techniques, such as the use of sonic or ultrasonic agitation devices.

The use of an *ex vivo* closed-end canal model more accurately simulates *in vivo* situations, in which the tooth's foramen and outer surface are sealed by the periodontal ligament and further embedded in alveolar bone. Gas entrapment along the end of a closed microchannel (Migun & Shnip 2002, Pesse *et al.* 2005) is a well-known physical phenomenon in engineering sciences. Although gas entrapment was created by placing small capillaries in a liquid in those studies and may be different from what is encountered in root canal irrigation (Senia *et al.* 1971), it is possible that similar gas entrapment may occur in the most 0–2 mm root canal during the delivery of an irrigant. Tay *et al.* (2010) compared the smear layer removal and debris clearance potential of NaOCl and EDTA delivered by a side-vented needle to single canal anterior teeth in a closed canal system versus an open canal system. Although the closed-end system utilized by Tay *et al.* (2010) was not as elaborate as the experimental set up employed in this study, it served the same purpose in preventing fluid and gas communication between the root canal and the external environment, thereby simulating the clinical scenario when a tooth is encased by the periodontal ligament in a bony socket (Gutaris *et al.* 2005, Burleson *et al.* 2007). In that study, an open canal system was also employed in which irrigants were allowed to drip through the apical foramen. The results of that study indicated that the canals were considerably cleaner at all levels (i.e. coronal third, middle third and apical third) of the canal when an open canal design was employed, and that the differences in smear scores and debris scores between the closed canal and open canal systems were most notable in the apical third of the canals. In the same study, the authors opined that it was difficult to use scanning electron microscopy for examining the apical 0.5–1 mm of the canal walls and suggested using light microscopy for critically examining the canal cleanliness (i.e. debris retention) at the different

canal levels in the critical zone in which the apical lock phenomenon appeared to exist inside a closed canal (i.e. 0–2 mm from the apical seat). Thus, light microscopy was employed in this study to examine this critical zone at 0.2 mm intervals for 10 canal levels (i.e. 1.0–2.8 mm from the anatomical apex, with working length established at 1.0 mm from the anatomical apex). As it has been confirmed that an open canal system overestimates canal debridement efficacy in *ex vivo* studies, the use of a dual-system experimental design was not repeated in this study. In a separate study that utilized a dual-system design, also it has been determined that there was no fluid extruded from an open canal system when the ANP technique was used during irrigation of single-rooted teeth.

Understandably, light microscopy does not have the resolution of scanning electron microscopy to identify smear layer remnants from the instrumented canal walls. Nevertheless, smear layers are not present in the noninstrumented isthmus regions. Thus, no attempt was made in this study to examine the efficacy of smear layer removal. The use of light microscopy in combination with Masson's trichrome staining, however, did permit easier recognition of sclerotic dentine and acellular cementum, which retained the red, acid fuchsin component of the trichrome stain (Flint *et al.* 1975). Conversely, this acidic dye component was displaced by the subsequently applied aniline blue dye component, which stained the collagen component of the tubular radicular dentine. The loose soft tissue debris within the canals and isthmi were predominantly stained with a blue hue. In some specimens, the entire root section was stained intensely red except for some tubular radicular dentine and the intermediate cementum that were stained with a blue colour (not shown). A detailed discussion of the histochemical mechanisms associated with dye retention is beyond the scope of this discussion. Previous studies using light microscopy (Paqué *et al.* 2006) and scanning electron microscopy (Tay *et al.* 2010) have shown that the apical 3 mm of the radicular dentine in anterior teeth was highly sclerotic. Thus, the red-stained radicular dentine in this study could represent also sclerotic dentine. It is noteworthy that in sections with highly sclerotic dentine, the blue-stained tubular dentine was always located along buccolingual axis of the mesial root, external to the mesiobuccal and mesiolingual canals. A similar phenomenon was also observed when scanning electron microscopy was used to examine the apical 5 mm of premolar teeth

with two canals. Patent tubular dentine was predominantly found along the buccolingual axis of the premolar roots and external to the buccal and palatal canals, whilst the rest of the radicular dentine was highly sclerotic. It is not known what exactly causes dentine sclerosis in the apical part of the roots and why the process of dentine sclerosis is slower along the longer buccolingual axis when compared with the shorter mesiodistal axis. This is an important issue that merits further research.

In the presence of apical gas entrapment associated with a closed canal system (Tay *et al.* 2010), both MDI with the repeated insertion of a close fitting gutta-percha cone to working length and ANP technique with the placement of the EndoVac micro-cannulus to working length resulted in relatively clean instrumented canal spaces. As there were no differences in the percentage of canal spaces occupied by debris between the two techniques at all canal levels, the first null hypothesis that there is no difference between the canal debridement efficacy of the two irrigant agitation techniques at different canal levels from the anatomical apex in a simulated closed canal system must be accepted. As there is fairly close adaptation between the gutta-percha cone utilized in the MDI technique and the canal walls, it is possible that some of the hard tissue debris may be impregnated on the surface of the gutta-percha cone during repeated insertions into the canal space. Repeated rubbing of a gutta-percha cone with irregular dentine chips impregnated on its surface against a canal wall that has been depleted of the smear layer may generate a new smear layer. This speculation has to be confirmed using scanning electron microscopy of the canal walls that have been treated with the MDI technique. The information would be of interest to those clinicians who ascribe to the philosophy of smear layer removal as their canal debridement objectives (Shahravan *et al.* 2007).

Contrary to the results obtained from the instrumented canals, both MDI and the ANP technique were unable to completely remove debris from the narrow isthmi present in the mesial root of mandibular molars. Debris ranging from 15 to 27 area % was found to be present at all the 10 canal levels of the MDI group, whilst debris ranging from 0.7 to 2.5 area % was observed at 7 of the 10 canal levels of the ANP group. As there was considerably more debris present in the MDI group, either at individual canal levels or when the data from the 10 levels were pooled together for statistical analysis, the second null hypothesis that there is no difference between the isthmus debridement

efficacy of the two irrigant agitation techniques at different canal levels from the anatomical apex in a simulated closed canal system must be rejected.

It is pertinent to note that debris removal was much more difficult in the narrow isthmus regions than in the instrumented canals. Although the two examples shown in Figs 5 and 6 were completely patent isthmi, up to 30% of the isthmi in each group were partially obliterated (not shown). These 'partial isthmi' were obliterated by sclerotic dentine in a three-dimensional manner so that completely obliterated, partially obliterated and completely patent versions of the same isthmus could be observed at different canal levels of the apical 1–2.8 mm region of the roots. This complicated morphology rendered it extremely difficult for the delivery of a large volume of irrigant with a high flow rate to the entire isthmus region even with the use of the ANP technique. It is worth mentioning that the presence of a completely patent isthmus did not result in better debris removal, as illustrated in the MDI group (Fig. 5). With the MDI technique, it is possible, as previously mentioned, that displacement of the apically entrapped gases within the canal space was achieved at the expense of displacing gases to the dead-ends of the isthmus regions. It is also possible that the gutta-percha cone, being closely adapted only to circular canal space, was incapable of displacing the gases that was originally present within the isthmus region. Irrespective of the cause, this resulted in a greater amount of debris remaining within the narrow isthmi in the MDI group. Using micro-CT to examine the distribution of isthmi in the apical 5 mm of the mesial root of mandibular molars, Mannocci *et al.* (2005) reported that isthmi recognizable by micro-CT were present at all canal levels with prevalence between 17% and 50%. As the micro-CT employed in that study had a lower resolution than what could be achieved using light microscopy, this study clearly demonstrated that even under the circumstance where a hairline-type isthmus was barely visible under the micro-CT, the isthmus could be identified as partially patent under light microscopy. Teixeira *et al.* (2003) reported that 22% of the isthmi present in mandibular first molars were completely patent whilst 37% were partially patent. Although only narrow isthmi were selected for examination in this study, it would be interesting to compare the effect of isthmus debridement between roots with narrow and wide isthmi using the ANP technique in a future study.

Within the limits of this study, it may be concluded that both the MDI technique and the ANP technique

produced fairly clean canals that had minimal debris recognizable by light microscopy. Both techniques did not completely remove light microscopy-recognisable debris from the narrow isthmus between the canals because of the difficulty in getting irrigating solutions to reach the isthmus and to create a strong enough current to flow through the isthmus. Although both techniques involve the placement of the agitation component directly to working length, the use of the ANP technique resulted in considerably cleaner isthmi when compared with MDI.

Acknowledgements

Set ups for the closed canal system for the simulation of the effect of apical gas entrapment were generously provided by Dr John Schoeffel. Preparation of histological sections was generously supported by the Department of Endodontics, School of Dentistry, Medical College of Georgia. The micro-CT scanner employed in one part of the study was acquired via Grant R21 DE019213-01 from the National Institute of Dental and Craniofacial Research (PI. Franklin R. Tay). The authors thank Dr Jongruhl Kim for demineralization of the root specimens, Mrs Donna Kumiski for cutting, staining and mounting of the histological sections, Dr Ulf Wikesjo for use of his light microscope for acquisition of the digitized images, Mr Thomas Bryan for assembly of the fixtures and for cleaning up after completion of the experiments by endodontic residents and Mrs Marie Churchville for secretarial support.

References

- Albrecht LJ, Baumgartner JC, Marshall JG (2004) Evaluation of apical debris removal using various sizes and tapers of ProFile GT files. *Journal of Endodontics* **30**, 425–8.
- Archer R, Reader A, Nist R, Beck M, Meyers WJ (1992) An in vivo evaluation of the efficacy of ultrasound after step-back preparation in mandibular molars. *Journal of Endodontics* **18**, 549–52.
- Baumgartner JC, Mader CL (1987) A scanning electron microscopic evaluation of four root canal irrigation regimens. *Journal of Endodontics* **13**, 147–57.
- Bergenholtz G, Spångberg L (2004) Controversies in endodontics. *Critical Reviews in Oral Biology and Medicine* **15**, 99–114.
- Boutsiokis C, Lambrianidis T, Kastrinakis E (2009) Irrigant flow within a prepared root canal using various flow rates: a Computational Fluid Dynamics study. *International Endodontic Journal* **42**, 144–55.

- Brito PR, Souza LC, Machado de Oliveira JC et al. (2009) Comparison of the effectiveness of three irrigation techniques in reducing intracanal *Enterococcus faecalis* populations: an in vitro study. *Journal of Endodontics* **35**, 1422–7.
- Burleson A, Nusstein J, Reader A, Beck M (2007) The in vivo evaluation of hand/rotary/ultrasound instrumentation in necrotic, human mandibular molars. *Journal of Endodontics* **33**, 782–7.
- Carver K, Nusstein J, Reader A, Beck M (2007) In vivo antibacterial efficacy of ultrasound after hand and rotary instrumentation in human mandibular molars. *Journal of Endodontics* **33**, 1038–43.
- Chávez de Paz LE, Bergenholtz G, Dahlén G, Svensäter G (2007) Response to alkaline stress by root canal bacteria in biofilms. *International Journal of Endodontics* **40**, 344–55.
- Chávez de Paz LE, Hamilton IR, Svensäter G (2008) Oral bacteria in biofilms exhibit slow reactivation from nutrient deprivation. *Microbiology* **154**, 1927–38.
- Chow TW (1983) Mechanical effectiveness of root canal irrigation. *Journal of Endodontics* **9**, 475–9.
- Desai P, Himel V (2009) Comparative safety of various intracanal irrigation systems. *Journal of Endodontics* **35**, 545–9.
- Estrela C, Sydney GB, Figueiredo JA, Estrela CR (2009) Antibacterial efficacy of intracanal medicaments on bacterial biofilm: a critical review. *Journal of Applied Oral Sciences* **17**, 1–7.
- Evans GE, Speight PM, Gulabivala K (2001) The influence of preparation technique and sodium hypochlorite on removal of pulp and predentine from root canals of posterior teeth. *International Endodontic Journal* **34**, 322–30.
- Flint MH, Lyons MF, Meaney MF, Williams DE (1975) The Masson staining of collagen – an explanation of an apparent paradox. *Histochemical Journal* **7**, 529–46.
- Fukumoto Y, Kikuchi I, Yoshioka T, Kobayashi C, Suda H (2006) An ex vivo evaluation of a new root canal irrigation technique with intracanal aspiration. *International Endodontic Journal* **39**, 93–9.
- Gao Y, Haapasalo M, Shen Y et al. (2009) Development and validation of a three-dimensional computational fluid dynamics model of root canal irrigation. *Journal of Endodontics* **35**, 1282–7.
- de Gregorio C, Estevez R, Cisneros R, Heilborn C, Cohenca N (2009) Effect of EDTA, sonic, and ultrasonic activation on the penetration of sodium hypochlorite into simulated lateral canals: an in vitro study. *Journal of Endodontics* **35**, 891–5.
- Gu LS, Kim JR, Ling J, Choi KK, Pashley DH, Tay FR (2009) Review of contemporary irrigant agitation techniques and devices. *Journal of Endodontics* **35**, 791–804.
- Gutarts R, Nusstein J, Reader A, Beck M (2005) In vivo debridement efficacy of ultrasonic irrigation following hand-rotary instrumentation in human mandibular molars. *Journal of Endodontics* **31**, 166–70.
- Haidet J, Reader A, Beck M, Meyers W (1989) An in vivo comparison of the step-back technique versus a step-back/ultrasonic technique in human mandibular molars. *Journal of Endodontics* **15**, 195–9.
- Hsieh YD, Gau CH, Kung Wu SF, Shen EC, Hsu PW, Fu E (2007) Dynamic recording of irrigating fluid distribution in root canals using thermal image analysis. *International Endodontic Journal* **40**, 11–7.
- Jiang LM, Verhaagen B, Versluis M, van der Sluis LW (2010) Evaluation of a sonic device designed to activate irrigant in the root canal. *Journal of Endodontics* **36**, 143–6.
- Mannocci F, Peru M, Sherriff M, Cook R, Pitt Ford TR (2005) The isthmuses of the mesial root of mandibular molars: a micro-computed tomographic study. *International Endodontic Journal* **38**, 558–63.
- McGill S, Gulabivala K, Mordan N, Ng YL (2008) The efficacy of dynamic irrigation using a commercially available system (RinsEndo) determined by removal of a collagen 'biomolecular film' from an ex vivo model. *International Endodontic Journal* **41**, 602–8.
- Migun NP, Shnip AI (2002) Model of film flow in a dead-end conic capillary. *Journal of Engineering Physics and Thermophysics* **75**, 1422–8.
- Moser JB, Heuer MA (1982) Forces and efficacy in endodontic irrigation systems. *Oral Surgery Oral Medicine Oral Pathology* **53**, 425–8.
- Nair PN (2006) On the causes of persistent apical periodontitis: a review. *International Endodontic Journal* **39**, 249–81.
- Nielsen BA, Baumgartner CJ (2007) Comparison of the EndoVac system to needle irrigation of root canals. *Journal of Endodontics* **33**, 611–5.
- Paqué F, Luder HU, Sener B, Zehnder M (2006) Tubular sclerosis rather than the smear layer impedes dye penetration into the dentine of endodontically instrumented root canals. *International Endodontic Journal* **29**, 18–25.
- Paqué F, Laib A, Gautschi H, Zehnder M (2009) Hard-tissue debris accumulation analysis by high-resolution computed tomography scans. *Journal of Endodontics* **35**, 1044–7.
- Pesse AV, Warriar GR, Dhir VK (2005) An experimental study of the gas entrapment process in closed-end microchannels. *International Journal of Heat and Mass Transfer* **48**, 5150–65.
- Peters OA (2004) Current challenges and concepts in the preparation of root canal systems: a review. *Journal of Endodontics* **30**, 559–67.
- Ricucci D, Siqueira JF Jr (2010) Fate of the tissue in lateral canals and apical ramifications in response to pathologic conditions and treatment procedures. *Journal of Endodontics* **36**, 1–15.
- Schoeffel GJ (2008) The EndoVac method of endodontic irrigation: part 2—efficacy. *Dentistry Today* **27**, 82, 84, 86–87.
- Senia ES, Marshall FJ, Rosen S (1971) The solvent action of sodium hypochlorite on pulp tissue of extracted teeth. *Oral Surgery Oral Medicine Oral Pathology* **31**, 96–103.
- Shahravan A, Haghdoost AA, Adl A, Rahimi H, Shadifar F (2007) Effect of smear layer on sealing ability of canal

- obturation: a systematic review and meta-analysis. *Journal of Endodontics* **33**, 96–105.
- Siqueira JF Jr, Araújo MC, Garcia PF, Fraga RC, Dantas CJ (1997) Histological evaluation of the effectiveness of five instrumentation techniques for cleaning the apical third of root canals. *Journal of Endodontics* **23**, 499–502.
- Tay FR, Gu LS, Schoeffel JG et al. (2010) The effect of vapor lock on root canal debridement using a side-vented needle for positive-pressure irrigant delivery. *Journal of Endodontics* **36**, 745–750.
- Teixeira FB, Sano CL, Gomes BP, Zaia AA, Ferraz CC, Souza-Filho FJ (2003) A preliminary in vitro study of the incidence and position of the root canal isthmus in maxillary and mandibular first molars. *International Endodontic Journal* **36**, 276–80.
- Usman N, Baumgartner JC, Marshall JG (2004) Influence of instrument size on root canal debridement. *Journal of Endodontics* **30**, 110–2.
- Vaudt J, Bitter K, Neumann K, Kielbassa AM (2009) Ex vivo study on root canal instrumentation of two rotary nickel-titanium systems in comparison to stainless steel hand instruments. *International Journal of Endodontics* **42**, 22–33.
- Walker TL, del Rio CE (1991) Histological evaluation of ultrasonic debridement comparing sodium hypochlorite and water. *Journal of Endodontics* **17**, 66–71.
- Weller RN, Niemczyk SP, Kim S (1995) Incidence and position of the canal isthmus. Part 1. Mesio Buccal root of the maxillary first molar. *Journal of Endodontics* **21**, 380–3.
- Williamson AE, Sandor AJ, Justman BC (2009) A comparison of three nickel titanium rotary systems, EndoSequence, ProTaper universal, and profile GT, for canal-cleaning ability. *Journal of Endodontics* **35**, 107–9.
- Yamada R, Armas A, Goldman M, Pin A (1983) A scanning electron microscopic comparison of a high volume final flush with several irrigating solutions. *Journal of Endodontics* **9**, 137–42.
- Zehnder M (2006) Root canal irrigants. *Journal of Endodontics* **32**, 389–98.