Efficacy of Three Supplementary Irrigation Protocols in the Removal of Hard-tissue Debris from the Mesial Root Canal System of Mandibular Molars

by

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Abstract

Removal of accumulated hard-tissue debris (AHTD) by two ultrasonically-assisted and one multisonic irrigation systems was assessed with micro-computed tomography (micro-CT). Twenty-four extracted mandibular molars having two mesial canals connected by an isthmus and converging to a single foramen were selected. After preparation of the mesial canals with WaveOne Gold instruments (Dentsply Sirona Maillefer, Ballaigues, Switzerland), anatomically matched specimens were assigned to three final irrigation protocols (n=8): intermittent-ultrasonic (IU), continuous-ultrasonic (CU) and GentleWave (GW) system (Sonendo Inc, Laguna Hills, CA). Datasets of the micro-CT scans were co-registered and the percentage reduction of AHTD was statistically compared using one-way ANOVA and post-hoc Tukey tests with 5% significance level. Mean percentage reduction of AHTD in canals and isthmuses was significantly higher for GW (96.4% and 97.9%, respectively) than for CU (80.0% and 88.9%, respectively) (P<0.05). AHTD reduction for IU (91.2% and 93.5%, respectively) did not differ significantly from GW and CU (P>0.05).
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Chapter 1
Introduction

1 Apical Periodontitis

Apical periodontitis (AP) is an inflammatory process leading to destruction of the periradicular tissues. Embodying a host defense response to microbial challenge of endodontic origin, it involves pathologic changes of the cementum, periodontal ligament and alveolar bone, that are radiographically manifested as a periapical radiolucency. The presence of micro-organisms in the root canals of teeth with inflamed and necrotic pulps was demonstrated over a century ago (1) while their etiological role in the development of AP was established half a century later in 1965. Kakehashi et al. (2) demonstrated that pulp necrosis and periapical destruction occurred when pulp tissues were exposed to the oral cavity in control rats with a conventional oral microbial flora, whereas no AP developed in germ-free rats. The essential role of micro-organisms in the development of AP was further confirmed in subsequent animal (3-5) and human (6, 7) studies.

AP is a biofilm-mediated infection (8, 9). Biofilms are dynamic communities of bacteria that are irreversibly attached and are embedded in a self-produced extracellular matrix (10). Biofilms are omnipresent and essential to human homeostasis (11). However, they are also responsible for most chronic infections and almost all persistent bacterial infections (11, 12). Biofilm formation is one of the main strategies for bacterial growth and survival. The ability of micro-organisms to interact and to establish a polymicrobial flora enhance their resistance to antimicrobial measures and their capability to induce more severe tissue destruction compared to their planktonic form and to mono-species infections (13).

Biofilms represent a protected mode of growth and are equipped with adaptive capacity to survive environmental stresses and to interfere with host defense mechanisms (14). The self-produced extracellular matrix of extracellular polymeric substance (EPS) constitutes the main component of biofilms, occupying 85-90% of their volume, and is composed of extracellular polysaccharides, proteins and DNA (15). Maintaining the integrity of biofilms, EPS protects against unfavourable environmental conditions such as desiccation, pH and oxidative stresses and osmotic shock (16). It also acts as an impermeable barrier, preventing harmful substances like antibiotics and
disinfectants from diffusing through the cell membrane (13). The protective effect of this diffusion barrier is further enhanced with the presence of extracellular substances and enzymes embedded within the matrix. Certain components of the extracellular matrix can react chemically and directly neutralize various antimicrobial agents (17).

The concept of biofilms in the root canal systems was recognized by Nair (8) in 1987. Using correlative light and transmission electronic microscopy, the presence of dense aggregates of bacteria condensed in single or multiple layers and attached to dentinal canal walls was described. An amorphous material was observed amongst the bacterial aggregates. Despite the presence of a diverse microflora in the oral cavity, only a limited assortment of species was recovered in the endodontic microflora (14). AP is a dynamic habitat-adapted infection of the root canal space. Bacterial dispersion in root canal biofilms varies according to the environmental and nutritional conditions within the root canal system (18). Obligate anaerobes dominate the endodontic flora of teeth with clinically intact crowns but having necrotic pulps and AP (7, 19, 20). In contrast, the root canal flora of teeth with exposed pulps and AP is less dominated by strict anaerobes (4, 6, 7). The type of bacterial metabolism is dictated by the availability of oxygen and the type of nutrients (e.g. carbohydrates and proteins). In addition, the endodontic microflora of teeth with untreated or primary AP differs significantly from that of post-treatment or secondary AP (21). Primary endodontic infections are typically polymicrobial with a predominance of gram-negative anaerobic rods. Various bacterial species dominate at different stages of the disease process. As the degradation of pulp tissue and the endodontic infection progress, there is a depletion of oxygen and nutrients in the apical portion of the root canal space. Slow growing obligate anaerobes with proteolytic capability dominate in the apical portion, whereas the more coronal aspect of the root canal is generally colonized by rapid growing facultative anaerobes (18). In post-treatment AP, the microbial flora is usually reduced in species diversity and quantity, and the bacterial species recovered are typically Gram-positive facultative bacteria (22).

The aim of endodontic therapy is to prevent or treat AP by disinfection of the root canal system, which is achieved through chemo-mechanical cleaning protocols (23). Although root canal treatments consistently yield favourable long-term outcomes, reduction or resolution of an apical radiolucency may not occur in all endodontically treated teeth. Persistent endodontic infections may be attributed to different factors including insufficient aseptic control, iatrogenic errors,
untreated canals, inadequate mechanical debridement, and ineffective coronal seal (24). Even when all the clinical principles are met, and all procedures performed to the highest standards, infection may still persist. There are factors beyond the root canals that can interfere with post-treatment healing of the periapical tissues, such as foreign body reaction to exogenous materials or endogenous cholesterol crystals, a cystic condition of the lesion and extraradicular infections (25). In addition, despite unprecedented advances in endodontic treatment technologies, the complex anatomy of the root canal system remains a substantial obstacle to achieving effective disinfection and removal of biofilms, with portions of root canal walls remaining inaccessible to instruments and antimicrobials (26, 27).

2 Anatomy of the root canal system

Various methodologies have been used to study the complex root canal morphology (28). Two-dimensional and three-dimensional evaluation techniques include plastic resin injection, cavity access and radiographs with files placed within the root canals, retrospective radiographic observation, clearing of samples with and without ink injection, sectioning and macroscopic evaluation, scanning electron microscopy (SEM), computed tomography (CT), spiral computed tomography (SCT), micro-computed tomography (micro-CT), and cone-beam computed tomography (CBCT) (29). Differences in study design, methodology and studied populations account for the reported anatomical variations.

2.1 Root canal morphology and configuration

In a classic study on the classification of root canal morphology using two thousand four hundred extracted human permanent teeth that were cleared and injected with hematoxylin dye, Vertucci et al. (30) identified eight pulp space configurations:

- **Type I** A single canal extends from the pulp chamber to the apex
- **Type II** Two separate canals leave the pulp chamber and join short of the apex to form one canal (2-1 configuration)
- **Type III** One canal leaves the pulp chamber, divides into two within the root, and then merges to exit as one canal (1-2-1 configuration)
- **Type IV** Two separate and distinct canals extend from the pulp chamber to the apex
Type V  One canal leaves the pulp chamber and divides short of the apex into two separate and distinct canals with separate apical foramina (1-2 configuration)

Type VI  Two separate canals leave the pulp chamber, merge in the body of the root, and redivide short of the apex to exit as two distinct canals (2-1-2 configuration)

Type VII One canal leaves the pulp chamber, divides and then rejoins within the body of the root, and finally redivides into two distinct canals short of the apex (1-2-1-2 configuration)

Type VIII Three separate and distinct canals extend from the pulp chamber to the apex

2.2 Isthmus

An isthmus is defined as a narrow communication between two canals in the same root that contains pulp-derived tissue (31, 32). It has also been described as a corridor (33), a lateral connection (34) and a transverse anastomosis (30). Hsu and Kim (35) classified isthmus configurations as follows:

Type I Two or three canals with no communications

Type II Two canals with a definite connection between them

Type III Three canals with a definite connection between them

Type IV Canals extend into the isthmus area

Type V A true connection or wide corridor between the 2 main canals

Type V was reported as the most frequent among mesial roots of mandibular molars (35).

2.3 Mandibular molars

Mandibular first molars are the most frequent endodontically treated teeth (36, 37). Mandibular first molars typically present with 2 roots, the mesial root characterized by a flattened mesiodistal dimension and a widened buccolingual dimension, and a distal root with one wide oval canal or 2 canals (29). The presence of a third root (i.e. radix entamoralis) averages 13% and is strongly
correlated with the ethnicity of studied populations (29). The most common canal space configuration, according to Vertucci’s classification (29), is type IV with an incidence of 52%, followed by type II with an incidence of 35%.

Mandibular first molars frequently present complex root canal configurations with intercanal communications. A systematic review (29) reported that isthmus communications are present in 55% of the mesial roots and in 20% of the distal roots. The incidence of type V isthmuses ranges from 23% to 77% in mesial roots and 8% to 55% in the distal roots (29). Using a stereomicroscope, Teixeira et al. (38) reported the isthmus incidence to be the greatest at 3 to 5 mm from the apex. In another micro-computed tomography study (39) evaluating the incidence of isthmuses in the apical 5 mm of the mesial root of mandibular molars, the presence of isthmuses at all levels ranged from 17% to 50%. It was also reported that the first millimeter from the apex contained fewer isthmuses, and the presence of isthmuses was most abundant at the third millimeter from the apex. When mandibular first molars were inspected clinically during apical surgery, isthmuses were observed in 83% in mesial roots, from which 29% were type V, and in 36% of distal roots, from which 21% were type V (40).

Considering the high incidence of isthmuses in root canal systems, this anatomical canal configuration should be addressed during nonsurgical and surgical endodontic interventions. In addition, isthmuses are subject to bacterial colonization similar to that of the main root canals (41) but are inaccessible to instruments. As a result, their disinfection critically relies on effective delivery of antibacterial solutions.

3  Disinfection in endodontics

3.1  Instrumentation

Instrumentation of the root canal system fulfills both mechanical and biological goals (42). According to Hülsmann et al. (43), adequate mechanical instrumentation of the root canal system should satisfy the following major criteria:

- Removal of vital and necrotic tissue from the main root canal(s)
- Creation of sufficient space for irrigation and medication
- Preservation of the integrity and location of the apical canal anatomy
- Avoidance of iatrogenic damage to the canal system and root structure
- Facilitation of canal filling
- Avoidance of further irritation and/or infection of the periradicular tissues
- Preservation of sound root dentine to allow long-term function of the tooth

The aforementioned mechanical and biological goals can be accomplished using instrumentation, either manual, automated, sonic or ultrasonic, laser irradiation, or non-instrumental modalities (43). Mechanical instrumentation alone has limited antibacterial effects. Byström and Sundqvist (20) demonstrated that mechanical instrumentation accompanied by irrigation with saline rendered only 53% of subsequent root canal samples free of bacterial growth, compared to 80% when mechanical instrumentation was combined with 0.5% sodium hypochlorite (NaOCl) irrigation. Similar results were obtained with the use of nickel titanium rotary files (44). In addition, Peters et al. (27) reported that 35 to 50% of the root canal space remained non-instrumented following routine mechanical preparation. Apical ramifications, lateral canals, and isthmuses connecting main root canals have all been shown to harbour bacterial biofilm-like structures (9, 41, 45, 46). As a result, chemical disinfection is essential for predictable and effective elimination of bacteria from infected root canals and inaccessible canal areas.

3.2 Irrigation

Irrigation in endodontics is indispensable as it possesses mechanical, chemical and biological properties (47). An ideal irrigation solution should be biocompatible, and allow for proper lubrication of the canals, flushing of the debris, dissolution of organic and inorganic tissues, and removal of the smear layer. Its antimicrobial effect should also possess a sustained effect and enable the inactivation of endotoxins and microorganisms in planktonic and biofilm states, without affecting the physical properties of dentin. Several irrigation solutions have been investigated for use in endodontics, of which NaOCl remains the most commonly used and the antibacterial solution of choice in endodontics.

3.3 Sodium hypochlorite (NaOCl)

Potassium hypochlorite was first chemically created by Claude Louis Berthollet and was later industrially produced in Javel, France in the 18th century, hence the name “eau de javel” (48). Hypochlorite solutions were initially used as bleaching agents. NaOCl was later recommended by
Labarraque to prevent childbed fever and other infectious diseases (49). Its antiseptic properties gained wide acceptance by the end of the 19th century, and the use of a buffered 0.5% NaOCl solution was used for the irrigation of open and infected wounds during World War I (50). NaOCl is an excellent non-specific proteolytic and antimicrobial agent. These properties, along with its low cost and ease of access, prompted the use of NaOCl in endodontics as recommended by Coolidge in 1919 (51).

NaOCl is a strong base and a nonspecific oxidizer. The mechanism of action of NaOCl facilitates organic tissue, lipid and fatty acid degradation and bacterial enzyme inactivation. It also interferes with cellular metabolism and phospholipid destruction. NaOCl exhibits a dynamic equilibrium (52):

\[
\text{NaOCl} + \text{H}_2\text{O} \rightleftharpoons \text{NaOH} + \text{HOCl} \rightleftharpoons \text{Na}^+ + \text{OH}^- + \text{H}^+ + \text{OCl}^-
\]

NaOCl acts as an organic and fat solvent that degrades fatty acids and transforms them into fatty acid salts (soap) and glycerol (alcohol). This saponification reaction also reduces the surface tension of the remaining solution. In the neutralization reaction, NaOCl neutralizes amino acids, forming water and salt. In water, NaOCl ionizes to produce Na+ and hypochlorite ion (OCl-), and establishes an equilibrium to form sodium hydroxide (NaOH) and hypochlorous acid (HOCl), a weak acid and an oxidizer. The presence of HOCl and OCl- are pH dependent. At high pH values of 9 and above, the hypochlorite ion predominates, whereas at neutral pH, the hypochlorous acid is mostly present (53). A chloramination reaction occurs when HOCl releases chlorine that then combines with amino groups (-NH₂) to form chloramines, which interfere with cell metabolism. Hypochlorous acid and hypochlorite ions disrupt oxidative phosphorylation and other membrane-associated activities and lead to amino acid degradation and hydrolysis (42, 52). Chlorine compounds are naturally generated by neutrophils and are part of the innate immune response in humans. The antimicrobial effectiveness of NaOCl also relies significantly on the availability of its chlorine content (OCl- and HOCl). Chlorine is a strong oxidant and causes an irreversible oxidation of sulphydryl groups (-SH) in bacterial enzymes, rendering the enzymes inactive and inhibiting their adverse actions.

The concentrations of NaOCl used in endodontics range from 0.5% to 8.25%. While the use of different concentrations has met with controversy, the tissue dissolving effect has been shown to be directly related to the concentration (54). In contrast, clinical and laboratory studies have failed...
to demonstrate significant differences in antibacterial efficacy related to concentrations of NaOCl (55-58). The lower and higher concentrations are equally effective in reducing the number of bacteria in infected root canals (57). Apparently, the volume and exchange frequency of the irrigation solution are more critical for disinfection; a larger volume of irrigation is recommended to compensate for lower concentrations (57). The presence of organic matter like inflammatory exudates, tissue remnants and microbial biomass consume NaOCl and results in weakening of its effect (58). Continuous replenishment and adequate time exposure are thus important for optimizing the effects of NaOCl (59).

3.4 Ethylenediaminetetraacetic acid (EDTA)

Various chlorine-releasing and auxiliary irrigation solutions have been advocated in endodontics, but none appears to have the same effectiveness as NaOCl at comparable concentrations. While possessing excellent antimicrobial and tissue dissolving properties, NaOCl is toxic to the periapical tissues and has little effect on the removal of inorganic matter like the smear layer. Although none of the currently available irrigation solutions can be regarded as ideal, studies have been conducted to optimize the properties of the most commonly used solutions. Goldman et al. (60) proposed the use of both NaOCl and REDTA, a commercial brand of ethylenediaminetetraacetic acid (EDTA), to remove the organic matter and smear layer observed by scanning electron microscopy in the root canal space. Subsequently, Byström et al. (56) have demonstrated in vivo that the combined use of 5% NaOCl and 15% EDTA was more efficient than the use of NaOCl alone, that is unable to remove inorganic matter.

A smear layer is an amorphous and thin layer composed of fragments of odontoblastic processes, bacteria and pulp remnants (61). It is formed during mechanical preparation when a metallic endodontic instrument, such as hand and rotary stainless steel and NiTi files, ultrasonic tips and burs, touches a mineralized dentin wall within the root canal (62). The smear layer has two confluent components, one adhering on the surface of the canal walls, and the other one extending into dentinal tubules up to a depth of 40 µm (63). There has been considerable disparity in the literature regarding the importance of removing the smear layer. Nonetheless, there is strong consensus on the need to remove it, mainly because the smear layer harbours bacteria and biofilms (61, 62). In addition, the smear layer covers the dentin surface and obscures the openings of dentinal tubules. It then acts as a barrier to disinfecting agents. An adequate seal between the root
filling material and the canal walls may also be compromised with a reduced penetration of the sealer (62).

McComb and Smith (64) were the first to demonstrate that REDTA removed the smear layer formed on the surface of instrumented root canals. EDTA is a strong chelating agent, with concentrations ranging from 10 to 17%. EDTA is considered to be more tissue-friendly compared to other acid-based chelating agents since it is most active at a neutral pH (65). Although EDTA does not have a direct effect on microbial viability, it produces bacteriologic inhibition and possesses antibacterial effects by attacking cell walls, preventing cell wall synthesis and interfering with biofilm cohesion (66). The chelating properties of EDTA arise from its ability to sequester di- and tri-cationic metal ions like calcium. It chelates the inorganic component of the dentin by binding with metal ions of cell walls. This mechanism of action extracts bacterial surface proteins and leads to cell death (42). A laboratory study (67) investigated the influence of irrigation time with EDTA and NaOCl on the removal of smear layer. In contrast to NaOCl, where its effectiveness is influenced by the frequency of replenishment and time exposure, all tested irrigation times with EDTA (1, 3 and 5 minutes) were equally effective in the removal of smear layer. In addition, Sen et al. (68) found no significant difference in the removal of smear layer by different concentrations (1%, 5%, 10%, 15%) of EDTA.

Although the combined use of NaOCl and EDTA is advocated (56, 60), the chemical interactions between the two irrigation solutions are well documented (53, 69). Mixing of NaOCl with EDTA results in an acid/base neutralization reaction between the two. This chemical interaction results in a rapid and drastic decrease and loss of free available chlorine (OCl- and HOCl). Although the chelating ability and antibacterial effects of EDTA are maintained, the loss of free available chlorine significantly reduces the dissolving properties of NaOCl. Clinically, copious irrigation with NaOCl is recommended to wash out remnants of EDTA when used in an alternating irrigation regimen.

3.5 Irrigation devices

From manual irrigation techniques to machine-assisted agitation systems, irrigation methods have been refined over the years to enhance the delivery of irrigation solutions to mechanically inaccessible areas of the complex root canal system. Manual irrigation techniques include the
traditional syringe-needle delivery, as well as the manual dynamic agitation technique that uses a well-fitting gutta-percha master cone to agitate the irrigation solution in a repetitive up-and-down motion within the root canal. In addition, NaviTip FX (Ultradent Products Inc, South Jordan, UT), a 30-gauge irrigation needle covered with a brush, was introduced commercially as an adjunct for debridement of the canal walls and agitation of the irrigation solutions. (70)

Machine-assisted agitation systems include devices using sonic and ultrasonic energy. The newer GentleWave system (Sonendo Inc, Laguna Hills, CA) uses multisonic energy. Sonic devices such as Vibringe (Vibringe B. V. Corp, Amsterdam, Netherlands) and EndoActivator (Dentsply Tulsa, Tulsa, OK) operate at a lower frequency (2-3 kHz) compared to ultrasonic systems (25-40 kHz) (71). CanalBrush (Coltene Whaledent, Langenau, Germany) is an endodontic microbrush that can be used manually or sonically by attaching the brush to a contra-angle handpiece to facilitate removal of debris from canal extensions and irregularities (70).

Various file systems such as the Self-Adjusting File (SAF) (ReDent, Nova, Ra’anana, Israel) and the XP-endo Finisher file (FKG Dentaire, La Chaux de Fonds, Switzerland) have been introduced to achieve concurrent root canal cleaning, shaping, irrigation and agitation.

3.6 Apical negative pressure

The RinsEndo (Durr Dental, Bietigheim, Germany), EndoVac (SybronEndo, Orange, CA), INP needle (Mixnus Fine Engineering Co Ltd, Nagano, Japan) and Canal CleanMax (Maxium Dental, Secaucus, NJ) are systems that combine canal irrigation and a suction mechanism to avoid extrusion of irrigation solutions into the periapical tissues. Irrigation using an intracanal aspiration technique was first demonstrated by Fukumoto et al. (72) as an attempt to deliver irrigation solutions to the apical portion of the canal while minimizing extrusion beyond the apical foramen. While the RinsEndo works based on suction under hydrodynamic pressure, the EndoVac system and the INP needle uses apical negative pressure to draw irrigation solutions down the root canal, then back up into the suction unit (73). The term “apical negative pressure” refers to the condition in which the enclosed volume of irrigation inside the root canal space exerts a lower pressure than its external surroundings. In contrast to the conventional positive pressure syringe-needle irrigation, apical negative pressure devices in endodontics have the ability to suction, thereby drawing and delivering the irrigation solution passively to the apex (70).
3.7 Vapor lock

Senia et al. first described the vapor lock effect in the root canals in 1971 (74). Since roots are enclosed by the periodontium and the surrounding bony structures, the root canals then behave like closed-end channels, resulting in gas entrapment in the apical portion during irrigation (75). Tay et al. (75) demonstrated in vitro that the presence of an apical vapor lock effect adversely affected debridement efficacy through conventional syringe-needle delivery. The simulation of a closed-end system in laboratories studies is commonly achieved by sealing the roots with hot glue and embedding them in polyvinylsiloxane to restrict fluid flow through the apical foramen during chemo-mechanical cleaning procedures. The formation of a vapor lock represents a major obstacle in conventional syringe-needle irrigation. It limits penetration of the disinfecting solutions and precludes the apical portion from contact and disinfection by the irrigation solutions (70). In contrast, sonically- and ultrasonically-activated irrigation solutions improve the penetration of irrigation by breaking the vapor lock and moving the solutions apically and laterally (76).

3.8 Ultrasonic irrigation

The use of ultrasonics in endodontics as an adjunct to mechanical debridement was first reported by Richman in 1957 who adapted an ultrasonic scaler for root canal therapy and root resection (77). Martin later introduced and popularized the use of ultrasonics to assist in irrigation during root canal therapy (78). The bactericidal effects of ultrasonics in the root canal system using a titanium vibrating ultratip was determined quantitatively, and a bactericidal synergism was demonstrated when irrigation with ultrasonics was coupled with a bactericidal irrigation solution (78). Various studies (79-83) further confirmed the effects of ultrasound to improve the cleaning of the canal by removing significantly more smear layer and debris, and by increasing the tissue-dissolving and antimicrobial effects of NaOCl.

Ultrasound is acoustic or sound waves with a frequency above the human hearing limit, which is approximately 20 kHz (78). The term “endosonics” was introduced by Martin and Cunningham (84). It is defined as an ultrasonic synergistic system of root canal instrumentation and disinfection, where the files are driven to oscillate in a characteristic pattern of nodes and anti-nodes along their lengths at a frequency of 25 to 30 kHz. Although ultrasonically driven files yield inconsistent canal preparations, ultrasonic energy has proven to be highly effective for the irrigation of root canals
In current clinical practice, ultrasonically-activated irrigation is a commonly used adjunct for final irrigation to aid in improved cleaning and disinfection (86).

The literature suggests that the effectiveness of ultrasonics is achieved through acoustic streaming, cavitation and heating (84). Acoustic streaming is defined as a rapid and steady movement of fluid in a circular or vortex-like motion around a vibrating file (87). It produces great shear forces with the greatest stresses occurring at points of maximum displacement like the file tip and antinodes along the file length (87). Cavitation is the formation of cavities or bubbles in a fluid medium through tensile forces induced by high-speed flows or flow gradients (85). When the acoustic pressures are great enough, the expansion, contraction and distortion of the bubbles create implosion of thousands of microbubbles resulting in localized areas of pressure and heat generation. The radiating shock waves can force the irrigation solution into all dimensions. They also result in an effective scrubbing action and in dislodgement of debris from the root canal (78).

Two types of cavitation, stable and transient, have been reported during ultrasonic irrigation of the root canal system. As described by Roy et al. (88), stable cavitation is a collection of large bubbles driven in a low-velocity amplitude or pulsation. In contrast, the velocity amplitude found in transient cavitation is extremely high, resulting in an energetic and turbulent fluid motion with the associated effect of shock wave generation. Ahmad et al. (89) challenged the concept of transitional cavitation and concluded that acoustic streaming was the main mechanism involved in cleanliness of the root canal system when using ultrasonic irrigation systems. Heat generated by ultrasonics is found to be within the therapeutic range and it does not cause lysis of normal cells even after 15 minutes of ultrasonic irrigation (90, 91). More importantly, the heat produced enhances the efficacy of NaOCl. Cunningham and Balekjian (92) demonstrated that the heat generated by ultrasonic irrigation increased the tissue-dissolving efficacy of 2.5% NaOCl to a level comparable to that of 5% NaOCl.

Two types of ultrasonic irrigation are described in the literature. The first type is the simultaneous combination of ultrasonic irrigation and instrumentation, previously termed active ultrasonic irrigation (AUI). The second type functions without simultaneous instrumentation and is known as passive ultrasonic irrigation (PUI). In PUI, energy is transmitted by means of ultrasonic waves from an oscillating file or smooth wire to an irrigation solution in the root canal (85). The acoustic energy can then induce acoustic streaming and cavitation of the irrigation solution (93). Originally, the term “passive” was used to describe the noncutting action of the ultrasonically activated files.
Weller et al. (82) were the first to document the use of a smooth and tapered stainless steel instrument that was ultrasonically activated to working length in resin blocks and in extracted teeth, where maximum surface contact with the canal walls was advised. Subsequent studies demonstrated the influence of constrained oscillatory pattern caused by file-wall contact on the debridement efficiency of PUI (94, 95). Jensen et al. (83) later proposed to passively activate the file, which implies no intentional instrumentation, planing or contact with canal walls to maximize the effects of acoustic streaming. This technique with no intentional file-wall contact to avoid damping of the file motion has then become the contemporary description of “passive” ultrasonic irrigation. However, Boutsiosoukis et al. (96) demonstrated that unintentional file-to-wall contact was unavoidable. Due to its extensive occurrence, the term “ultrasonically-activated irrigation” (UAI) was proposed to better describe this irrigation activation technique.

3.9 Intermittent and continuous ultrasonically-activated irrigation

The delivery of irrigation solutions and ultrasonic agitation can either be intermittent or continuous (91). While the continuous ultrasonically-activated irrigation (CU) is delivered via an ultrasonically-activated irrigation needle, the intermittent ultrasonically-activated irrigation (IU) requires a vibrating instrument within the canal and replenishment of the solution with a syringe after each activation cycle. In a laboratory study investigating heat generation by UAI (91), a continuous flow of irrigation solution resulted in a smaller and more uniform temperature rise both inside and outside the root canal space, possibly due to a greater volume of irrigation delivered. Although all temperature changes were within physiological ranges, a time-temperature relationship was established, and it was recommended that the canal be flushed with an irrigation solution after every 30 second cycle of intermittent activation. Chlorine, the active component of NaOCl responsible for tissue dissolution and antimicrobial activity, is consumed more rapidly during an intermittent flush, and the amount of irrigation that is activated is smaller when compared to a continuous replenishment of irrigation solution (58, 97). During IU, the irrigation solution is directly injected into the root canal space. The efficacy of IU is dependent on the ability of the ultrasonic file to penetrate within 1 to 2 mm from the working length, which can be difficult to achieve in curved canals (97). In contrast, when using the CU technique, the irrigation solution is delivered in an activated state through the ultrasonically-energized needle, avoiding the need to insert the delivering needle into the apical portion of the root canal (97). However, additional coronal flaring of the root canal may be necessary for the irrigation solution to flow into the canal.
and to avoid creating an irregularly shaped canal preparation (98). The efficiency of CU is time dependent, and is not influenced by the irrigation flow rate and volume (99). Passarinho-Neto et al. (100) confirmed these findings, and demonstrated that longer applications (1 minute versus 3 and 5 minutes) of CU resulted in reduced residual debris when the volume of irrigation was the same in all the experimental groups. The authors suggested that the greater increase in temperature of NaOCl with longer irrigation time allowed for better debris removal. Another laboratory study yielded the same results; while removal of dentin debris using the IU technique was as effective after 3 minutes as 1 minute, in the CU group it was significantly less efficient after 1.5 minutes than 3 minutes (98). It was suggested that the greater temperature rise of NaOCl induced by IU improved its tissue dissolution properties, allowing a reduced irrigation time to achieve the same efficiency.

The use of UAI with an ultrasonic tip activated close to the working length has been reported to be safe (101, 102). The effect of acoustic streaming during UAI moves the irrigation solutions coronally, thus preventing the solutions from extruding through the apical foramen. In addition, the pulp and periapical tissues act as a natural barrier, inhibiting extrusion of debris and irrigation solutions. Nonetheless, laboratory studies have reported the potential extrusion of irrigation solution using the CU technique, jeopardizing the safety of the procedure. This could be due to a greater volume of irrigation solution within the root canal space and the pressure that is generated (101, 103, 104). However, it remains unknown whether the resistance exerted by the various materials placed at the apical foramen to simulate the periapical tissues is comparable to that exerted by the periapical tissues in vivo. In addition, the apical resistance may differ in vivo depending on the stages of root development and on the condition of the periapical tissues (normal or pathological) (101). To avoid apical extrusion of irrigation solutions during CU, Castelo-Baz et al. (105) combined the advantageous properties of CU and apical negative pressure, and introduced the concept of continuous apical negative pressure ultrasonic irrigation (CANUI). The device allows continuous replenishment of fresh irrigation that is simultaneously aspirated.

3.10 GentleWave system

The GentleWave (GW) system (Sonendo Inc, Laguna Hills, CA) was introduced in the United States in 2016. According to the manufacturer, it is an apical negative pressure irrigation device requiring minimal instrumentation of the root canal system (106, 107). The device is comprised of
a console, a procedure instrument which is a single-use tip attached to a handpiece, and a central unit that contains three individual irrigation solution containers, one waste canister, a degassing system and a pressure generator. The system delivers an energized flow of irrigation solutions from the central unit to the procedural instrument. The programmed irrigation regimen begins with 3% NaOCl followed by 8% EDTA, with a rinse of distilled water in between and at completion. According to the manufacturer (108), a spray is released from the tip at approximately 45 mL/min and at a pressure of 9000 psi. The interaction between the continuous flow of irrigation and the stationary fluid inside the pulp chamber creates a strong shear force that induces a cavitation cloud. The implosion of cavitation bubbles generates multisonic energy produced by a broad spectrum of acoustic waves, as well as acoustic streaming with a vortical flow pattern. The hydrodynamic effects are further enhanced by the use of degassed irrigation fluids which can minimize energy loss, reduce the possibility of apical vapor lock and optimize fluid delivery throughout the entire root canal system. Fluid within the root canal space is continuously collected and removed from the chamber through a five-point vented suction system built in the sealing lid of the procedural instrument (109).

The GentleWave system was previously called the Multisonic Ultracleaning System. The system applies advanced fluid dynamics, acoustics, and tissue dissolution chemistry to remove tissue and debris from the entire root canal system simultaneously (109, 110). Thus far, laboratory studies have demonstrated promising results by GW as compared with conventional irrigation systems. Haapasalo et al. (109) demonstrated an 8-fold faster tissue dissolution rate in bovine tissue samples with GW compared to other irrigation modalities, including UAI, EndoVac, and conventional syringe-needle irrigation. Vandrangi and Basrani (108) have subsequently conducted a series of pilot studies to assess the efficacy of GW in disrupting inoculated E. faecalis and removing debris and smear layer in root canal systems of molars using scanning electron microscopy and histological analysis. The report (31) suggested good efficacy of GW in areas of anatomical complexities and in the apical thirds of the root canal system, when compared to conventional protocols. GW’s greater cleaning capacity, reduction in residual debris and NaOCl penetration depth into dentinal tubules were further demonstrated in various laboratory studies (111, 112). A recent publication (113) demonstrated the absence of organic tissue remnants or dentin debris left after irrigation with GW in non-instrumented root canals. GW was also shown to yield greater bacterial reduction compared to ultrasonic systems (114).
Ma et al. (115) assessed the ability of different irrigation regimens, including syringe-needle irrigation, ultrasonics irrigation and GW, to remove calcium hydroxide (Ca(OH)$_2$) from the canals of mandibular molars evaluated with micro-CT imaging. The study confirmed the effective circulation of the irrigation solutions at all parts of the root canal system as GW was the only method able to efficiently remove Ca(OH)$_2$ along the entire canal length and inside root canal complexities, with residual Ca(OH)$_2$ observed in only 1 out of 31 canals. Wohlgemuth et al. (116) evaluated the effectiveness of GW in the retrieval of fractured instrument fragments with minimal instrumentation. The study reported an overall retrieval success rate of 61% in the apical level and 83% in the mid-root level, all without the need to excessively enlarge the root canals.

Since GW delivers energized irrigation solutions at a high flow rate, the risk of periapical extrusion as a measure of safety in clinical usage was assessed in vitro. Charara et al. (106) used a pressure-regulated apparatus to simulate periapical back pressure in mandibular molars. Apical extrusion of irrigation solutions was quantified and compared among GW, conventional syringe-needle and EndoVac. The study reported no incidence of extrusion in any of the canals irrigated with GW and EndoVac. The results were not influenced by the size or length of preparation. An absence of extrusion suggested that the devices generated apical pressures within the parameters set in the study. Similarly, Haapasalo et al. (107) measured the apical pressure generated during irrigation of the palatal and distobuccal root canals of maxillary molars using an analogous pressure-regulated device. It was once again demonstrated that GW generated negative pressure at the apical foramen irrespective of the size of canal preparation.

To date, three prospective cohort studies evaluated the performance of GW in vivo. The first two reports (117, 118) evaluated the six- and twelve-month healing outcome after endodontic treatment using GW. Based on clinical signs and symptoms and radiographic PAI scores, teeth were classified as healed, healing, or diseased. Teeth classified as healing or healed were considered as a success and accounted for the cumulative success rate of treatment. The cumulative success of endodontic therapy was 97% at 12 months, with 92% and 5% of the necrotic pulp cases classified as healed and healing, respectively. However, in these studies, only 23% of the total sample had preoperative AP, which is a well-established outcome predictor (119, 120). The subsequent report (121) addressed this concern by assessing the 12 month healing outcome in teeth with a Periapical Index (PAI) (122) score greater than 3. A reduction in PAI scores occurred in 98% of the teeth that received treatment with GW, with 82% and 16% of the teeth classified as healed and healing,
respectively. Interestingly, the complete healing rate (82%) of chronic AP at 12 months using GW is considerably higher than the 50% previously reported (123, 124) for conventional endodontic treatment. These results may be extrapolated to project even higher complete healing rates in the longer term after treatment with GW. However, a larger sample size is necessary to provide higher level of evidence of the long-term performance of GW. Also, taking into consideration the fact that the aforementioned in vivo and in vitro studies were in part sponsored by the manufacturer, independent studies on GW are warranted.

4 Micro-computed tomography

4.1 Background

Micro-computed tomography (micro-CT) was introduced in the 1980s for use in nonclinical settings. In contrast to the conventional tomography used in the medical field, the term “micro” depicts its miniaturized design and its indication to image smaller specimens. In addition, its remarkably improved resolution over conventional imaging techniques allows for detailed computerized analysis and manipulation (125).

Apart from radiographic examination, previous methods developed to study the three-dimensional anatomy of the complex root canal system resulted in complete destruction of the teeth or limited the procedure to a few well-defined cross-sections of the roots. The potential application of micro-CT imaging in endodontics was first investigated by Nielsen et al. (125), more than a decade after its development. The ability of micro-CT imaging to evaluate the morphological changes in surface area and volume before and after instrumentation and root filling was demonstrated using four maxillary first molars at an isotropic resolution of 127 µm. The ability to represent the tridimensional anatomy of the internal and external structures in a non-destructive manner was considered revolutionary in endodontic research. Bjørndal et al. (126) subsequently performed a qualitative and quantitative analysis of the relationship between the external and internal morphology of the crown and root complex, as well as the correlation between the shape of the outer root surface and that of the root canal using micro-CT at a higher isotropic resolution of 33 µm. The study further confirmed the exceptional accuracy and reliability of this non-destructive technique. The applicability of conventional x-ray computerized tomography on human teeth in
vivo was initially suggested by Tachibana and Matsumoto (127). However, the resolution was inadequate for detailed image processing of certain aspects of the root canals. In addition, concerns were raised regarding the radiation dosage and time of exposure.

4.2 Mechanism of action

A micro-CT scanner is comprised of a micro-focus x-ray source, a motorized rotational stage with a customized subject holder, a detector array, and a host computer equipped with a system control mechanism and software resources for reconstruction, visualization and analysis of the samples (128). During micro-CT imaging, the x-ray source emits radiation continuously to the specimen attached to the sample stage. A charge-coupled-device (CCD) digital camera detects the attenuated intensity of the x-ray beam and produces electrical signals which are used to form high resolution images while the tooth rotates on its own axis in a step-by-step manner. Transversal slices or cross-sectional 2D images are then acquired by the detector. The projection raw data are subsequently input into the host computer and reconstructed into a virtual tridimensional model using multiple algorithms available in specific software programs (129).

4.3 Main features

This imaging modality presents several advantages in comparison with conventional methods (130). Its image quality and resolution were previously unattainable. Most importantly, its non-destructive nature allows samples to remain unaltered for further experimentation and future scans. The technique is also highly accurate and reproducible, allowing quantitative and qualitative analysis of the studied specimens. Furthermore, specific areas of interest can be selectively isolated by segmentation for further assessment. Image segmentation is a binarization process in which surrounding structures are removed manually and/or automatically from the image based on the difference in radiodensity. In endodontic research, this is commonly performed to analyze the internal anatomy of teeth where the pulp space is segmented from the surrounding enamel, dentin and cementum. Nonetheless, data acquisition, reconstruction and analysis are time consuming and expensive. A further limitation is that only hard tissues can be visualized whereas soft tissues remain invisible. In addition, the high radiation dose emitted typically restricts the application of micro-CT imaging to nonliving objects or to small animals in laboratory settings.
4.4 Applications in endodontics

Since micro-CT imaging is predominantly used in nonliving specimens, many features such as the exposure time, radiation energy and output, number of x-ray focal spots and detectors are optimized in comparison with conventional CT. With further development and improvement in the speed of data collection, resolution and image quality, the use of micro-CT imaging has gained popularity in research over the last decades. Some applications of micro-CT in endodontic research include the analysis of internal anatomy of teeth, instrumentation of the root canals, root canal fillings, retreatment, physical and biological properties of materials. Several promising applications of micro-CT in endodontics are being investigated (130). For example, the solubility rate of a material can be analyzed by assessing the pre- and post-immersion volume. This method can provide a more accurate representation of volume loss compared to the conventional solubility test commonly used in endodontic research. Moreover, micro-CT has the potential to verify the deformation of instruments after multiples uses. Over the years, attempts have been made to analyse biological properties with micro-CT. It has been suggested that the volume of apical lesions can be assessed with scanning of fixed tissues using micro-CT.

5 Accumulated hard-tissue debris

As previously discussed, it is well established that mechanical instrumentation is an indispensable phase of root canal therapy. Besides the removal of organic and inorganic tissues, mechanical instrumentation also facilitates the delivery of irrigation solutions throughout the root canal system. Nonetheless, canal instrumentation also presents potential negative effects, such as modification of the original canal anatomy, the production of smear layer and the occurrence of iatrogenic errors (131). In addition, hard-tissue debris produced by cutting of the root canal walls accumulate in the inaccessible niches of the root canal systems, including the isthmuses where such are present. Accumulation of hard-tissue debris is undesirable, as it may harbour persistent microorganisms while preventing access to antibacterial irrigation solutions (131, 132).
5.1 Assessment by micro-CT imaging

In 2009, Paqué et al. (131) reported on accumulated hard-tissue debris (AHTD) within the isthmuses of mesial roots in mandibular molars after instrumentation, depicted by high-resolution micro-CT imaging with an isotropic resolution of 20 μm. Debris were depicted as radiopaque entities filling the voxels previously identified as soft tissue, liquid or air (radiolucent or black) in the preoperative scan. Qualitative analysis depicted AHTD packed in almost the entire isthmus volume, as well as in areas of the main canal that had not been instrumented. The specimens were subsequently sectioned, and the root sections submitted for scanning electron microscopy imaging to validate the presence of dentin debris. While this first report using micro-CT imaging to assess AHTD drew attention to this occurrence, no irrigation was used throughout the study to maximize the amount of AHTD. Paqué et al. (132) later investigated the impact of sequential irrigation procedures on AHTD using the previously validated methodology. The sequential irrigation protocol consisted of irrigating the canals with 1% NaOCl followed by a flush with 17% EDTA during instrumentation. The final NaOCl application was ultrasonically activated using the intermittent flush method after the cleaning and shaping procedures. As previously reported, tridimensional reconstructions of the mesial root canals showed AHTD packed in isthmuses, fins, ramifications and accessory canals, but a significant reduction of AHTD was observed when the canals were irrigated during instrumentation. Further significant reduction of AHTD occurred using UAI after instrumentation. However, half of the amount of AHTD remained even after the final irrigation protocol. This study demonstrated the accumulation and partial removal of AHTD despite the application of a frequently recommended instrumentation and irrigation regimen.

Endal et al. (133) confirmed the presence of a considerable amount of hard-tissue debris packed into the isthmus areas of mandibular molars despite continuous irrigation during and after instrumentation. The report also suggested that AHTD may adversely impact on the ability of root fillings to seal the canal, because penetration of root canal sealers into areas of the root canal system occupied by debris may be prevented. Multiple laboratory studies subsequently assessed the efficacy of different irrigation systems in the removal of AHTD from the root canal and isthmus area using micro-CT imaging. Versiani et al. (134) compared positive and negative pressure irrigation systems, while Freire et al. evaluated the efficacy of UAI and EndoVac (135). Leoni et al. (136) compared the efficacy of four final irrigation protocols (UAI, XP-endo finisher, conventional syringe-needle and the Self-Adjusting File), and Keles et al. (137) assessed the
performance of the Self-Adjusting File and EndoVac. Recently, Verstraeten et al. (138) investigated the efficacy of laser-activated irrigation and UAI. The potential influence of the depth of insertion of irrigation needle tips on the removal of AHTD was also evaluated (139). However, none of the investigated systems and irrigation regimens was able to completely eliminate AHTD from root canals and isthmuses. Despite differences in methodology among the studies, removal of AHTD was reported in a range of only 51%-94%. These findings underline the limited efficacy of current irrigation protocols in the removal of AHTD.
Chapter 2
Rationale, Aim, Hypothesis

Rationale

Mechanical preparation of the mesial root canal system of mandibular molars may hinder disinfection by packing hard-tissue debris within the isthmus area; therefore, effective disinfection relies on the development of irrigation adjuncts for debris removal and the effective delivery of antibacterial solutions. The GentleWave (GW) system was recently approved for clinical use in endodontics and independent evaluation is warranted. To date, only one study demonstrated histologically the efficacy of the GW system in debris removal from the isthmuses of maxillary and mandibular molars (111). The use of nondestructive assessment methods is warranted to investigate the ability of GW to enhance cleansing of isthmuses.

Aim

To assess the efficacy of GW, in comparison to intermittent and continuous ultrasonically-activated irrigation, in the removal of accumulated hard-tissue debris from root canals and isthmuses within mesial roots of mandibular molars using micro-computed tomographic imaging.

Null Hypothesis

There would be no difference in the reduction of accumulated hard-tissue debris among the GW, intermittent and continuous ultrasonically-activated irrigation groups.
Efficacy of 3 Supplementary Irrigation Protocols in the Removal of Hard Tissue Debris from the Mesial Root Canal System of Mandibular Molars

ABSTRACT

Introduction: Instrumentation of the mesial root canal system of mandibular molars may hinder disinfection by packing hard tissue debris within the isthmuses. The removal of accumulated hard tissue debris (AHTD) by 3 supplemental irrigation systems, 2 ultrasonically activated and 1 multisonic, was assessed with micro-computed tomographic imaging.

Methods: Twenty-four extracted mandibular molars with 2 mesial canals connected by an isthmus and converging to a single foramen were selected. After preparation of the mesial canals with WaveOne Gold instruments (Dentsply Mallefer, Ballaigues, Switzerland), anatomically matched specimens were assigned to 3 final irrigation protocols (n = 8): intermittent ultrasonic (IU) with an ultrasonically energized 200-μm wire (Iritisafe; Satelec, Bordeaux, France), continuous ultrasonic (CU) with an ultrasonic irrigation needle (ProUltra PiezoFlow, Dentsply Mallefer), and GentleWave (GW) system (Sonendo Inc, Laguna Hills, CA). Specimens were scanned (SkyScan 1176; Bruker-microCT, Kontich, Belgium) at 17.18-μm pixel size before and after preparation and irrigation protocols. Data sets were coregistered, and the percentage reduction of AHTD calculated within the canals and isthmus for each specimen was statistically compared using 1-way analysis of variance and post hoc Tukey tests with a 5% significance level.

Results: The mean percentage reduction of AHTD in canals and isthmuses was significantly higher for GW (96.4% and 97.9%, respectively) than for CU (80.0% and 88.9%, respectively) (P < .05). AHTD reduction for IU (91.2% and 93.5%, respectively) did not differ significantly from GW and CU (P > .05).

Conclusions: GW achieved greater efficacy in the removal of AHTD from the mesial root canal system of mandibular molars compared with CU but not IU. The efficacy of CU and IU was comparable. (J Endod 2019; 45:1–7.)

KEY WORDS

Hard tissue debris; isthmus; micro-computed tomography; multisonic irrigation; ultrasonically activated irrigation

BASIC RESEARCH – TECHNOLOGY

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IRIGATION PROTOCOLS IN REMOVAL OF HARD TISSUE DEBRIS

Preparation of the mesial root canal system of mandibular molars may hinder disinfection by packing hard tissue debris within the isthmus area. Therefore, its disinfection relies on the development of irrigation adjuncts for debris removal and the effective delivery of antibacterial solutions.

Significance

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Mandibular first molars are the most frequent endodontically treated teeth. These teeth frequently present complex root canal configurations, with isthmuses communications present in 55% of the mesial roots and 20% of the distal roots. The isthmus incidence is the greatest at 3–5 mm from the apex, where it was clinically observed during apical surgery in 83% of mesial roots and 36% of distal roots of mandibular first molars.

Considering the high incidence of isthmuses in the root canal system and their inaccessibility to mechanical instrumentation, their disinfection then critically relies on effective delivery of antibacterial solutions. From traditional syringe needle delivery to machine-assisted agitation systems, irrigation methods have been refined over the years to enhance the delivery of irrigation solutions to mechanically inaccessible areas of the complex root canal system. The application of irrigation solutions and ultrasonic agitation during ultrasonically activated irrigation can either be intermittent or continuous.

While the continuous ultrasonically activated irrigation (CU) method is delivered via an ultrasonically activated irrigation needle, the intermittent ultrasonically activated irrigation (IU) requires a vibrating instrument within the canal and replenishment of the solution with a syringe after each activation cycle.

The GentleWave (GW) system (Sorden Inc, Laguna Hills, CA) is a novel apical negative-pressure distension device requiring minimal root canal instrumentation as per the manufacturer. The system applies advanced fluid dynamics, acoustics, and tissue dissolution chemistry to remove tissue, debris, and biofilms from the entire root canal system simultaneously. The device was recently approved for clinical use in endodontics, and independent evaluation is warranted. To date, only 1 study showed histologically the efficacy of the GW system in debris removal from the isthmuses of maxillary and mandibular molars. The use of nondestructive assessment methods is warranted to investigate the ability of the GW system to enhance cleansing of isthmuses. Therefore, the aim of the present study was to assess the efficacy of the GW system in comparison with intermittent and continuous ultrasonically activated irrigation in the removal of AHTD from root canals and isthmuses within mesial roots of mandibular molars using micro-computed tomographic (micro-CT) imaging. The null hypothesis tested was that there would be no difference in the reduction of AHTD among these 3 supplementary irrigation protocols.

### MATERIALS AND METHODS

#### Sample Size

The sample size was estimated based on preliminary data obtained from 5 specimens. Following the same instrumentation and final irrigation protocols as described later, 2 specimens were assigned to the GW and CU groups and 1 specimen to the IU group. The effect size of the IU group was established from the one previously reported by Leoni et al. Using G*Power 3.1.9.2 software (Heinrich Heine Universitat, Dusseldorf, Germany) for 1-way analysis of variance and the data from the pilot study, a minimal total sample of 18 specimens would be expected to provide analysis with 99% power and a 5% level of significance to statistically substantiate differences between the experimental groups. A total of 24 specimens were included in the final analysis.

#### Specimen Selection

The study protocol was approved by both institutional ethics boards of the University of Toronto and the University of Sao Paulo (protocol #35314). Initially, 50 extracted mandibular molars with moderately curved mesial roots (10–20°, Schneider’s method) in both mesiodistal and buccolingual directions were imaged with a micro-CT scanner (SkyScan 1176; Bruker-microCT, Kontich, Belgium) at 17.18 μm (pixel size), 90 kV, 278 μA, 180° rotation around the vertical axis, and a rotation step of 0.4° using a 0.5-mm-thick aluminum filter. The acquired projection images were reconstructed (NRecon v.1.6.10.4, Bruker-microCT) with a beam-hardening correction of 10%, smoothing of 2, ring artifact correction of 3, and an attenuation coefficient ranging from 0.006–0.04, resulting in the acquisition of approximately 550 slices per root. Then, 24 teeth presenting 2 independent canals in the mesial root connected by an isthmus from the middle to the apical third and exiting in a single foramen (Vertucci type II configuration) were selected. None of the teeth had root fillings, root canals, cracks, fractures, internal or external resorption. To ensure anatomic similarity among the specimens, length (L), volume (V), surface area (SA), and Structure Model Index (SMI) of the mesial root canals before the experimental procedures were calculated (CTAn v.1.15, Bruker-microCT) (Table 1). The volume of interest was selected extending from the cementoenamel junction level to the apex of the mesial root set by integration of all cross sections.

#### Root Canal Preparation

The mesial root canals in all specimens were prepared by 1 operator (R.C.) experienced in the use of reciprocating instruments. After access cavity preparation, the mesial canals were negotiated with size 10 K-type files (Dentsply Mallefer, Ballugaes, Switzerland), and emergence of the tip at the apical foramen was verified under 10× magnification (Carl Zeiss, Oberkochen, Germany). The working length (WL) was established 0.5 mm short of the foramen. Next, the foramen was sealed by covering the apical tip of the mesial roots with hot glue to simulate a closed-ended canal system. A glide path was established to the WL with a ProGlider instrument (Dentsply Mallefer), and the root canals were sequentially enlarged with WaveOne Gold Small and Primary instruments (Dentsply Mallefer) to the WL activated in a reciprocating motion (ProMark Endo Motor, Dentsply Mallefer). To facilitate debris accumulation in the isthmus area, irrigation and aspiration throughout the preparation procedures were performed only at the orifice level with a total of 5 mL distilled water per canal using a 30-G ProFile Endo irrigation needle (Dentsply Mallefer) adapted to a disposable plastic syringe.

Each canal was slightly dried with 1 absorbent paper point (WaveOne Small, Dentsply Mallefer), and the specimens were submitted to a further scan and analysis following the aforementioned parameters. Postoperative scans were coregistered with their respective preoperative data set using the affine registration module of the 3D Slicer 4.10 software (available from http://www.slicer.org), and postoperative 3D parameters (volume, surface area, and SMI) were also acquired (Table 1). Then, spatially registered surface models of the roots were compared regarding the unprepared area of the root canal (Table 1) calculated by the formula $SA/SA_{ref} * 100$, where $SA_{ref}$ represents the unprepared canal surface area and $SA$, the root canal surface area before preparation, to ensure the consistency of the instrumentation protocol. A further analysis of the matched images was also performed to calculate hard tissue debris accumulated within the mesial root canal system after instrumentation procedures using CTAn v.1.15 software (Bruker micro-CT). Quantification of AHTD was performed by the difference between nonprepared and prepared root canal space using postprocessing procedures. The presence of a material with density similar to dentin in regions previously occupied by air in the nonprepared root canal space was considered debris and quantified by intersection between images before and after canal instrumentation. The
total volume of AHTD was calculated in cubic millimeters (mm³) and expressed as the percentage of the total canal system volume and the isthmus area after preparation (Table 1).

**Final Irrigation Protocols**

Aiming to enhance the internal validity of the experiment, the mesial root canals were matched to create 8 groups of 3 based on the morphology of the root canal system (length, volume, surface area, and SMI), the unprepared canal surface, and the percentage volume of AHTD after preparation. Then, 1 specimen from each group was randomly assigned to 1 of the following 3 experimental groups (n = 8) according to the final irrigation protocols, which followed the manufacturers’ directions:

1. **Group 1: IU;** a noncutting, 200-µm stainless steel ultrasonic file (mF ultrasonic; Satelec, Bordeaux, France) driven by the P5 Newtron ultrasonic system (Acteon North America, Mount Laurel, NJ) at a power setting of 9 mm with 6% NaOCl followed by 17% EDTA for 2 minutes, and a final rinse with distilled water for 15 seconds in a flow rate of 50 mL/min.

2. **Group 2: CU;** a ProUltra PiezoFlow ultrasonic irrigation needle (ProUltra, Dentsply Mallefer) was connected to the P5 Newtron ultrasonic system (Acteon North America) at a power setting of 5 mm up-and-down motion. The final irrigation protocol began with 6% NaOCl for 10 seconds followed by 17% EDTA for 30 seconds and a final rinse with 6% sodium hypochlorite (NaOCl) for 20 seconds. Irrigation was performed in a flow rate of 15 mL/min per canal.

3. **Group 3: GW;** before the final irrigation protocol, an occlusal platform was fabricated of a resin material (SoundSeal, Sironendo Inc) and a preformed plastic matrix to ensure an airtight seal between the access cavity and the procedural instrument. The final irrigation protocol began with 3% NaOCl for 5 minutes followed by distilled water for 30 seconds, 8% EDTA for 2 min, and a final rinse with distilled water for 15 seconds in a flow rate of 50 mL/min.

**Statistical Analysis**

Normal distribution of the data was assessed, and 3-dimensional morphology of the root canals (length, volume, surface area, and SMI), untouched canal surface, and percentage of AHTD after preparation and irrigation protocols were expressed as the mean and standard deviation and compared among groups using 1-way analysis of variance and post hoc Tukey tests with a 5% level of significance.

**RESULTS**

The degree of homogeneity of the 3 experimental groups was confirmed regarding pre- and postoperative morphologic parameters (canal length, volume, surface area, and SMI), unprepared canal surface, and the volume of AHTD after canal preparation (Table 1, *P* > .05). Qualitative observation of the scanning electron microscopic images corresponded well to the radiopaque areas within the isthmuses and the AHTD depicted on the micro-CT tridimensional reconstruction of the same specimens (Fig. 1).

**Scanning Electron Microscopy**

In order to validate the method, after the postirrigation scans, axial cross sections at the coronal, middle, and apical thirds of the root canals in all groups (Fig. 2) were scanned at 40× magnification on the scanning electron microscope (S-3400N; Hitachi, Tokyo, Japan) up to 420× magnification to corroborate the presence of debris in the isthmus areas after the final irrigation protocol (Fig. 1A-E). Brieﬂy, after removal of the distal roots at the cementsumamel junction level, grooves were cut on the mesial roots with a diamond-coated disc close to but without exposing the canals and isthmus areas at levels predetermined from the corresponding micro-CT scans where residual AHTD was present. The specimens were then split horizontally with a sharp chisel and mallet. The unprocessed specimens were imaged at an accelerating voltage of 5 kV and at a working distance of 15 mm.

**TABLE 1 - Mean (± Standard Deviation) of 3-dimensional Parameters Evaluated in the 24 Mesial Roots of Mandibular Molars before (BF) and after (AF) Root Canal Preparation**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental step</th>
<th>Group 1: IU (n = 8)</th>
<th>Group 2: CU (n = 8)</th>
<th>Group 3: GW (n = 8)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (mm²)</td>
<td>BF</td>
<td>10.5 ± 1.4</td>
<td>9.4 ± 1.3</td>
<td>9.9 ± 0.6</td>
<td>.2</td>
</tr>
<tr>
<td>Volume (mm³)</td>
<td>BF</td>
<td>4.0 ± 1.6</td>
<td>3.9 ± 2.3</td>
<td>3.6 ± 1.3</td>
<td>.9</td>
</tr>
<tr>
<td></td>
<td>AF</td>
<td>11.7 ± 3.2</td>
<td>8.2 ± 3.0</td>
<td>9.4 ± 1.8</td>
<td>.05</td>
</tr>
<tr>
<td>Surface area (mm²)</td>
<td>BF</td>
<td>44.3 ± 11.1</td>
<td>47.2 ± 23.0</td>
<td>47.0 ± 12.0</td>
<td>.1</td>
</tr>
<tr>
<td></td>
<td>AF</td>
<td>66.2 ± 13.1</td>
<td>58.0 ± 20.4</td>
<td>58.4 ± 9.2</td>
<td>.5</td>
</tr>
<tr>
<td>Structure Model Index</td>
<td>BF</td>
<td>1.9 ± 0.5</td>
<td>1.7 ± 0.7</td>
<td>1.5 ± 0.3</td>
<td>.5</td>
</tr>
<tr>
<td></td>
<td>AF</td>
<td>2.6 ± 0.6</td>
<td>2.2 ± 0.4</td>
<td>2.3 ± 0.2</td>
<td>.2</td>
</tr>
<tr>
<td>Untouched area (%)</td>
<td>AF</td>
<td>14.5 ± 4.3</td>
<td>16.8 ± 9.1</td>
<td>14.0 ± 6.0</td>
<td>.7</td>
</tr>
</tbody>
</table>
**DISCUSSION**

Apical periodontitis is a biofilm-mediated disease, and the inability to impact on biofilms within areas of the root canal system that are inaccessible to conventional chemomechanical disinfection protocols may compromise treatment outcomes. The pursuit for improved disinfection efficacy in the anatomically complex root canal system has in recent years focused on the effects within the isthmus areas as a potential target for biofilm eradication. Bacteria with polymicrobial flora organized as biofilms have been identified within the isthmus areas of human mandibular first molars, both immediately after completion of single-visit endodontic treatment and in a tooth associated with posttreatment apical periodontitis. This study assessed the ability of root canal disinfection protocols to remove hard tissue debris from uninstrumented canal irregularities and isthmuses as indication of possible access to biofilms within these rather inaccessible areas.

The experimental design used herein aimed to evaluate the irrigation properties of the tested irrigation devices under standardized conditions, thus disregarding selected clinical application guidelines. Accordingly, although the manufacturer of GW recommends canal instrumentation up to only size 20/06, in this study all mesial canals were instrumented to size 25/07 with minimal irrigation to standardize the volume and distribution pattern of AHTD. In addition, in accordance with a previously established methodology, the Small and Primary WaveOne Gold reciprocating instruments were used in sequence to produce sufficient amounts of AHTD that will allow quantification of its removal efficacy. The volume of produced AHTD, approximately 15% of the total canal volume, was comparable with the 19% reported in a previous study in which the WaveOne reciprocating instruments were used.

In the present investigation, the reduction of AHTD differed significantly among the tested final irrigation protocols; therefore, the null hypothesis was rejected. The GW system removed AHTD by 96.4% within the mesial root canals and by 97.9% within the isthmus areas. These results supported the efficacy of GW in cleansing the complex root canal system in the mesial canals of molars. The IU group reduced AHTD by 91.2% and 93.5% from the canals and isthmus areas, respectively. Its efficacy was statistically comparable with the GW system and appeared superior to that reported in previous micro-CT studies in which similar sequential irrigation steps showed a reduction of 50.8% and 55.6%, respectively. The greater efficacy of IU reported in the present study could be attributed to the higher activation power setting in this study compared with the previous ones. In contrast, the GU group showed the lowest AHTD reduction despite its comparable efficacy to IU, which is in accordance to previous reports.

However, it was inferior to that of GW. One limitation of the present study is the use of a different irrigation solution sequence in the ultrasonically activated irrigation experimental groups compared with the GW group. Although its clinical significance remains to be elucidated, dentin erosion has been observed in vitro when NaOCl is used as a final irrigation solution after demineralization agents. The use of NaOCl as a final rinse in the ultrasonically activated irrigation groups could have potentially created cleaner root canal walls with fewer dentin debris by allowing a deeper penetration of NaOCl into areas previously covered by the smear layer. Although AHTD removal efficacy was comparable for the GW system and IU, the greater penetration of irrigation delivered by the GW system compared with ultrasonically activated irrigation systems was previously suggested. Ultrasonic irrigation devices rely on the transmission of acoustic energy from an oscillating file in which the file motion is likely to be impeded as the root canal narrows in the apical portion. In contrast, the GW system uses a broad spectrum of sound waves to distribute fluids throughout the entire root canal system. Compared with ultrasonic energy that is dispersed at a single frequency, multisonic energy emitted by the GW system enables effective delivery of energized irrigation into micro-sized dentinal tubules at a high flow rate. The interaction between the continuous flow of irrigation solution and the...
stationary fluid inside the pulp chamber creates a strong shear force that induces a cavitation cloud. The implosion of cavitation bubbles generates multisonic energy produced by a broad spectrum of acoustic waves as well as acoustic streaming with a vortical flow pattern. The hydrodynamic effects are further enhanced by the use of degassed irrigation fluids, which can minimize energy loss and optimize fluid delivery throughout the entire root canal system.

Although the clinical implications of AHTD remain unknown, dentin debris have been shown to significantly alter the biological efficacy of intracanal disinfectants. In addition, dentin debris exhibit inhibitory effects on commonly used irrigation solutions by diminishing free available chlorine and antibacterial properties of NaOCl. Furthermore, AHTD may protect microorganisms clogged in inaccessible areas by providing a spatial barrier between the bacteria and antimicrobial irrigation. AHTD may also interfere with the seal provided by the root filling. The aforementioned potential concerns highlight the need for the development of measures to prevent and to disrupt AHTD to enhance access to biofilms inside inaccessible areas of the root canal system in the quest for improving long-term prognosis. Further investigation is warranted to define the relationship between hard tissue debris and biofilms. With further methodologic refinement in micro-CT imaging, future studies should also aim to image and quantify biofilms within the root canal system.

**CONCLUSION**

Within the limitations of this in vitro study, none of the tested irrigation protocols was able to significantly reduce AHTD as compared to AF. The use of supplementary irrigation protocols was able to further reduce AHTD by up to 91.2%, but no statistical differences were observed between groups 1 and 2. microscopic examination of AHTD was able to visualize the presence of hard tissue debris and biofilms within the root canal system. The results of this study highlight the importance of developing effective measures to prevent and to disrupt AHTD to enhance access to biofilms inside inaccessible areas of the root canal system in the quest for improving long-term prognosis. Further investigation is warranted to define the relationship between hard tissue debris and biofilms. With further methodologic refinement in micro-CT imaging, future studies should also aim to image and quantify biofilms within the root canal system.

**TABLE 2** - Mean (± Standard Deviation) of Accumulated Hard Tissue Debris Evaluated in the 24 Mesial Roots of Mandibular Molars after Preparation (AF) and the Supplementary Irrigation Protocol (IR)

<table>
<thead>
<tr>
<th>Experimental step</th>
<th>Group 1: IU (n = 8)</th>
<th>Group 2: CU (n = 8)</th>
<th>Group 2: GW (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root canal Volume (mm³)</td>
<td>AF 0.7 ± 0.4</td>
<td>0.5 ± 0.4</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>IR 0.07 ± 0.08</td>
<td>0.08 ± 0.06</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>Reduction (%)</td>
<td>IR 91.2 ± 5.2ab</td>
<td>80.0 ± 20.6b</td>
<td>96.4 ± 4.2a</td>
</tr>
<tr>
<td>Isthmus Volume (mm³)</td>
<td>AF 0.4 ± 0.4</td>
<td>0.3 ± 0.2</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>IR 0.03 ± 0.07</td>
<td>0.03 ± 0.04</td>
<td>0.005 ± 0.007</td>
</tr>
<tr>
<td>Reduction (%)</td>
<td>IR 93.5 ± 5.0ab</td>
<td>88.9 ± 5.3b</td>
<td>97.9 ± 2.1a</td>
</tr>
</tbody>
</table>

Different superscript bold letters in the same line mean statistical significant difference between groups (1-way analysis of variance post hoc Tukey test, P < .05).

**FIGURE 2** – Distal views of representative 3-dimensional reconstructions of the mesial root canal systems of 3 representative mandibular molars before (in green) and after (in red) preparation with a reciprocating system and after 3 supplementary irrigation protocols. AHTD is depicted in black.
render the mesial root canals and isthmus areas of mandibular molars free of dentin debris. The GW system showed better efficacy in the removal of AHTD from the mesial canals and isthmus areas when compared with CU but not IU. The efficacy of IU and CU irrigation systems was comparable.

**ACKNOWLEDGMENTS**

The authors thank Dentsply Maillefer for providing the WaveOne Gold instruments and the ProUltra PiezoFlow devices and SonEndo Inc for delivering the GentleWave system to our facilities to conduct this independent study.

**REFERENCES**

16. Chan et al.
<table>
<thead>
<tr>
<th></th>
<th>Reference</th>
<th>Details</th>
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</table>
Chapter 4

Discussion

Isthmus and biofilms

AP is a biofilm-mediated disease (8, 9) and the inability to impact on biofilms within areas of the root canal system that are inaccessible to conventional chemo-mechanical disinfection protocols may compromise treatment outcomes (41). The pursuit of improved disinfection efficacy in the anatomically complex root canal system has in recent years focused on the effects within the isthmus areas, as a potential target for biofilm eradication (41, 45). Nair et al. (45) illustrated in vivo the presence of biofilms in inaccessible areas of the root canal system immediately after completion of a single-visit endodontic treatment. The apical portion of the mesial roots of mandibular first molars with distinct AP were removed surgically after a standard instrumentation with either nickel-titanium files or stainless steel hand K-type files and irrigation regimen. Residual intracanal bacteria were depicted by correlative light and transmission electron microscopy in 14 of the 16 treated teeth. The numerous accessory canals and anastomosing segments that were left non-instrumented and clogged with microorganisms illustrated once again the formidable challenge presented by the anatomical complexity of root canal systems. The isthmus areas were also colonized with microbes and often contained islands of fibro-dentinal structures. The isolated bacteria revealed a polymicrobial flora with a multilayered condensation that resembled biofilms. Carr et al. (41), using transmission electron microscopy, also reported the presence of a complex multispecies biofilm at the entire length of the isthmus of an endodontically retreated mandibular molar associated with post-treatment AP. This case report also illustrated the ability of microbial communities to thrive and prosper in extremely harsh and nutrient-deficient environments more than a decade after completion of initial and repeated endodontic treatments. These findings highlight the need for the development of measures to enhance access to biofilms inside inaccessible areas of the root canal system in the quest for improving long-term prognosis.

Relevance of AHTD

Although the clinical implications of AHTD remain unknown, dentin debris have been demonstrated to significantly alter the antimicrobial efficacy of intracanal disinfectants including the commonly used irrigation solutions and intracanal medication (144, 145). It was previously reported (144, 146) that the presence of dentin debris resulted in a reduction of free available
chlorine and antibacterial properties of NaOCl. A hypochlorite-compatible chelator like etidronic acid was reported to be able to reduce but not completely prevent the formation of AHTD during rotary instrumentation (132, 146, 147). It was suggested that the chemical interaction between the chelator and the inorganic surface of dentin debris enables the detachment of bacteria and the release of organic tissues that are subsequently dissolved by NaOCl (146). Furthermore, AHTD may protect microorganisms clogged in inaccessible areas by providing a spatial barrier between the bacteria and antimicrobial irrigation (147). AHTD may also interfere with the seal provided by the root filling (133).

**GentleWave system: concept and safety**

The concept behind GW is very similar to the non-instrumental hydrodynamic technique (NHT) introduced by Lussi et al. (148) in the early 1990s. The ability to clean the complex root canal system without conventional hand or rotary instrumentation was achieved by a device that was able to build up controlled cavitation inside the root canal space. Alternating pressure fields were generated within an overall reduced pressure range. The reduced pressure within the root canal allowed for the development of micro- and macroscopic bubbles that would expand and collapse when a quick pressure rise was induced. Cavitation and hydrodynamic turbulences would then build up, allowing the irrigation solution to penetrate the root canal system into all dimensions and to be more easily exchanged by fresh irrigation solutions. This first report on NHT demonstrated its capacity to improve canal cleanliness compared with hand instrumentation, especially in the middle and apical thirds of curved root canals. Attin et al. (149) later evaluated the cleaning ability of NHT in vivo, and concluded that the percentage of roots with remaining debris increased with increasing depth towards the middle and apical parts of the root canals. More importantly, the application of NHT resulted in a greater amount of remaining debris as compared to studies using rotary nickel-titanium instruments. The authors suggested that the presence of periapical tissues does not guarantee the maintenance of a closed system in vivo, which is crucial to achieving the hydrodynamic turbulence necessary to clean the root canals.

According to the manufacturer of GW, the generation of hydrodynamic cavitation and the induction of an acoustic field of broadband frequencies are key features to enhanced dissolution and removal of organic and inorganic matter while conserving natural tooth structure. Although the safety of the device has been demonstrated in laboratory studies (106, 107), in a personal
communication with the manufacturer (March 2018), it was proposed that bleeding from the periapical tissues into the root canal should be expected \textit{in vivo} after completion of the irrigation regimen. Although no hypochlorite accidents related to the usage of GW have been reported to date, it is conceivable that the hydrodynamic irrigation provokes irritation of the pulpal and/or periapical tissues and possible extrusion of irrigation solutions. Blood is a nutrient-containing fluid, it can thus promote bacterial growth (150). In addition, persistent blood flow into the root canals can reduce the chlorine concentration of NaOCl and impair its cleansing properties (149). Further studies are warranted to assess the safety of GW \textit{in vivo}.

\textbf{Study methodology}

To address the aim of the present study, mandibular first molars with Vertucci type II configuration were selected. As previously discussed, this root canal system configuration favours the accumulation of AHTD due to the presence of an isthmus (131, 134, 137). In addition, it is the second most commonly found configuration in mandibular first molars (30). Furthermore, as reported by Hsu and Kim (35), true isthmuses are most frequently found in the mesial roots of mandibular molars.

Standardization and matching of the specimens using micro-CT imaging prior to assignment to the experimental groups enhanced the internal validity of this \textit{in vitro} experiment (151). In the present investigation, the degree of homogeneity of the specimens in the three experimental groups was assessed and confirmed regarding pre- and postoperative morphological parameters (canal length, volume and surface area, and structure model index), non-instrumented canal surface and the volume of AHTD after canal preparation (Table 1; $P > 0.05$).

The experimental design used herein aimed to evaluate the irrigation properties of the tested irrigation devices under standardized conditions, thus disregarding selected clinical application guidelines. Accordingly, although the manufacturer of GW recommends canal instrumentation up to only size 20/.06, in this study all mesial canals were instrumented to size 25/.07 with minimal irrigation, so as to standardize the volume and distribution pattern of AHTD. In addition, in accordance with a previously established methodology (136), the small and primary WaveOne Gold reciprocating instruments were used in sequence to produce sufficient amount of AHTD that will allow quantification of its removal efficacy. The volume of produced AHTD,
approximately 15% of the total canal volume, was comparable to the 19% reported in a previous study (142) where the WaveOne reciprocating instruments were used.

**Study results**

In the present investigation, none of the tested irrigation protocols was able to render the mesial root canals and isthmus areas of mandibular molars free of AHTD. However, reduction of AHTD differed significantly among the tested final irrigation protocols; therefore, the null hypothesis was rejected. GW removed AHTD by 96.4% within the root canal lumens and by 97.9% within the isthmus areas. These results supported the efficacy of GW in cleansing the complex root canal system in the mesial canals of molars (111).

The IU group reduced AHTD by 91.2% and 93.5% from the canals and isthmus areas, respectively. Its efficacy was comparable to that of GW and appeared superior to that reported in previous micro-CT studies (132, 135). A review paper (70) on irrigation agitation techniques and devices has suggested that agitation techniques including manual and machine-assisted agitation systems improve the apical cleaning efficacy when compared with conventional syringe-needle irrigation, due to enhanced irrigation dynamics. However, studies using micro-CT imaging to assess the efficacy of intermittent ultrasonically-assisted irrigation systems on the removal of AHTD have reported that only half of the debris created during instrumentation can be removed using similar sequential irrigation steps as used in the present study (132, 135). In contrast, Leoni *et al.* (136) demonstrated that IU reduced the volume of AHTD by 94.1%, also assessed by micro-CT imaging. The better efficacy of AHTD reduction reported in the latter study could be influenced by the selection of mandibular molars with a Vertucci type I configuration, compared to Vertucci type II configuration used in the previous studies (132, 135). The greater efficacy of IU reported in the present study could also be attributed to the higher activation power setting used.

While AHTD removal efficacy was comparable for GW and IU, the greater penetration of irrigation delivered by GW compared to ultrasonically-activated irrigation systems was previously suggested (112). Ultrasonic irrigation devices rely on the transmission of acoustic energy from an oscillating file, where the file motion is likely to be impeded as the root canal narrows down towards the apical portion (85, 94, 95). In contrast, GW utilizes a broad spectrum of sound waves to distribute fluids throughout the entire root canal system. Compared to ultrasonic energy that is
dispersed at a single frequency, multisonic energy emitted by GW enables effective delivery of energized irrigation into micro-sized dentinal tubules at a high flow rate (108, 109).

In the present study, the continuous ultrasonically-activated irrigation (CU) group showed the lowest AHTD reduction efficacy in canals and isthmuses (79.97% and 88.85%, respectively) despite its comparable efficacy to IU. Van der Sluis et al. (98) confirmed that both intermittent and continuous ultrasonically-activated irrigation methods were efficient in removal of dentin debris. However, the efficiency of ultrasonically-activated irrigation with a continuous flow is time dependent. While 3 minutes of ultrasonic activation is as effective as 1 minute with the intermittent flush technique, 1.5 minutes of activation is less efficient than 3 minutes with the continuous flush technique. Tanomaru-Fllho et al. (143) also showed no difference in cleaning efficiency among the two ultrasonically-activated irrigation techniques.

**Limitations and future directions**

Although the concept of smear layer has been supported by laboratory studies, there is a lack of consensus on which procedures can reliably remove it, due to the qualitative and non-reproducible character of most in vitro smear layer removal studies (152). In the present study, the smear layer, which has a thickness of 0.5 to 2 μm (62), could not be identified or differentiated from the radiopaque areas within the root canal and isthmus areas captured at an isotropic resolution of 17.18 μm. As a result, the current methodology was not sensitive enough to capture the smear layer or debris with a particle size smaller than 17.18 μm. Although micro-CT imaging can provide highly accurate and quantifiable comparison of pre- and post-instrumentation images without destruction of the specimens, its resolution needs to be further increased up to the nanometer scale to depict smear layer.

Ultra-high spatial resolution nano-CT currently represents the highest spatial image resolution attainable in a laboratory tomographic device (153). Besides its higher spatial resolution, nano-CT also offers a scanning duration that is significantly lower than that of conventional micro-CT imaging. In addition, nano-CT is equipped with a more precise system design with advanced technical properties, such as a flat-panel detector with higher contrast resolution, a granite base and air-bearing rotating unit, an air-conditioned cabinet and a vibration insulator to ensure the stability of the specimen during the data acquisition process (154). Its potential applications in
the field of endodontics appear promising. To date, nano-CT imaging has been used to assess the presence of voids in root fillings (154), as well as to investigate the internal architecture of external cervical resorption (155). The high contrast sensitivity enables nano-CT to illuminate low-density differences and poor contrast between dentin and other structures with similar radiodensity. Despite all its advantages, the use of nano-CT imaging is limited due to its high cost and the restriction concerning the size of the specimens. Future research is needed to further explore its applications in endodontics.

In addition, the clinical relevance of AHTD remains unclear and further investigation is warranted to define the relationship between hard-tissue debris, biofilms and long-term healing outcomes. The concepts that debris could shelter bacteria and inhibit the activity of topical antiseptics are deduced from *in vitro* studies only. Furthermore, with further methodological refinement in micro-CT imaging, future studies should aim to image and quantify biofilms within the root canal system. Lastly, long-term clinical outcome studies in teeth treated with GW are warranted.
Chapter 5

Conclusion

Within the limitations of this *in vitro* study, none of the tested irrigation protocols was able to render the root canal and isthmus areas of mandibular molars free of dentin debris. The GentleWave system showed better efficacy in removal of accumulated hard-tissue debris from the mesial canals and isthmus areas when compared to continuous ultrasonically-assisted irrigation, but not to intermittent ultrasonically-assisted irrigation. The efficacy of intermittent and continuous ultrasonically-activated irrigation systems was comparable.
References


Tables

**Table 1.** Mean (± SD) of 3D parameters evaluated in 24 mesial roots of mandibular molars before (BF) and after (AF) root canal preparation.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental Step</th>
<th>Group 1 – IU (n=8)</th>
<th>Group 2 – CU (n=8)</th>
<th>Group 3 – GW (n=8)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>BF</td>
<td>10.5 ± 1.4</td>
<td>9.4 ± 1.3</td>
<td>9.9 ± 0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Volume (mm³)</td>
<td>BF</td>
<td>4.0 ± 1.6</td>
<td>3.9 ± 2.3</td>
<td>3.6 ± 1.3</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>AF</td>
<td>11.7 ± 3.2</td>
<td>8.2 ± 3.0</td>
<td>9.4 ± 1.8</td>
<td>0.05</td>
</tr>
<tr>
<td>Surface area (mm²)</td>
<td>BF</td>
<td>44.3 ± 11.1</td>
<td>47.2 ± 23.9</td>
<td>47.0 ± 12.0</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>AF</td>
<td>66.2 ± 13.1</td>
<td>58.0 ± 20.4</td>
<td>58.4 ± 9.2</td>
<td>0.5</td>
</tr>
<tr>
<td>SMI</td>
<td>BF</td>
<td>1.9 ± 0.5</td>
<td>1.7 ± 0.7</td>
<td>1.5 ± 0.3</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>AF</td>
<td>2.6 ± 0.6</td>
<td>2.2 ± 0.4</td>
<td>2.3 ± 0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Untouched area (%)</td>
<td>AF</td>
<td>14.5 ± 4.3</td>
<td>16.8 ± 9.1</td>
<td>14.0 ± 6.0</td>
<td>0.7</td>
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</tbody>
</table>
Table 2: Mean (± SD) of AHTD evaluated in 24 mesial roots of mandibular molars after preparation (AF) and supplementary irrigation protocols (IR)

<table>
<thead>
<tr>
<th>Experimental Step</th>
<th>Group 1 – IU (n=8)</th>
<th>Group 2 – CU (n=8)</th>
<th>Group 3 – GW (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root Canal Volume (mm³) AF</td>
<td>0.7 ± 0.4</td>
<td>0.5 ± 0.4</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>IR</td>
<td>0.07 ± 0.08</td>
<td>0.08 ± 0.06</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>Reduction (%) IR</td>
<td>91.2 ± 5.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>80.0 ± 20.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>96.4 ± 4.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Isthmus Volume (mm³) AF</td>
<td>0.4 ± 0.4</td>
<td>0.3 ± 0.2</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>IR</td>
<td>0.03 ± 0.07</td>
<td>0.03 ± 0.04</td>
<td>0.005 ± 0.007</td>
</tr>
<tr>
<td>Reduction (%) IR</td>
<td>93.5 ± 5.0&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>88.9 ± 5.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>97.9 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Different superscript bold letters in the same line mean statistical significant difference between groups (one-way ANOVA post-hoc Tukey test; P < 0.05).
Figures

**Figure 1.** Representative 3D reconstructions of the mesial root canal systems of 8 representative mandibular molars before (in green) and after (in red) preparation with a reciprocating system and after supplementary irrigation with the GentleWave system. Accumulated hard-tissue debris (AHTD) is depicted in black.
Figure 2. Representative 3D reconstructions of the mesial root canal systems of 8 representative mandibular molars before (in green) and after (in red) preparation with a reciprocating system and after supplementary irrigation with continuous ultrasonically-assisted irrigation. Accumulated hard-tissue debris (AHTD) is depicted in black.
**Figure 3.** Representative 3D reconstructions of the mesial root canal systems of 8 representative mandibular molars before (in green) and after (in red) preparation with a reciprocating system and after supplementary irrigation with intermittently ultrasonically-assisted irrigation. Accumulated hard-tissue debris (AHTD) is depicted in black.
Figure 4. Validation of the method used in this study demonstrated by the correlation between micro-CT and scanning electron microscopy images regarding the presence of hard-tissue debris within the mesial root canal system. (A) Representative 3D model of a mesial root canal system after experimental procedures depicting the presence of AHTD (in black); (B) axial cross-section slice at 9.9 mm from the apex of the representative specimen; (C-E) corresponding SEM images of the same slice in (B) at different magnifications (35×, 150× and 250×, respectively).
Figure 5. Validation of the method used in this study demonstrated by the correlation between micro-CT and scanning electron microscopy images regarding the presence of hard-tissue debris within the mesial root canal system. (A) Representative 3D model of a mesial root canal system after experimental procedures depicting the presence of AHTD (in black); (B) axial cross-section slice at 4 mm from the apex of the representative specimen; (C-D) corresponding SEM images of the same slice in (B) at different magnifications (85× and 180×, respectively).