Chemical Evaluation of the Apical Extrusion of Sodium Hypochlorite Using the Gentlewave® System

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DEDICATION

This thesis is dedicated to my wife, Alicia, and to my parents, Tom and Irma.

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INTRODUCTION

The disinfection of root canal systems has traditionally been achieved physically, through instrumentation, and chemically, through the use of irrigating solutions (1). Achieving the goal of complete debridement within the root canal space while conserving tooth structure is a delicate balance. Enlarging root canal systems to improve chemical debridement can reduce the bacterial load present (2). On the other hand, this enlargement has the potential to not only cause immediate iatrogenic errors, such as zipping, perforations, or transportation, but also to structurally weaken teeth due to dentin removal (3,4).

Sodium hypochlorite (NaOCl) is one of the most common irrigants utilized in endodontic therapy. NaOCl creates an alkaline environment that effectively dissolves tissues, microbes, and microbial byproducts (5–9). However, the use of NaOCl with traditional needle irrigation alone is ineffective at debriding isthmuses and lateral canals (10). In order to improve the efficacy of chemical debridement, many devices have been invented and employed in endodontics such as sonic instruments, ultrasonic instruments, negative apical pressure devices, and lasers (11–14).

The GentleWave® system (GWS) (Sonendo Inc., Laguna Hills, CA) is a new irrigation device that may allow clinicians to accomplish a more complete debridement of root canal systems while also conserving radicular tooth structure. Early research has demonstrated the ability of the GWS to dissolve tissue and debride isthmuses effectively (15,16). The GWS also appears to have the ability to induce negative apical pressure, but it cannot remove gutta-percha during retreatments (17–19). Additional contraindications

to using the GWS include roots located immediately adjacent to the maxillary sinus, open apices, perforations, resorption, or fractures in teeth.

The GWS uses NaOCl for part of its protocol, and, like other irrigation techniques, NaOCl should ideally be confined to root canal spaces during this process (1). Extrusion of NaOCl can cause destruction of apical tissues, and, in some circumstances, NaOCl accidents can occur (20). The negative pressure the GWS system creates at the apical foramen has been previously evaluated using voltage readings and pressure devices (17,18). However, the irrigation fluid used in these studies was distilled water. The possible apical extrusion caused the GWS has not been previously evaluated using actual recommended irrigants (NaOCl and EDTA). The extrusion of NaOCl specifically has also not previously been evaluated with the GWS. The purpose of this study was to chemically assess the relative amounts of NaOCl extruded after irrigation with the GWS compared to conventional needle irrigation using an *in vitro* model. These data will be important in assessing both the safety and limitations of the GWS.

REVIEW OF THE LITERATURE

The objective of endodontic therapy is to restore and maintain normal apical tissue health so patients can achieve a functional and asymptomatic dentition. Removal of necrotic tissue and microbial irritants appears to increase the success rate of endodontic treatment (21,22). Disinfection of the root canal space has been achieved through a combination of mechanical and chemical debridement. Mechanically, endodontic files are used to enlarge root canals to both debride and facilitate chemical debridement (1).

A variety of irrigants have been used to assist in chemically debriding root canals. Some of these irrigants include iodine, chlorhexidine, quaternary ammonium compounds, Ethylenediaminetetraacetic acid (EDTA), formaldehyde derivatives, and NaOCl. The ideal properties of a root canal irrigating solution include: (1) a broad antimicrobial spectrum and high efficacy against anaerobic and facultative microorganisms organized in biofilms, (2) the ability to dissolve necrotic pulp tissue remnants, (3) the ability to inactivate endotoxin, and (4) the ability to prevent the formation of a smear layer during instrumentation or dissolve this layer once it has formed (23). Ideally, an irrigant would also be safe to use both locally and systemically (10). Unfortunately, an irrigant that possesses all of these qualities has not been discovered.

Of the currently available irrigation solutions utilized for disinfecting root canal systems, NaOCl appears to be the most ideal (23). Berthollet, in the 18^{th} century, was the first person to produce a chlorine solution, derived from potassium hypochlorite (23). In World War I, Dakin and Carrel used 0.5% NaOCl to irrigate infected wounds (24). NaOCl creates an alkaline environment with a pH of approximately 11-12. NaOCl becomes ionized in water and dissociates into Na^+ and OCl^-; these ions establish an

equilibrium with hypochlorous acid (HOCl), which is thought to be the antibacterial component after NaOCl dissociation (10). NaOCl has been used in a variety of concentrations, from 0.5% to 6%; however, the higher concentrations seem to have greater efficacy (8,25).

NaOCl has effective tissue dissolution capacity, especially when compared to other irrigants (5–7). In combination with EDTA, 5.25% NaOCl also removes superficial debris and smear layer contents better than other concentrations or combinations of irrigants (26). NaOCl is effective at removing biofilms, especially at higher concentrations (8,9). Its antibacterial efficacy can also extend into dentinal tubules (27). The GWS uses a concentration of 3-3.1% NaOCl.

During endodontic therapy, clinicians must balance the goal of complete debridement with conservation of tooth structure. Increasing the apical size of canals, such as to file sizes of 60 or 80 (0.6-0.8mm), achieves superior disinfection (2). However, endodontic hand and rotary files have a tendency to straighten within curved canals, which can result in zipping, elbows, and increased removal at the outer portion of curvatures (3). Even a size 8 or 10 file can cause transportation of major foramina in teeth (28). Root canal configurations are often round, oval, flattened, or irregular (29). Enlarging these alternative shapes to a large circular size risks causing iatrogenic errors.

Many clinicians choose to enlarge canals to three file sizes larger than the first file to bind in the canal at working length. However, this method does not assure complete dentin removal at root apices and leaves areas in canals unprepared (30). In fact, with micro-CT analysis, it has been determined that 35% or more of canal walls remain

untouched after instrumenting canals with variety of different endodontic rotary files (31,32).

Enlarging canals has the potential to increase the risk of tooth fracture. Endodontically treated teeth are more prone to flexure and fracture due to decreased central tooth structure, and the force required to fracture endodontically treated premolars is 30% lower than uninstrumented teeth (33,34). Using finite element analysis (FEA), it has been determined that a reduction in dentin wall thickness can lead to increased fracture susceptibility (4). Roots enlarged with files of greater tapers are significantly weaker than roots instrumented with lesser tapered files (35).

Due to the limitations in mechanical debridement alone, many different irrigation and activation techniques have been developed. Traditionally, irrigants have been delivered to root canal spaces with syringes and metal needles. These needles have been made in a variety of sizes and tip designs. However, conventional needle irrigation alone is ineffective in debriding accessory canals, isthmuses, and apical portions of root canal spaces (10). Using a Computational Fluid Dynamics model, it has been demonstrated that side-vented needles deliver irrigants approximately 1-1.5mm beyond the tip of the needles; open-ended needles delivered irrigants 2mm or more beyond the tip of the needles (36). Higher pressures were observed at the apical foramina when using the open-ended needles compared to the side-vented needles. Another limitation of needle irrigation is the presence of a “vapor lock” and fluid stagnation in the apical portion of canals, which restricts apical disinfection of canals (37).

In order to increase the efficacy of irrigants within root canal spaces, sonic and ultrasonic instruments were introduced to endodontics in the 1950s (11). In the mid-

1990s, a subsonic handpiece, the Micromega 1500, was shown to clear dye effectively from plastic teeth (38). Over a decade later, a new subsonic instrument, the Endoactivator, was able to reduce the bacterial load in teeth more than conventional needle irrigation alone (39). Passive ultrasonic irrigation is believed to clean canals via acoustic streaming (12,40). Passive ultrasonic irrigation can be performed with a small file or smooth wire in an ultrasonic handpiece. *In vivo* studies have demonstrated a higher likelihood to obtain negative canal cultures after the usage of passive ultrasonic irrigation following hand and rotary instrumentation when compared to hand and rotary instrumentation alone (41,42). Passive ultrasonic irrigation has been shown to be more effective than sonic irrigation as well (12,40).

Other methods of chemical debridement have also been used to effectively clean root canal systems. Diode and Er:YAG lasers have been shown to achieve disinfection in dentinal tubules after colonization with E. faecalis biofilms (14,43). The EndoVac irrigation system (Discus Dental, Culver City, CA), uses a cannula connected to a high vacuum suction and a delivery tip. The cannula simultaneously exerts a negative pressure within the canal while, at the same time, drawing irrigation solutions to the apex (44). When comparing the efficacy of debriding the apical 1 mm of root canals, the EndoVac irrigation system removed significantly more debris compared to traditional needle irrigation (45).

Sonic irrigation, ultrasonic irrigation, lasers, and the EndoVac systems have all been shown to improve debridement of root canals. However, root canals must be shaped to an adequate size to accommodate these devices. For example, the smallest EndoVac cannula is 35 (0.35mm) at the tip (44). Ultrasonic and sonic irrigation devices can be

difficult to use around curved canals, and these devices also work more effectively in larger canals (12,40). Additionally, an in vitro model using extracted teeth has shown that ultrasonic NaOCl activation can result in uncontrolled removal of dentin, even when used at manufacturer recommended settings (46).

Technology has emerged to attempt to accomplish the competing objectives of full debridement and conservation of tooth structure. The GentleWave® system (GWS) produces a broad spectrum of sound waves below and above the ultrasonic spectrum while delivering de-gassed irrigation solutions throughout the root canal system (Figure 1) (15,16). It is hypothesized that these soundwaves cause cavitations in the irrigation solutions that induce shear stresses along the root canal walls. The GWS delivers high-speed streams of irrigants through a handpiece (Figure 2). The rate of irrigant delivery is approximately 50ml per minute. The time of each irrigant delivery depends on the pre-operative diagnosis and is occasionally changed by the company via software updates. At this time, the order and time of irrigant delivery for most cases is: (1) 60 seconds distilled water (leakage test), (2) 240 seconds 3% NaOCl, (3) 30 seconds distilled water, (4) 90 seconds EDTA, and (5) 15 seconds of distilled water.

During the irrigation process of the GWS, the irrigant streams collide with a concave plate at the terminus of the handpiece, which is positioned 1 mm or more occlusal to the pulpal floor in molars (Figure 2). The handpiece for anterior and premolar teeth positions the plate inside the handpiece. After collision with the plate, the irrigants are deflected around the chamber and into the root canals. The irrigants are continuously removed through small suction holes in the handpiece. The GWS is able to generate negative pressure in part due to the “closed-loop” system created with a resin platform

built by the clinician that serves as a gasket between the tooth and the handpiece (Figure 2).

Figure 1: Photograph of the GWS console.

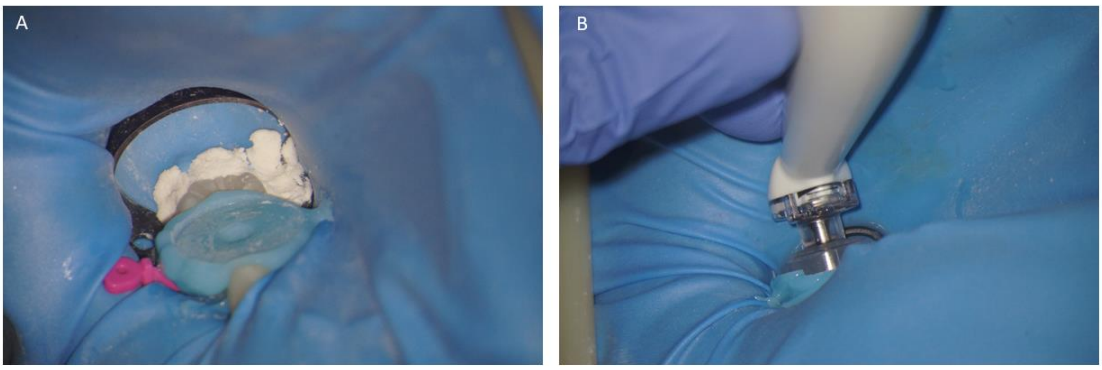


Figure 2: (A) Photograph of resin platform before placement of GWS. (B) Placement of GWS on platform.

The GWS is able to decrease the amount of residual debris in mesiobuccal and mesiolingual canals of mandibular molars compared to conventional rotary instrumentation and needle irrigation (15). Recent debris removal analysis by Chan et al. using microCT imaging revealed accumulated hard tissue debris removal was enhanced with the GWS compared to continuous ultrasonic irrigation (ProUltra PiezoFlow, Dentsply Maillefer; Charlotte, NC)); however, there was no difference between the GWS and intermittent, passive ultrasonic irrigation (Irrisafe wire, Satelec, Bordeaux, France) (47). It is worth noting that the Chan study instrumented canals to WaveOne primary size (size 25 apically). The manufacturer of the GWS recommends minimal instrumentation: apically to a size 20 with .06 taper. At this time, no study has examined the efficacy of debridement using the GWS with a minimal instrumentation size. Even with high volumes of NaOCl and EDTA introduced into canals with multisonic energy, minimal dentin erosion occurs (48). An example of a clinical case utilizing the GWS is shown in Figure 3.

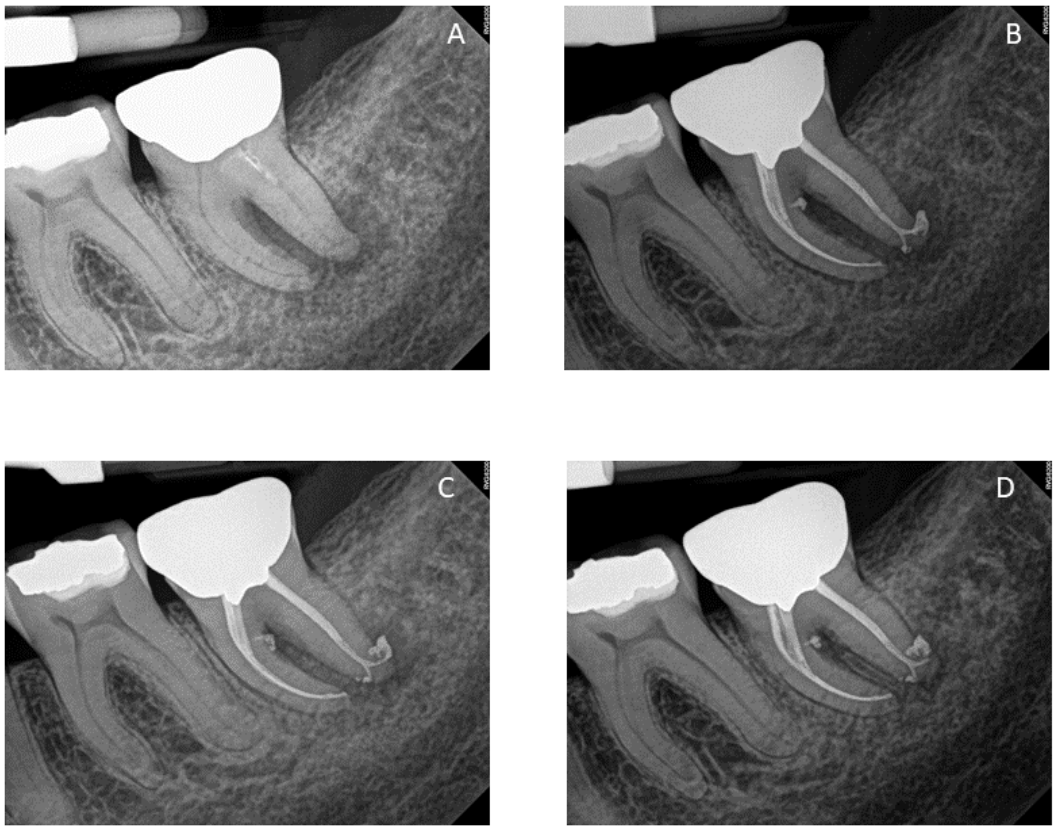


Figure 3: Radiographs of clinical case treated with the Gentlewave system in a single treatment visit. Panel A shows the pre-operative periapical radiograph. Panel B shows the immediate post-operative radiograph. Panel C shows the 6-week post-treatment radiograph. Panel D shows the 6-month post-treatment radiograph.

According to the GWS manufacturer, contraindications to using the device are roots adjacent to the maxillary sinus, open apices, perforations, resorption, or fractures in teeth. These contraindications may be due to concerns about irrigant extrusion. NaOCl is extremely cytotoxic to vital tissue, and can cause severe inflammatory reactions, which have been directly demonstrated in a rabbit model (49). It has been suggested that flare-ups may result from chemical or microbial injury to periradicular tissue (50). Extrusion

of NaOCl can cause a "NaOCl accident," in which sudden pain, profuse bleeding, and almost immediate swelling occurs in patients (20,51). Subsequent to the tissue damage caused by NaOCl, pain can last for many months in some cases. Scabbing, scarring, and nerve tissue damage can also occur after a NaOCl accident (20).

In a survey of Diplomates of the American Board of Endodontics, approximately 42% of the endodontists who responded reported experiencing a NaOCl accident at some point during their careers (52). This study also found a higher prevalence in female patients compared to males and in maxillary teeth compared to mandibular teeth. It has been suggested that the decrease in bone density and thinness of cortical bone surrounding maxillary teeth could contribute to this higher prevalence (20,52). Extreme pressure from needles inside canals can increase the likelihood of NaOCl accidents; this pressure can result from locking needles inside canals, which decreases the capacity of the irrigants to be evacuated coronally. The pressure created by the GWS is known to be high, but little is known about the effects of this pressure in the apical areas of the root canal system as will be discussed below.

Ideally, irrigants used during non-surgical endodontic treatment are confined to the root canal space in order to avoid iatrogenic damage to periapical tissues (1). Therefore, extrusion of irrigants and solvents have been studied for decades in endodontics in a variety of ways. An elegant study in 1977 examined the penetration of irrigating solutions *in vivo*: a radiopaque irrigating solution was used during endodontic treatment in patients (53). The teeth were radiographed after irrigation to assess the extent of the spread of the radiopaque solution. The solution seemed to be confined to the

canal spaces in vital cases, but the solution disseminated into the periapical tissues in necrotic cases.

A decade later, an *in vitro* model was used to determine debris extrusion after different root canal preparation techniques (54). Instrumentation was performed on extracted teeth and debris was weighed after it was desiccated. Due to the desiccation, this technique did not evaluate extrusion of irrigants but, rather, debris accumulation. Myers and Montgomery repeated this study model with updated root canal instrumentation techniques in 1991 (55). Multiple recent studies have also investigated debris extrusion using a weighing method or microCT comparisons (56–60).

As discussed above, a variety of ways to improve irrigation of root canal systems have been developed. In addition to measuring apical extrusion of debris, irrigant extrusion of these systems has also been examined *in vitro*. A collection vial was used to measure solutions extruded after irrigation was performed with the EndoVac, EndoActivator, manual irrigation, ultrasonic needle irrigation, and Rinsendo (61). This collection vial was weighed after irrigation with each device.

Mitchell and colleagues published two studies that utilized a novel method of detecting extrusion of NaOCl (62,63). This method capitalized on the high pH of NaOCl: extracted teeth were embedded in an agarose gel with a pH-sensitive dye called m-Cresol purple. M-Cresol purple is yellow at a pH of 7.4 (physiologic pH) and changes to purple at a pH of 9. After irrigation, extrusion of NaOCl could be visualized as purple areas in the gels due to its high pH. The purple areas were measured using ImageJ after taking standardized photographs. These measured areas were compared in order to estimate the relative amount of extrusion of different irrigation methods.

The studies performed by Mitchell et al. investigated NaOCl extrusion after using needle irrigation, EndoVac, EndoActivator, Rispi-Sonic file attached to a MicroMega, and passive ultrasonic irrigation (62,63). Due to this experimental model's unique detection of NaOCl, this design was repeated by other research groups in order to compare more irrigation methods. The extrusion of NaOCl after irrigation with needle irrigation, the EndoVac, EndoActivator, and PIPS (photoacoustic streaming) was compared (64). Additionally, this model was used to compare NaOCl extrusion of needle irrigation, the self-adjusting file (SAF), passive ultrasonic irrigation, or the EndoVac system. The studies by Mitchell et al., Yost et al., and Iriboz et al. found significantly less apical extrusion of NaOCl when using the EndoVac system compared to the other irrigation systems (62–65). However, as mentioned previously, the required root canal diameter needed to facilitate the EndoVac might predispose teeth to structural failure.

The method described by Mitchell et al. could not quantify the amount of extrusion of NaOCl. In order to accomplish this, Rodriguez-Figueroa et al. measured NaOCl extrusion of different irrigating systems by blending previous vial collection methods with the use of m-Cresol purple (66). Single-rooted, decoronated, extracted teeth were sealed to microcentrifuge tubes, and solutions were collected in the tubes after irrigation with NaOCl utilizing different systems. M-Cresol purple was added to the microcentrifuge tubes and the solutions in the tubes were read by a spectrophotometer. After creating a standard curve with known concentrations of NaOCl, Rodriguez and colleagues were able to quantify the relatives amounts of extrusion while also determining that passive ultrasonic irrigation and EndoVac systems could be used safely to within 1 mm of working length.

The negative pressure the GWS system creates at the apical foramen has been evaluated using voltage readings (17). Additionally, the extrusion of the GWS has been tested in teeth within an airtight chamber with incompressible fluid; the irrigation fluid in this study used water, and water displacement within the chamber was measured (18). However, the extrusion of the GWS has not been previously evaluated or quantified using actual recommended irrigants (NaOCl and EDTA). The extrusion of NaOCl specifically has also not previously been evaluated with the GWS.

SPECIFIC AIMS

1. To determine if NaOCl is extruded beyond the apex of mandibular molars after using the GWS.
2. To compare the GWS and conventional side-vented needle irrigation in regards to the relative amount of NaOCl extruded beyond the apex of mandibular molars.

HYPOTHESES

1. The GWS causes extrusion of NaOCl beyond the apex of mandibular molars.
2. The GWS causes an increased amount of apical extrusion of NaOCl compared to conventional side-vented needle irrigation.

NULL HYPOTHESES

1. The GWS does not cause extrusion of NaOCl beyond the apex of mandibular molars.
2. There is no difference in the amount of apical extrusion of NaOCl between the GWS and conventional side vented needle irrigation.

MATERIALS AND METHODS

Sample Preparation

The methods and use of extracted human teeth were granted an exemption for records and tissue specimens by the University of Minnesota Institutional Review Board (IRB ID: STUDY00004735; Assurance of Compliance: FWA00000312). The exemption was granted because human tissues utilized were deidentified and previously collected.

Pilot Study and Design Considerations

A pilot project was undertaken using the model by Mitchell et al. (62). Four maxillary molar True Teeth (DELabs; Santa Barbara, California) were accessed. All canals were prepared with Vortex Blue files (DENTSPLY; Charlotte, NC) using crown-down instrumentation. The final preparation sizes of two of the teeth were 20/06 and the sizes for the other two teeth were 40/04. A 0.2% agarose gel (Sigma; St. Louis, MO) was prepared. M-Cresol purple (Acros Organics; New Jersey) was added to the gel so the final concentration of m-Cresol purple was 0.005% in the gel. Four ml of the gel was added to each of four acrylic boxes that were 1-inch x 1-inch (Etsy; the Glass Connection). Each of the four True Teeth that were prepared were fixed to the lids of the acrylic boxes. Size 15/02 FlexoFiles (DENTSPLY; Charlotte, NC) were placed 1mm beyond working length in each True Tooth to prevent gel from migrating into the canal spaces. The lids were fixated and sealed to the acrylic boxes with Kool Dam (PulpDent; Watertown, MA), and placed in a 37 °C water bath.

The size 15 hand files were removed from each tooth immediately prior to irrigation. Sealing platforms were made with Kool Dam on two of the True Teeth before irrigation according to the GWS protocol. In the first tooth (prepared to size 20/06 in all

four canals), irrigation was performed with 5mL of 5.25% NaOCl using a 30-gauge side-vented needle (ProRinse Probes; DENTSPLY; Charlotte, NC). In the second tooth (prepared to size 20/06 in all four canals), irrigation was performed with GWS with the first water cycle and the first NaOCl cycle (4 minutes with 3% NaOCl); the GWS irrigation protocol was stopped after the NaOCl cycle. In the third tooth (prepared to size 40/04 in all four canals), irrigation was performed with 5mL of 5.25% NaOCl using a 30 gauge side-vented needle (ProRinse Probes; DENTSPLY; Charlotte, NC)). In the fourth tooth (prepared to size 40/04 in all four canals), irrigation was performed with the GWS with the first water cycle and the first NaOCl cycle (four minutes with 3% NaOCl); the GWS irrigation protocol was stopped after the NaOCl cycle. The gels were photographed immediately before irrigation and 5 minutes after irrigation at a standardized distance of 60mm from the camera (Nikon D3200; Tokyo, Japan) (Figure 4). Figure 5 shows a close-up photograph of the True Tooth prepared to a 40/04 after irrigation with the GWS, displaying the appearance of the gel having been suctioned up into the tooth.

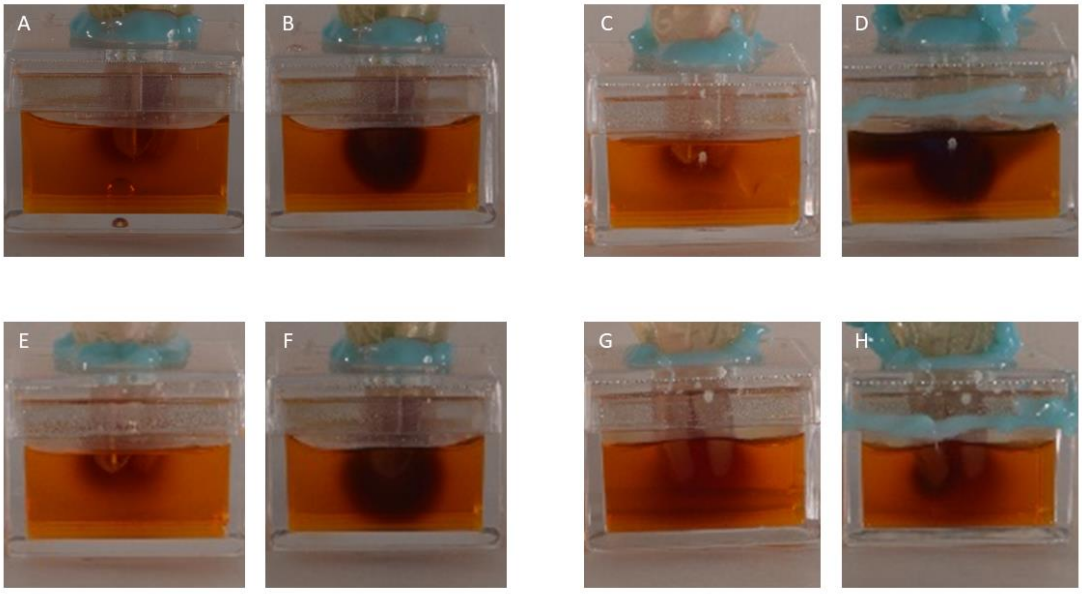


Figure 4: Panel A is a photo of a True Tooth prepared to 20/06 before irrigation with a side-vented needle, and Panel B is a photo of this tooth after irrigation. Panel C is a photo of a True Tooth prepared to 20/06 before irrigation with the GWS, and Panel D is a photo of this tooth five minutes after irrigation. Panel E is a photo of a True Tooth prepared to 40/04 before irrigation with a side-vented needle, and Panel F is a photo of this tooth after irrigation. Panel G is a photo of a True Tooth prepared to 20/06 and before irrigation with the GWS, and Panel H is a photo of this tooth five minutes after irrigation.

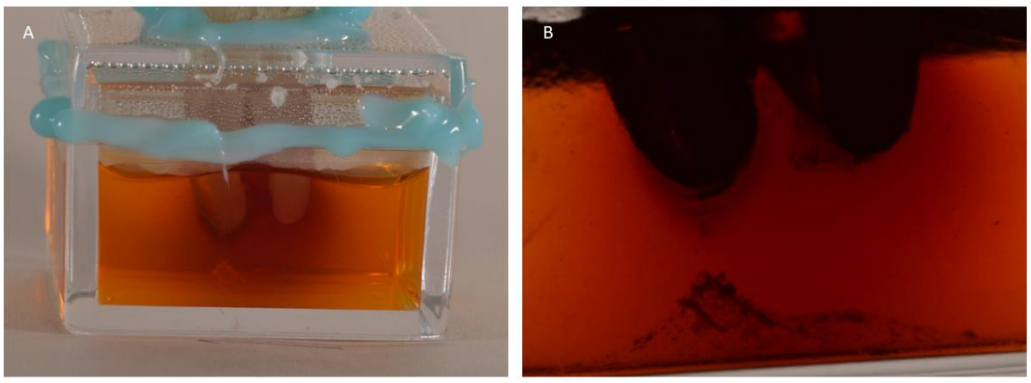


Figure 5: Photograph of the True Tooth prepared to a 40/04 after irrigation with the GWS, displaying the appearance of the gel having been suctioned up into the tooth.

ImageJ was used to quantify the number of pixels in the area of color change before and after irrigation (data not shown). After completing this pilot project, it was determined that the quantification of the area of the color change was too subjective to reliably evaluate the relative extrusion of NaOCl. Additionally, the physical alteration of the gel in the 40/04 sample by the GWS made the quantification of pixels less accurate (Figure 5).

Chemical Assessment of NaOCl Extrusion

In order to more objectively quantify the relative extrusion of NaOCl, a modification of the model used by Rodriguez et al. was undertaken (66). Mandibular first and second molars were collected from the Oral Surgery and Periodontal clinics at the University of Minnesota School of Dentistry and stored in 0.1% Thymol. Teeth were discarded if resorption, root cracks, existing crowns, or severe caries extending past the occlusal surface were present. Endodontic accesses were prepared in each of the molars and #8 FlexoFiles (DENTSPLY; Charlotte, NC) were introduced into the canals. Patency was visually confirmed. In the events when patency could not be achieved, molars were discarded. All canals were prepared to within 0.5mm of the apex with Vortex Blue files (DENTSPLY; Charlotte, NC) with a crown-down technique using distilled water for irrigation. Patency was confirmed with 10K files. The final preparation size of all mesial canals was 30/04 and the final preparation size of all distal canals was 40/04. This process was repeated until 17 molar specimens were obtained.

The teeth were fixated and sealed to the lids of 1-inch x 1-inch clear acrylic boxes (Etsy; the Glass Connection; Milton, VT). 4ml of distilled water was added to each clear acrylic box, and the lids with the molars were placed on the boxes. All the apices of the

molars were fully submerged in distilled water. Each of the 17 specimens underwent 5 irrigation protocols:

Read 1: irrigation with 10ml 3% NaOCl with 30-gauge side-vented needle (ProRinse Probes; DENTSPLY; Charlotte, NC)

Read 2: irrigation with 10ml distilled water with 29-gauge end-vented needle (Navitip; Ultradent; South Jordan, Utah) [negative control]

Read 3: irrigation with the GWS

Read 4: irrigation with 10ml distilled water with 29-gauge end-vented needle (Navitip; Ultradent; South Jordan, Utah) [negative control]

Read 5: irrigation with 10ml 3% NaOCl with 29-gauge end-vented needle (Navitip; Ultradent; South Jordan, Utah) [positive control]

In reads 1, 2, 4, and 5, teeth were irrigated with a volume of 10ml of either NaOCl or distilled water (as specified above). This volume was chosen based on research by Yamada et al. (26). In read 1, the needles were placed short of the binding point or 2 mm from the working length, and irrigants were expressed over a period of 2 minutes with constant movement of the irrigation syringes coronally and apically. In reads 2, 4, and 5, irrigation needles were purposely placed at binding points in the canals, and irrigants were expressed over a period of 2 minutes with constant movement of the irrigation syringes coronally and apically. Figure 6 displays the irrigation apparatuses. In read 3, the GWS was activated in the NaOCl cycle for 15 seconds or until the liquid in the boxes was suctioned to the level of the root apices due to the apparent negative pressure from the GWS (Figure 6B and 6C). Groups 2 and 4 served as negative controls to ensure no residual NaOCl remained in the teeth or acrylic boxes between reads 1, 3, and 5. After

each sample was irrigated in each read, 1000 µL of the solution from each box was added to an Eppendorf tube (Falcon® 14 mL Round Bottom PP Test Tube, Sterile; Fisher Scientific; Pittsburgh, PA), which was vortexed for 10 seconds. Group 5 served as a positive control, as NaOCl extrusion was anticipated in this group, due to placing end-vented needles at the binding point in canals.

Figure 6: Irrigation apparatuses. Panel A displays irrigation and suction for reads #1, 2, 4, and 5. Panel B shows a molar and liquid in an acrylic box before GWS irrigation and Panel C displays the apparatus after GWS irrigation with remaining solution at the level of the apices of the molar.

In order to prepare a standard curve so that the relative amounts of extruded NaOCl could be determined, known volumes of 3% NaOCl (0, 20, 40, 200, 400, 600, 1000, 2000 µL) were added to distilled water so that the total volume was 4 mL. These volumes corresponded to 3% NaOCl solution dilutions of 0%, 0.5%, 1%, 5%, 10%, 15%, 25%, 50%, respectively. 1000 µL of each of these solutions was added to Eppendorf tubes.

Standard curve solutions were prepared for each Read (5 total standard curves were made). 7.5 µL of 1% m-creosol purple was added to each 1000 µL solution (the total concentration of m-Cresol purple in each solution was 0.75%). The tubes were vortexed for 10 seconds each.

Each solution was added to three wells on a 96 well plate (Thermo Scientific Nunc; Nunc 96 MicroWell™ Plates; Pittsburgh, PA), with each well having 250 µL of each solution (Figure 7). Therefore, each solution was analyzed in triplicate. 25 minutes after the addition of m-Cresol purple to each solution, a spectrophotometric analysis was completed (Chromate, Model 4300 Microplate) with a 570 µm filter (Figure 8). Pilot data revealed that a gradient in the optical density values is created 25 minutes after the addition of m-Cresol purple (Figure 8). This distinct gradient is not present at time points other than 25 minutes after adding m-Cresol purple (Figure 8).

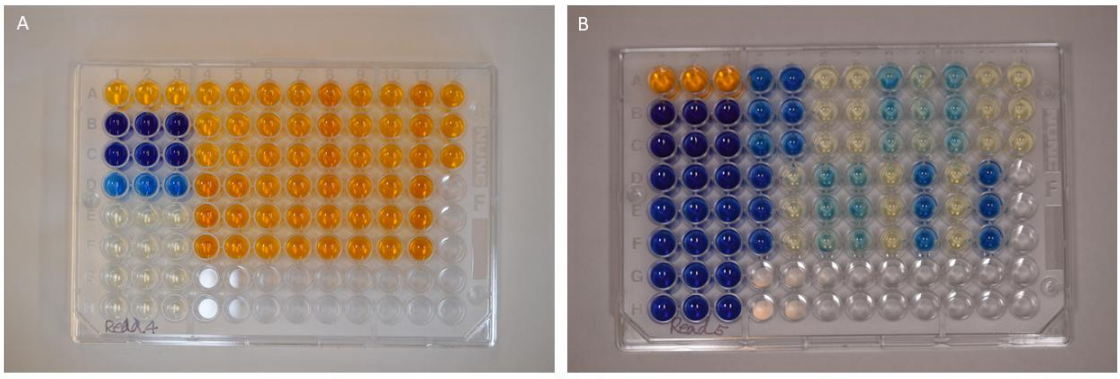


Figure 7: Photographs of 96 well plates used for Reads 4 and 5. The three columns on the left-hand side of panels A and B represent wells for known amounts of NaOCl.

µl NaOCl in 4000µl solution	Percentage of 3% NaOCl
0	0
20	0.5
40	1
200	5
400	10
600	15
1000	25
2000	50

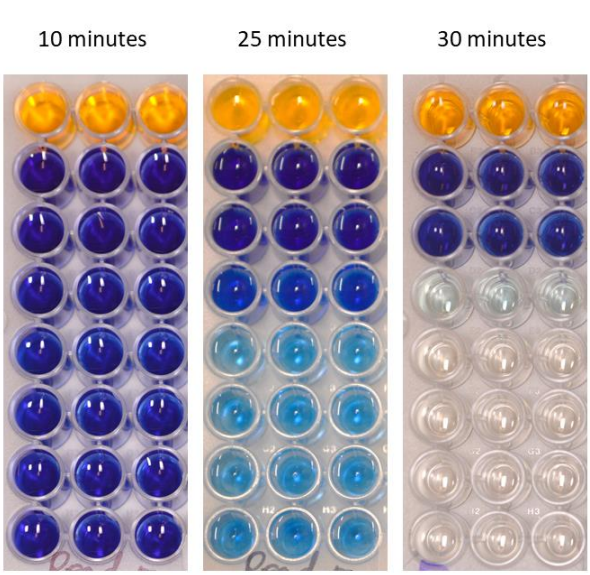


Figure 8: Photograph of wells used for standard curves. 10, 25, and 30 minutes after m-Cresol purple was added to the solutions, photographs were taken as shown above. Spectrophotometry readings were taken 25 minutes after m-Cresol purple was added to the solutions.

The optical density (OD) values for the standard solutions with known percentages of 3% NaOCl were used to create standard curves. The values were equated in "percentage of 3% NaOCl" in order to accommodate the difference in solution amounts present after irrigation with the GWS (Figure 6). The standard curves were created by utilizing a logarithmic trendline for the values from the standard solutions in Reads 1-5 in Excel (Microsoft; Redmond, WA). The equations from each trendline were then used to determine the unknown percentage of 3% NaOCl in samples from the mean ODs from each sample in Reads 1-5. Statistical analysis was performed on Read 1 (NaOCl with side-vented needle) and Read 3 (GWS) (this was the primary outcome measure). In 14 of 17 samples in Read 3, the estimated percentages of 3% NaOCl in the samples were above the highest known amount of 3% NaOCl in the standard solutions.

Statistical Analysis

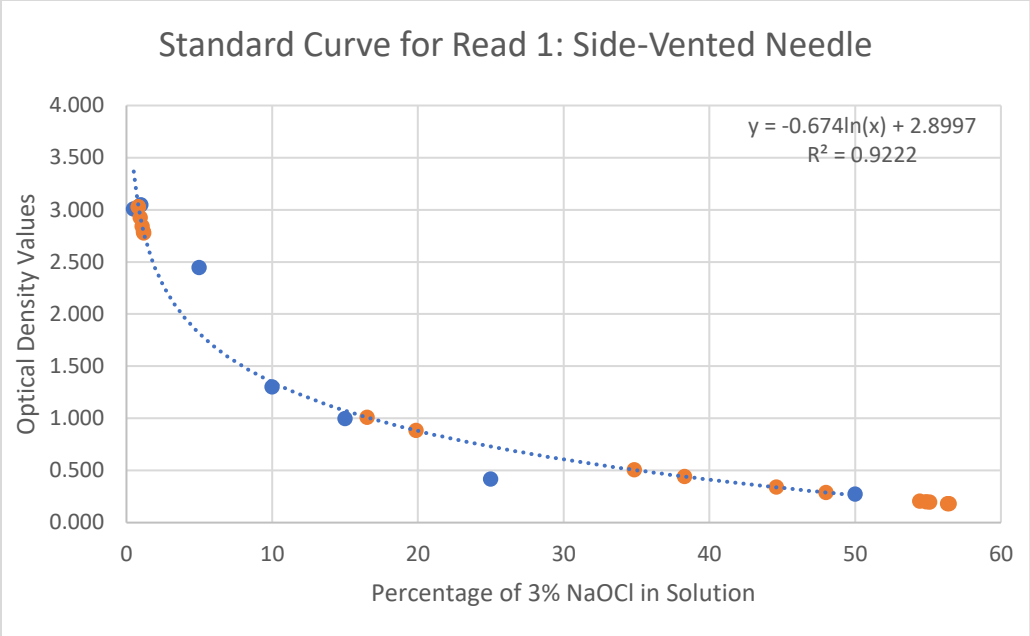
In order to more accurately model the results based on the known amounts in the standard curves created, solutions were categorized based on whether they were above or below the highest value in the standard curve (the highest amount of 3% NaOCl in standard solutions was 50%). Generalized linear mixed model was conducted to model the binary outcome (>50% vs <=50%). Analyses were performed in SAS 9.4. The level of significance was set to $p < 0.05$.

RESULTS

Reads 2 and 4 (irrigation with distilled water) served as controls to ensure residual NaOCl did not remain in any of the teeth or acrylic boxes. All of the solutions in these controls (from all 17 teeth in reads 2 and 4) had optical density values indicating no detectable NaOCl was extruded or present. The average of the estimated percentage of 3% NaOCl present in the solutions of the 17 samples in Read 1 (side vented irrigation) was 28.6% (SD 23.9%), in Read 3 (GWS irrigation) was 57.4% (SD 18.3%), and in Read 5 (end-vented irrigation; positive control) was 104.4% (SD 6.3%) (Figures 9, 10, and 11). Some of the values of the estimated percentages of 3% NaOCl in the samples were above the highest known amount of 3% NaOCl concentration in the standard solutions (the highest standard solution made was 2000 μL 3% NaOCl in a total solution volume of 4000 μL; therefore 50% of the solution was 3% NaOCl). Therefore, the means of the estimated percentage of 3% NaOCl present in the solutions of Reads 1, 3, and 5 were not statistically compared.

In order to more accurately model the results based on the known amounts in the standard curves created, solutions were categorized based on whether they were above or below the highest value in the standard curve (Table 1). Five out of 17 (29%) samples in the side-vented irrigation group (Read 1) had samples where greater than 50% of the solution was 3% NaOCl. 14 out of 17 (82%) samples in the GWS group (Read 3) had samples where greater than 50% of the solution was 3% NaOCl. 17 out of 17 (100%) samples in the end-vented irrigation group (Read 5) had samples where greater than 50% of the solution was 3% NaOCl (Table 1); this group served as the positive control. A significant difference was observed between the side-vented irrigation group and the

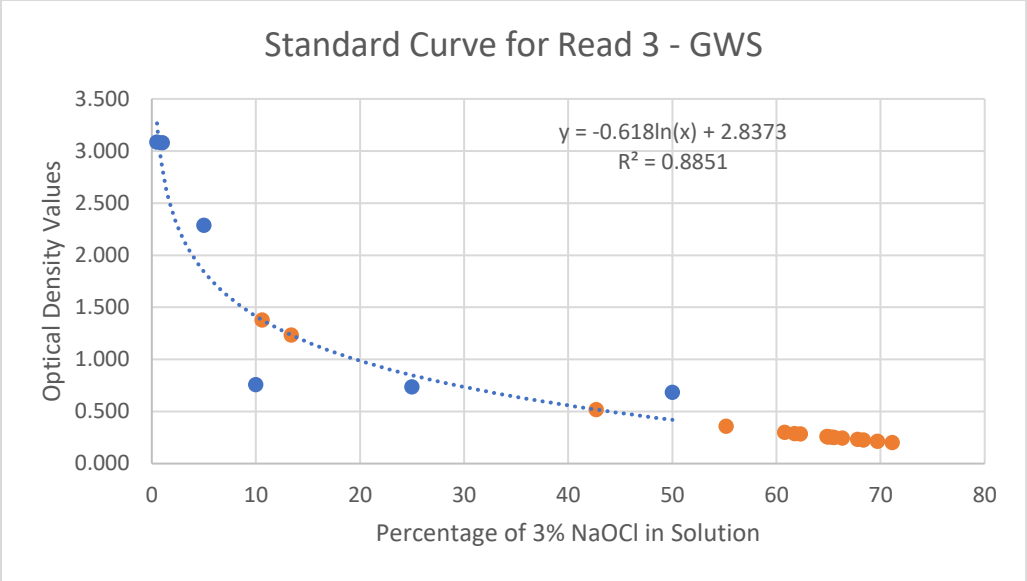
GWS irrigation group in terms of the number of samples where greater than 50% of the solution was 3% NaOCl.



Standard Data		
µl 3% NaOCl	Percentage of 3% NaOCl	Avg OD Value
0	0	0.070
20	0.5	3.009
40	1	3.047
200	5	2.445
400	10	1.300
600	15	0.997
1000	25	0.418
2000	50	0.275

Sample	Average Optical Density Value	Estimated Percentage of 3% NaOCl in Solution
S1	0.290	48.013
S2	0.885	19.879
S3	2.790	1.176
S4	0.182	56.357
S5	3.028	0.827
S6	0.197	55.116
S7	0.206	54.439
S8	0.181	56.468
S9	3.029	0.825
S10	1.009	16.530
S11	0.443	38.300
S12	0.506	34.848
S13	2.844	1.087
S14	2.776	1.201
S15	0.340	44.602
S16	2.926	0.961
S17	0.200	54.872

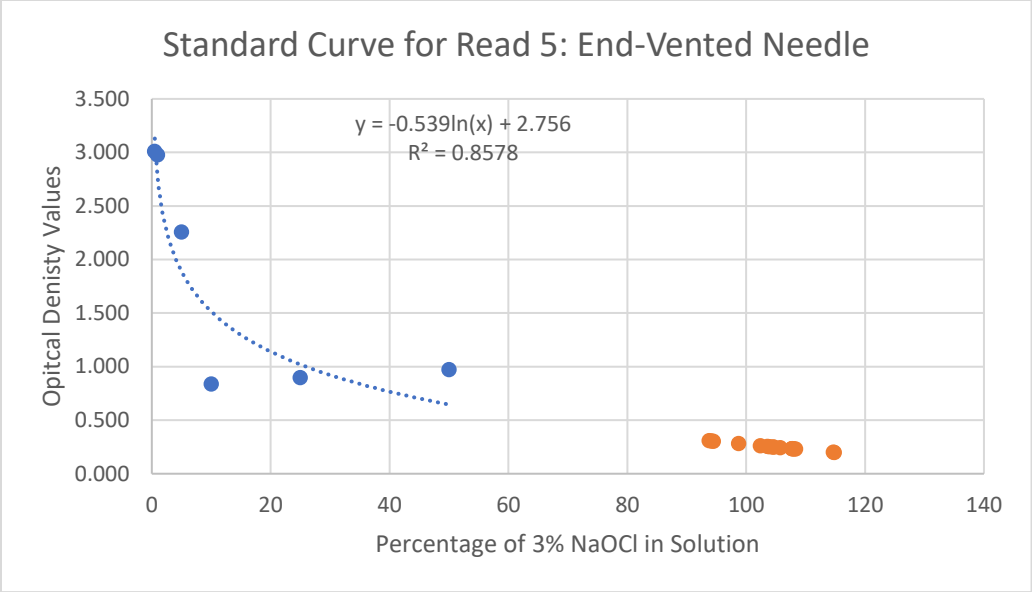
Figure 9: Standard Curve and Sample Values for Read 1: Side-Vented Needle. Blue points represent the standard solutions' OD values and corresponding values of the percentage of 3% NaOCl in solution; the 0 µl data were not included. The blue line is the logarithmic trendline created from the standard solution data. Orange points are representative of data from samples.



Standard Data		
µl 3% NaOCl	**Percentage of 3% NaOCl**	**Avg OD Value**
0	0	0.049
20	0.5	3.085
40	1	3.078
200	5	2.288
400	10	0.758
1000	25	0.735
2000	50	0.685

Sample	Average Optical Density Value	Estimated Percentage of 3% NaOCl in Solution
S1	1.378	10.599
S2	0.284	62.308
S3	0.245	66.331
S4	0.359	55.158
S5	0.299	60.781
S6	0.254	65.337
S7	0.214	69.706
S8	0.252	65.549
S9	0.231	67.814
S10	0.289	61.739
S11	0.517	42.691
S12	0.202	71.111
S13	0.258	64.950
S14	0.259	64.845
S15	0.226	68.365
S16	1.234	13.395
S17	0.258	64.950

Figure 10: Standard Curve and Sample Values for Read 3: GWS. Blue points represent the standard solutions' OD values and corresponding values of the percentage of 3% NaOCl in solution; the 0 µl data were not included. The blue line is the logarithmic trendline created from the standard solution data. Orange points are representative of data from samples.



Standard Data		
µl 3% NaOCl	Percentage of 3% NaOCl	Avg OD Value
0	0	0.064
20	0.5	3.011
40	1	2.978
200	5	2.258
400	10	0.837
1000	25	0.897
2000	50	0.972

Sample	Average Optical Density Value	Estimated Percentage of 3% NaOCl in Solution
S1	0.199	114.818
S2	0.200	114.676
S3	0.234	107.666
S4	0.233	107.799
S5	0.231	108.334
S6	0.281	98.737
S7	0.232	108.066
S8	0.306	94.145
S9	0.250	104.517
S10	0.261	102.406
S11	0.253	103.937
S12	0.255	103.616
S13	0.308	93.796
S14	0.304	94.495
S15	0.233	107.799
S16	0.244	105.752
S17	0.249	104.646

Figure 11: Standard Curve and Sample Values for Read 5: End-Vented Needle (positive control). Blue points represent the standard solutions' OD values and corresponding values of the percentage of 3% NaOCl in solution; the 0 µl data were not included. The blue line is the logarithmic trendline created from the standard solution data. Orange points are representative of data from samples.

	Read 1 (side-vented needle)	Read 3 (GWS)	Read 5 (end-vented needle) [positive control]	Read 1 vs 3 P value
Samples where mean percentage of extruded NaOCl was above 50%; n, (%)	5 (29%)	14 (82%)	17 (100%)	0.0005

Table 1: Relative amounts of extruded 3% NaOCl.

In 15 of 17 samples in read 3, the GWS suctioned the distilled water in the acrylic boxes to the level of the root apices (Figure 6, Panels B and C). This suctioning was the main reason the values of 3% NaOCl were reported in percentages of solution, instead of actual volume amounts. The distilled water level remained at the same level in 2 of 17 samples in read 3 (GWS irrigation). All 17 samples were videoed as they underwent irrigation with the GWS. Although extrusion was not quantified or tabulated from these videos, extrusion of irrigants and circulation of solutions was evident.

DISCUSSION

At this time, all studies that have been published about the GWS have been fully or partially funded by the GWS parent company, Sonendo (15,16,70,17–19,47,48,67–69). The GWS has been approved by the FDA, however more research needs to be completed to test the safety of the device. A critical component of the safety of an endodontic irrigation protocol is determining whether chemicals are extruded beyond the root canal space. A foundational principle of cleaning and shaping is keeping these procedures confined to the roots themselves (1). In the current study, mandibular molars were suspended in distilled water. Teeth were irrigated with three different methods: side-vented needle irrigation, the GWS, and end-vented needle irrigation. The distilled water in which the teeth were suspended was collected and analyzed using spectrophotometry and a pH indicator. The estimated percentage of 3% NaOCl in the distilled water was significantly higher in the GWS compared to the side-vented needle irrigation group. These findings suggest the GWS causes extrusion of NaOCl beyond the apex of mandibular molars; these data also suggest the GWS causes an increased amount of apical extrusion of NaOCl compared to conventional side-vented needle irrigation.

An in vivo method of measuring extrusion would likely not be ethical to test on patients using today's IRB standards (53). Additionally, using debris collection methods after desiccation would be unable to measure solution extrusion (54,55). The method used by Rodriguez et al. was initially considered to evaluate the extrusion of NaOCl using the GWS. However, multiple challenges existed in using this model to evaluate the GWS.

First, the Rodriguez et al. model sealed teeth in microcentrifuge tubes, and the apices of the roots were suspended in air. Any fluid that accumulated in the microcentrifuge tubes was collected and examined. The GWS requires a "closed-loop" system – therefore, an air leak, such as suspending root apices in the air of a microcentrifuge tube, would not accommodate the closed-loop, air-tight system that the GWS requires (17,18). This finding was confirmed during the design of this study.

Second, the microcentrifuge tubes fit single rooted teeth well, but the tubes do not accommodate molars. As stated above, the irrigant stream in the GWS collides with a plate at the terminus of the handpiece, which is positioned 1 mm or more occlusal to the pulpal floor in molars. The handpiece for anterior and premolar teeth positions the plate inside the handpiece. The position of the plate closer to the pulpal floor in molars places the irrigant collision point closer to the apices. Therefore, the aim was to evaluate extrusion utilizing molars.

After considering the limitations of applying the model used by Rodriguez et al to evaluate the extrusion of the GWS, the agarose gel model developed by Mitchell et al. was considered (62,66). Two additional research groups had used this model successfully to compare NaOCl extrusion with different irrigation systems (64,65). A pilot project was undertaken using this agarose gel model as described above. However, after the pilot project, it was determined that the quantification of the area of the color change using the agarose gel model was too subjective to reliably evaluate the relative extrusion of NaOCl. Additionally, the physical alteration of the gel by the GWS made the quantification of pixels less accurate.

Two previous studies have investigated the potential extrusion of the GWS (17,18). Both studies used air-tight, custom pressure vessels to quantify the amount of pressure at the apical extent of the canals. This method of measurement has been previously described by Park et al. (71). In the Charara et al. study, this pressure was elegantly kept at a constant 5.88 +/- 0.15 mm Hg to stimulate periapical back pressure (18). This pressure level was derived from a study investigating central venous pressure of human patients, since the exact pressure at the apices of teeth is unknown (72,73). The static fluid pressure of water in the 1-inch acrylic boxes utilized in the current study is estimated to have ranged between 1-2mm Hg. Future research is needed to determine different apical pressures, as they might vary in cases with intact periodontal ligaments versus teeth with apical tissue destruction, as demonstrated by Salzgeber and Brilliant (53). The lower pressure in this study might have additional relevance in cases where the sinus floor is thin, eroded, or perforated. The control groups in the Charara et al. study utilized 30-gauge open-ended needles (Navitip; Ultradent; South Jordan, Utah) and the EndoVac; a conventional side-vented needle was not utilized. The current study used a 29-gauge open-ended needles as the positive control and included a side-vented needle as the comparison group.

Both previous studies that investigated the potential extrusion of the GWS utilized water as an irrigant and investigated extrusion via physical characteristics (pressure) (17,18). Conversely, the present study is the first to chemically assess actual NaOCl extrusion of the GWS. Spectrophotometers can be used to measure the diffusivity of light at specific wavelengths; they produce optical density values that correspond to amounts of substances in solutions. Spectrophotometric analysis can accurately determine the pH

in solutions by using acid-base indicators (74). Spectrophotometry in this study was used to assess the amount of color change of a pH indicator in solutions surrounding the apices of molars after irrigation, similarly to Rodriguez et al. (66).

A limitation of this study was the quantification of the exact amounts of NaOCl extruded apically. As stated above, the exact pressure of the apical tissues is unknown, and likely changes depending upon the anatomical position and extent of possible apical tissue destruction. The relatively low pressure in this study's model may have allowed for excessive extrusion that would possibly not manifest clinically. Therefore, the results of this study should focus on the relative comparison of extruded NaOCl, instead of the exact amount of NaOCl extruded.

Another limitation of this study was the fact that some of the values of the estimated percentages of 3% NaOCl in the samples were above the highest known amount of 3% NaOCl concentration in the standard solutions. In order to more accurately model the results based on the known amounts in the standard curves created, solutions were categorized based on whether they were above or below the highest value in the standard curve. The values of the standard curve also restricted the ability to compare the GWS to the end-vented needle group, since many of the values in both groups were higher than the highest standard curve value.

An additional limitation of this study is the difficulty in comparing standard needle irrigation with the GWS. The GWS irrigates all canals at the same time. Side-vented needles can only irrigate one canal at a time, which makes the comparison of these methods challenging. The rate of irrigation during the GWS is approximately 50ml per minute. GWS cycle times range from 7 minutes 15 seconds to 8 minutes 45 seconds. The

rate and amount of GWS irrigation are difficult to compare to standard irrigation, which often uses 10-15 ml of irrigant over a period of 45-90 minutes (26).

More variability existed in the estimated percentages of 3% NaOCl extruded in the side-vented group compared to the GWS and the end-vented group. Since irrigant replacement occurs approximately 1-1.5mm beyond side vented needles, the increased variability in the side-vented group may have been due to teeth with naturally longer and more curved canals (36).

In 15 of 17 samples, the distilled water in the acrylic boxes was suctioned to the level of the root apices after using the GWS (Figure 6, Panels B and C). Anecdotally, this suggests a strong negative apical pressure of the GWS. These observations are supported by previous research (17,18). The distilled water level remained at the same level in 2 of 17 samples in the GWS group. In order for the GWS to de-gas the irrigation fluids, a closed-loop system must be established. One hypothesis is that the 2 samples in which the fluid levels did not change may have been in acrylic boxes with an "air-tight" seal of the lid to the bottom of the box. The negative pressure may have been able to suction air through the junction of the lid and the box in 15 of 17 samples where the water was suctioned. Nevertheless, during the time of GWS irrigation before the water level reached the apices of the teeth, a "closed-loop" system existed. Future research could investigate using this model with a sealed acrylic box. In the current study, the GWS was activated in the NaOCl cycle for 15 seconds or until the liquid in the boxes was suctioned to the level of the root apices. Future studies with a sealed acrylic box should be able to use the GWS for the full irrigation cycle.

Despite the apparent strong negative apical pressure of the GWS, significantly more NaOCl extrusion was present after irrigation with the GWS compared to the side-vented needle group. Perhaps, the negative pressure is able to withdraw NaOCl from the tissues rapidly before significant tissue destruction can occur. This might create a constant turnover or exchange of fluids in the apical tissue whereby the overall negative pressure overrides the extruded NaOCl. The presence of NaOCl beyond the apex coupled with the strong apical negative pressure may explain the apical bleeding that occurs after the use of the GWS, as reported by users and the parent company itself.

When using the GWS, the manufacturer recommended preparation size is 20/06. However, the GWS is currently recommended for a variety of canal sizes and for non-surgical root canal retreatment. The apical sizes during retreatment cases can vary widely. Additionally, the mean cross-sectional diameter 1 mm from the apex of the mesial canals of mandibular molars is 200-400 microns and the diameter in distal canals is 400-700 microns (75). Therefore, the GWS is currently recommended and being used clinically for apices larger than the company supported preparation size. The standard preparation size used in this study of all mesial canals was 30/04 and the final preparation size of all distal canals was 40/04. Future research is needed to investigate NaOCl extrusion using the GWS after different preparation sizes to guide clinicians on possible indications and limitations of its use.

As stated previously, the irrigant stream in the GWS collides with a plate inside the handpiece for anterior and premolar teeth. The collision at the plate in the molar handpiece occurs inside the chamber. Because of this position, the molar handpiece is thought to be more powerful than the anterior/premolar handpiece, due to the action of

the multisonic energy occurring closer to the apices in molars. Therefore, this study focused on evaluating extrusion of the GWS in molars. However, future studies should investigate the extrusion effects in anterior and premolar teeth as well.

It is widely accepted that the etiology of apical periodontitis is the presence of microbes, and the GWS appears to be effective in debriding root canal systems to reduce residual debris in canals (15,76). A common method of root canal debridement is the use of ultrasonic irrigant activation, which has been shown *in vitro* and *in vivo* to be more effective at removing debris and microbes compared to traditional needle irrigation alone (12,40–42). However, a recent systematic review indicated that even passive ultrasonic irrigation has not been shown improve outcomes or the healing rate of apical periodontitis compared with syringe irrigation (77). As Seltzer and Bender identified 54 years ago, cognitive dissonance still exists within endodontics: clinician-centered outcomes of canal debridement and extrusion may not correlate, linearly or otherwise, with patient-centered outcomes (78). Furthermore, no literature has been published linking the GWS to better outcomes compared to traditional irrigation methods. With the lack of outcome data on the GWS coupled with the possible increased risk of NaOCl extrusion, further unbiased research regarding the safety of the device is warranted before indiscriminate and widespread use of the technology occurs. The results presented above indicate that the GWS extrudes NaOCl apically. Future research should investigate whether these effects positively or negatively affect the outcomes of cases irrigated with the GWS.

CONCLUSIONS

This study utilized a method of chemically assessing the extrusion of NaOCl after irrigation with side-vented needle irrigation, the GWS irrigation, and end-vented needle irrigation. Within the limitations of this study mentioned above, it can be concluded that: the GWS causes significantly more apical extrusion of sodium hypochlorite compared to conventional side-vented needle irrigation. More research is warranted to explore the safety and the dynamic mechanisms of action of this irrigation system.

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