THE EFFECT OF AGITATION ON THE PENETRATION DEPTH OF SODIUM HYPOCHLORITE INTO DENTINAL TUBULES

by

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Abstract

Objective: The aim of this study was to compare the difference in irrigant penetration into dentinal tubules of 6% NaOCl using the EndoActivator®, ProUltra® PiezoFlow™ and EndoVac® and to compare them with the standard side-vented ProRinse® needle.

Methods: Sixty extracted anterior teeth with single canals were accessed conventionally, the pulp tissue removed and canal patency verified using minimal instrumentation. Crystal Violet dye was placed in the canals for 5 days followed by instrumentation of the canals to standard shape with ProTaper rotary files to size F4 using 1ml of 6% NaOCl used between each file. The teeth were divided into four groups and each agitation system was used with 6% NaOCl as per manufacturers recommendations. Each tooth was mounted in acrylic and cut into 1 mm thick section perpendicular to the long axis of the tooth using the Isomet® Linear Precision Saw (Censico International Pvt. Ltd.) The sections were analyzed with a Nikon® Eclipse® Microscope at 40x magnification and NaOCl penetration was measured with the NIS Elements™ Software (Nikon Corporation).

Results: The maximum penetration depth for the ProRinse® side-vented needle, EndoActivator and EndoVac irrigation methods occurred in the coronal third of the canal. However, the maximum penetration depth for the ProUltra® PiezoFlow™ Ultrasonic System occurred in the middle third. With regard to NaOCl Penetration area, the coronal and middle thirds showed better area penetration than the apical third in all irrigation groups.

Conclusions: The irrigation methods may affect the highest penetration depth of NaOCl into dentinal tubules at different areas of the root canal position.
Preface

Dr. Markus Haapasalo devised the concept and design of this research project, “The Effect of Agitation on the Penetration Depth of Sodium Hypochlorite in Dentinal Tubules”.

This project was performed with the guidance of both Dr. M. Haapasalo and Dr. Y. Shen.

Shannon Davis performed the research project, including all of the sample preparation, microscopic image photography and preparation of the thesis manuscript with editing by Dr. Markus Haapasalo and Dr. Ya Shen.

The relative contribution of collaboration for this project was:
Dr. Shannon Davis 60%, Dr. Ya Shen 20%, and Dr. Markus Haapasalo 20%.

This study was approved by the University of British Columbia Office of Research Services, Clinic Research Ethics Board (Certificate Number: H12-00300).

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N..............................................................30 Gauge ProRinse® Side Vented Irrigation Needle
PUS.............................................................ProUltra® PiezoFlow” Ultrasonic Irrigant System
EA...........................................................................EndoActivator® Sonic Irrigant System
ISO........................................................................International Standards Organization
EV........................................................................EndoVac® Irrigant Agitation System
EDTA.................................................................Ethylenediaminetetraacetic Acid
CUI........................................................................Continuous Ultrasonic Irrigation
CFD........................................................................Computational Fluid Dynamics
PUI........................................................................Passive Ultrasonic Irrigation
MDI........................................................................Manual Dynamic Irrigation
Microscope................................................................Nikon® Eclipse® Microscope
ANP......................................................................Apical Negative Pressure
NaOCl......................................................................Sodium Hypochlorite
MDT......................................................................Master Delivery Tip
H₂O₂......................................................................Hydrogen Peroxide
NiTi........................................................................Nickel Titanium
CHX......................................................................Chlorhexidine
Ga.........................................................................Gauge
Acknowledgements

I would like to thank everyone who has made my time at UBC a positive experience.
Thank you to both Dr. Haapasalo and Dr. Shen for your research ideas, showing me how to work in the research lab cutting teeth, learning to take microscopic pictures and also for providing me with feedback and assistance throughout my project. Thanks also to Zhejun who helped me numerous times in the lab when my equipment stopped working and also for showing me how to improve my microscopic images. I’d like to thank to Dr. Coil who was not only on my research committee but sat by my side during my first surgery, guiding me with patience and understanding. I appreciated the time you were in the clinic with us. Your positive attitude and calm demeanor were excellent attributes in a mentor.
I wish to thank Dr. Almeida for being on my committee, offering worthwhile suggestions and feedback and for also truly listening to what I had to say. You are certainly more than just an educator. You are a person who does not do what is easy, you do what is right and for that I have tremendous respect for you.
I also want to thank Lois, Shauna and Francisco for always going beyond their job description to assist us by either finding that missing piece of dental equipment, to share a laugh at the end of the day or to offer a shoulder to cry on during difficult days. Thanks for always doing your best and supporting us. We will not forget it.
Thanks to my fantastic classmates for the times you helped me and for the times we shared. I was blessed to have Marina in my class, as she is a gracious individual always abundantly helpful and kind. Thanks Mark & Ellen for sharing your study notes. You saved me countless hours of time by providing an organized review, which was immensely helpful. Thanks also to Christine for helping me with my research statistics.
I’d also like to thank my remaining classmates: There isn't one of you who didn't help me in some way by assisting me during a surgery, sending me research articles, lending me your notes or by some small act of kindness. Thank you Les, Agmar, Abdullah, Bassim, Rene, Frédéric, Mohsen, Houman, Wei, Neda and James.
Thanks also to all the support staff who made my life easier-Peter, who always helped me with my IT issues and to Theresa and Lisa who kept our lives “on track”.

x
Dedication

I have been given this opportunity to fulfill my lifelong desire of becoming an endodontist and for that I have been tremendously blessed. Only through God’s grace has this opportunity been provided to me. I am eternally thankful.

I dedicate this body of work to my husband, Kelsey. Although others have guided me along my path to achieve this goal it was you who made it possible by putting your career on hold to follow me to Vancouver and for keeping things together at home while I was consumed by my studies. Thank you for being my best friend and for supporting me in realizing my dream. Although we have had numerous challenges on our BC adventure I am happy that those difficult moments only strengthened our relationship and has made our union even stronger.

I also dedicate this to my daughter, Kaitlyn who was only six years old when we moved here but adjusted happily because we have been together as a family. Thank you Kaitlyn for your happy, chirpy little voice each morning awakening us to your positive energy and busy little spirit.

Lastly, I dedicate this to my mother, Katie Violet Fiddler. Thanks for making me believe that I could do anything I wanted, if I worked for it. I believed you and have achieved my dream.
Chapter 1: Literature Review

1.1 Introduction

In 1891, W.D. Miller hypothesized a correlation between bacteria and apical periodontitis (Ring, 2002) that was later confirmed in 1965 (Kakehashi et al., 1965). Kakehashi showed that pulp necrosis would develop in rats that had germs within their root canal system but pulp necrosis did not occur in germ free rats. Later studies (Sundqvist G., 1979; Moller et al., 1981) further confirmed the bacterial etiology of apical periodontitis. It is now generally accepted that bacteria are the major etiologic factor in the development of apical periodontitis due to widespread evidence (Molander et al., 1998; Lin et al. 2006; Fabricus et al., 2006). Further, literature has shown that teeth obturated with negative bacterial cultures have a better healing prognosis than those obturated after a positive bacterial culture (Sjögren et al., 1997).

The fundamental aim of endodontic treatment is therefore the prevention or eradication of apical periodontitis followed by obturation of the canal system. Obturation with sealer and gutta-percha further reduces remaining microorganisms in two ways: by antimicrobial activity of the sealer (Heling et al., 1998; Siqueira et al., 2000) and by rendering them harmless by entombing them (Sundqvist et al., 1998), where the bacteria are deprived of nutrition and space to multiply. The eradication of apical periodontitis is achieved by removal of pulp tissue, bacteria, and bacterial byproducts. This is achieved in two key steps: mechanical debridement of the canal followed by canal irrigation.

Mechanical instrumentation is generally achieved through the used of hand and rotary instrumentation (Siqueira et al., 1999) and is critical to bacterial reduction of infected
canals, where instrumentation alone has been shown to reduce the number of microorganisms by up to 1000 fold (Byström & Sundqvist, 1981). Canals cannot, however, be rendered bacteria free with instrumentation alone (Ørstavik et al., 1991; Shuping et al., 2000; Young et al., 2007). The effectiveness of instrumentation in removing bacteria is hindered by the composition of the microbial flora (Kayaoglu et al., 2004), local factors which may favor bacterial growth (oxygen and nutrient availability), host defenses and whether or not the bacteria are mature and act synergistically (biofilm) (Siqueira et al., 2002). The complexity of the root canal system, with prevalent system types in different teeth and race, further decreases the outcome of mechanical instrumentation (Alavi et al., 2002; Gulabivala et al., 2005). In fact, up to thirty five percent of the canal walls are untouched after mechanical instrumentation (Byström et al., 1981; Peters et al., 2001; Mayer et al., 2002). An area of particular interest are the dentinal tubules as they offer bacteria a microenvironment beyond host defense mechanisms and beyond systemically administered antibiotics where they are able to thrive (Oguntebi, 1994; Love et al., 2002; Chivatxaranukul et al., 2008). Thus, the irrigation phase of canal cleaning is more important than ever. Fortunately, mechanical instrumentation of the canal shapes the canal and enlarges it so that irrigants can gain access to the canal to further reduce the bacterial load. The importance of mechanoochemical canal preparation was recognized even before Kakehashi documented the association between bacteria and apical periodontitis (Stewart, 1955).

Endodontic irrigation functions to remove dentinal debris generated by mechanical instrumentation, to dissolve inorganic and organic components from the canal and to aid in lubrication during instrumentation (Haapasalo, 2005). Of all irrigant functions its most
crucial purpose is the supplementary elimination of bacteria and bacterial remnants (such as the endotoxin of gram negative bacteria) from the canal. Together mechanical instrumentation and irrigation are the basis of mechanochemical preparation, which work to eliminate apical periodontitis.

Irrigation of the canal is achieved by instrumenting the canal to an apical size that allows for adequate irrigant access (Ram, 1977; Falk et al., 2005), by using the correct diameter of needle for the canal taper (Chow, 1983) so that the needle reaches the depth (Hsieh et al., 2007) where a large volume of irrigant that is frequently replenished can be used (Siqueira et al., 2002). Irrigation strategies designed to eliminate canal bacteria should also eliminate microorganisms within dentinal tubules as research has shown that canal bacteria are able to penetrate tubules to a depth of 1000 μm (Haapasalo et al., 1987). One such bacterium is Enterococcus faecalis, a gram-positive facultative anaerobe, which can produce a dense infection of the dentinal tubules and is associated with persistent apical inflammation (Sundqvist et al., 1998; Molander et al., 1998).

1.2 Endodontic Treatment

1.2.1. Mechanical Instrumentation

Technological advancements aimed at improving the cleaning and shaping of the canal have led to a movement away from the ISO standard 2 percent taper (0.02mm per mm) stainless steel hand instrument. New instruments (hand and rotary instruments) made with innovative metallurgical properties and novel designs have led to faster canal preparation-utilizing instruments possessing greater flexibility (Glossen et al., 1995; Young et al., 2007). In vitro studies show that NiTi instruments lead to more centered
preparations with less canal straightening, reducing the risk of iatrogenic complications due to the inflexibility of stainless steel files (Gambill et al., 1996; Baumann, 2004).

Canal instrumentation results in mechanical debridement of the canal and the removal of both vital and necrotic tissue. Instrumentation also provides a continuously tapering funnel from the coronal aspect of the canal to the apical constriction, which creates space for irrigants to work within the canal (Khademi et al., 2006; Zehnder et al., 2011). Ideally these irrigants will reach the lateral canals, intercanal connections, fins and deltas that are just some of the canal configurations that exist (Hess et al., 1925; Cambruzzi et al., 1983). Canal variations have been shown to have more influence on changes that occur during preparation than the instrumentation techniques themselves (Peters et al., 2001; Vertucci, 2005). This means that despite our best efforts to clean all parts of the canal, our instruments are unable to negotiate the canal complexities that harbor bacteria (Al-Ali et al., 2012).

Numerous studies show overwhelming evidence that none of our current mechanical instrumentation methods completely eradicate bacteria (Shuping et al., 2000; Siqueira et al., 2002). A study by Byström and Sundqvist (1981) showed that even after four treatments of mechanical instrumentation and irrigation with saline, bacteria remained in half of the cases. In fact, studies have shown that despite the newer and faster instruments on the market that have been designed with improved metallurgic properties and claims of better canal cleaning there is no significant difference that has been shown in the reduction of canal bacteria (Hülsmann et al., 1997; Siqueira et al., 1999; Yin et al., 2010).
For this reason, irrigation of the canal needs to be performed with thoughtful
diligence so that the correct type of irrigants are utilized (Berber et al., 2006;
Mohammadi et al., 2008; Zhang et al., 2010), in the correct way so as to optimize their
contact with microorganisms that remain in the canal after instrumentation and so that
they may reach areas of the canal untouched by instrumentation (Abou-Rass et al., 1981;
Palazzi et al., 2011).

1.2.2. Endodontic Irrigation

Endodontic irrigation flushes dentinal debris, formed during instrumentation, out of
the canal. Various irrigants work differently, some removing organic remnants and
others removing inorganic remnants from the canal. Irrigation also reduces canal
bacteria and the efficacy of bacterial reduction depends upon the type of irrigant used
(Camps et al., 2009; Wang et al., 2012).

During instrumentation a deposit of dentin filings and pulp tissue remnants is
produced along canal walls called smear layer (Torabinejad et al., 2002). This smear
layer can prevent irrigants from reaching the dentinal tubules but is easily penetrated
by bacteria and may offer protection to biofilms along the canal walls (Akpata et al.,
1982; Love et al., 2002). Further, the remaining smear layer may predispose root fillings
to leakage (Kouvas et al., 1998). Therefore, the ideal irrigant would be capable of
penetrating and removing smear layer, have the ability to effectively kill microbes
within dentinal tubules yet would be systemically nontoxic and not damaging to
periodontal tissues. Some of the ideal properties of an irrigant would include the
following:

• Facilitate debris removal from canal
• Act as a lubricant to reduce instrument friction during preparation
• Facilitate dentin removal (lubricant)
• Dissolve inorganic tissue (dentin)
• Penetrate to the canal periphery & into tubules
• Dissolve organic matter (dentin collagen, pulp tissue, biofilm)
• Kill bacteria and yeast (also in biofilm)
• Not irritate or damage vital periapical tissue (not caustic or cytotoxic)
• Not weaken tooth structure

(Haapasalo et al., 2010)

None of the irrigants available on the market possess all of these properties so different irrigants are used to achieve these aims. Commonly more than one irrigant is used, usually one after the other with the aim of reducing organic and inorganic matter and dentinal debris (Abbott et al., 1991; Yoshida et al., 1995). The most widely used irrigant is NaOCl as it satisfies more of the ideal irrigant properties than any other irrigant. NaOCl can dissolve organic and necrotic tissue (Naenni et al., 2004), is a broad-spectrum antiseptic (Zehnder, 2006) and can inactivate endotoxin (da Silva et al., 2004). NaOCl does not remove smear layer, however, and for that reason other irrigants are utilized. EDTA is one other irrigant commonly employed to remove smear layer so that dentinal tubules can be exposed (Hülsmann et al., 2003; Zehnder et al., 2005; Mello et al., 2010). There are numerous irrigants on the market and some of these include CHX, MTAD, H₂O₂ and Qmix. Further details about NaOCl will be discussed, as it was the irrigant used in this research study.
Because canal bacteria still persist after mechanochemical treatment of the canal, (Siqueira et al., 2000; Chavez et al., 2003; Sakamoto et al., 2007) adjunctive treatments have been sought as limitations of standard needle irrigation have been identified (Gu et al., 2009). Some of the drawbacks of manual needle irrigation include weak mechanical flushing action, inaccessibility to canal irregularities, delivery of irrigant limited to 1mm beyond needle tip (Ram, 1977), placement limited to canal taper (Chow, 1983), risk of apical extrusion (Hülsmann et al., 2000) and the inability to overcome the presence of gas bubbles ahead of the advancing irrigant front known as the ‘vapor lock effect’ (Gu et al., 2009; Tay et al., 2010). As a result, better irrigation methods have been sought.

In the early 1990’s, Keir reported improved canal debridement (Keir et al., 1990) when he utilized canal brushes in an active brushing and rotary motion. In that study the brush bristles were claimed to extend into canal fins and deltas not normally reached by instrumentation alone. Although the EndoBrush® was not able to reach full working length due to its size, it was one of many studies that encouraged research for the development of new irrigant agitation systems.

Root canal irrigation is either performed as a manual technique or with a machine-assisted agitation device. Manual irrigation techniques include positive pressure irrigation (commonly done with a syringe and side-vented needle), manual pumping of a gutta percha point in an irrigant filled canal also known as manual dynamic irrigation (MDI) (Huang et al., 2008, McGill et al., 2008) and the use of brush-covered needles (Ribeiro et al., 2011). Machine assisted agitation devices are numerous. Some of these mechanical systems include continuous irrigation during instrumentation, sonic (Sabins et al., 2003) and ultrasonic (Mayer et al., 2002) systems, apical negative pressure
devices (de Gregorio et al., 2010; Cohenca et al., 2010), laser methods of irrigation (de Groot et al., 2009; Peters et al., 2011) and electrochemically-activated solutions (Solovyeva et al., 2000; Marais et al., 2001).

Sonic and ultrasonic agitation methods, as well as apical negative pressure irrigation will be discussed in detail and compared with standard, side-vented needle irrigation as these irrigation methods pertain to the research at hand.

1.2.3. Effect of Agitation on Irrigation

Various techniques have been proposed for canal irrigation, some of them simply extruding irrigant into the canal and others used in different ways to agitate the irrigants with the goal of improving canal disinfection (Jiang et al., 2010; Jiang et al., 2012). Research has demonstrated that the constant renewal of irrigant with agitation cycles avoids saturation, precipitation of particles, and favors enhanced debris removal from the canal (Nadalin et al., 2009; Caron et al., 2010; Ribeiro et al., 2011).

The chemical removal of organic tissue by NaOCl occurs by the release of hypochlorous acid, which reacts to insoluble proteins by formation of soluble polypeptides, amino acids and other by-products (Baumgartner et al., 1987). Only by the regular exchange of the irrigant may this chemical reaction be enhanced. Large volumes of irrigant coupled with its frequent exchange not only removes superficial debris but replenishes the chemical activity of NaOCl which becomes depleted upon reacting with organic tissue (Baumgartner et al., 1987).

Sustained irrigant replenishment and mechanical agitation is directly related to the dissolution capacity of dental tissues by NaOCl, as has been demonstrated by research
With the obvious benefits of irrigant agitation, better debris removal and irrigant exchange, several new types of irrigant agitation systems have been developed. Ones pertinent to the research at hand will be discussed.

1.2.4. Sodium Hypochlorite

The most widely used irrigant in endodontics (Siqueira et al., 1998; Mohammadi et al., 2008), NaOCl degrades tissues by the following chemical reactions: chloramination, amino acid neutralization and saponification (Estrela et al., 2002; Gomes-Filho et al., 2008). To state it simply, NaOCl is capable of degrading organic tissue, neutralizing amino acids and breaking down fatty acids (which helps to reduce surface tension).

The use of NaOCl as a root canal irrigant is primarily responsible for the elimination of most microorganisms in an infected canal system (Ringel et al., 1982; Kuruvilla et al., 1998). In fact, it has been said that only NaOCl is capable of rendering bacteria nonviable and eradicating biofilm (Clegg et al., 2006). It is somewhat ironic that the most antibacterial irrigant in endodontics is also the most inexpensive irrigant (with a reasonably long shelf life) (Clarkson et al., 1998; Clarkson et al., 2001). Studies have verified that there is a significant reduction in canal bacteria after instrumentation with NaOCl compared with saline (Ørstavik et al., 1991; Shuping et al., 2000; Arias-Moliz et al., 2009).
1.2.4.1 Properties

The properties of NaOCl can be affected by changes to its pH (normally a pH of 12), concentration, exposure time, and temperature (Christensen et al., 2008; Stojicic et al., 2010). These factors, which affect NaOCl properties, can be summarized as follows:

• Lowering the pH of NaOCl below 7.5 results in reduced tissue dissolution (Christensen et al., 2008; Rossi-Fedele et al., 2011).

• Decreasing the concentration of NaOCl results in less tissue dissolution—the best antibacterial power is found at a concentration of 5.25% (Hand et al., 1978) or full strength (which is now 6%).

• Increased contact time results in better tissue dissolution (Moorer et al., 1982).

• Higher temperatures also lead to improved tissue dissolution (Berutti et al., 1996; Zou et al., 2010).

• Higher volumes and continuous replenishment are required as NaOCl becomes deactivated via the dissolution process (Baker et al., 1975; Moorer et al., 1982; Lima et al., 2001).

• Agitation of NaOCl leads to increased debridement and antimicrobial capabilities (Cameron et al., 1987; Sjögren et al., 1987).

Research has shown that the concentration of NaOCl is not a significant factor in terms of its antibacterial effect (Byström et al., 1985). More important are the volume, contact time, and its ability to reach pulp tissue and bacteria within the complex recesses of the canal anatomy (Haapasalo et al., 2010). Continuous replenishment and agitation further increase the efficacy of NaOCl yet are not a guarantee of complete microbial elimination of the canal (Lima et al., 2001).
1.2.4.2. Limitations

NaOCl has a high surface tension (75 dynes/cm), which may affect its ability to penetrate dentin (Giardino et al., 2006), does not remove the inorganic components of smear layer and can be ineffective versus resistant biofilm bacteria. Research has shown poorer in-vivo antimicrobial performance compared with in-vitro testing; diminished clinical performance is likely due to canal complexity and inactivating substances such as periapical exudate, pulp tissue, dentin and bacterial biomass (Haapasalo et al., 2007). Unlike other irrigants, NaOCl is not substantive (White et al., 1997) so it must be in contact with tissues or bacteria to be effective.

Some of the most concerning aspects of NaOCl are its toxicity and the potential complications that can occur with its extrusion into periradicular tissues (Hülsmann et al., 2000). NaOCl also has a detrimental effect on dentin elasticity and flexural strength (Sim et al., 2001; Marending et al., 2007), which may contribute to the development of vertical root fracture (Qian et al., 2011).

When NaOCl is mixed with CHX a brownish precipitate forms. Recent research has shown that this precipitate, one thought to be cytotoxic and possibly carcinogenic (Basrani et al., 2007; Basrani et al., 2010) is not parachloroanaline (Thomas et al., 2010); yet one should avoid mixing these reagents together as the resulting precipitate is difficult to remove and may hinder canal cleaning.
1.3 Methods of Irrigant Delivery

1.3.1. Needle Irrigation

1.3.1.1. Properties

Conventional irrigation with syringes is still widely accepted by both general practitioners and endodontists. This type of irrigation is accomplished through the use of various sized needles or cannulas that are used to dispense irrigant into the canal. Needles can be open-ended or closed-ended with side-vented channels (Kahn et al., 1995). Due to concerns about the risk of irrigant extrusion and doubts regarding fluid dynamic efficacy, side-vented needles were developed with a closed-end (Fig. 1.A.). It was hoped that side-vented needles would improve fluid hydrodynamics and be safer (Hauser et al., 2007). As with all needles, it is important that they not bind within the canal but instead that the needle remain loose during irrigation to allow debris to be flushed coronally and to prevent irrigant extrusion (Brown et al., 1995; Desai et al., 2009).

Figure 1. A. Side-Vented Needle.
Boutsoukis et al. (2007) IEJ Vol. 4(9).

Figure 1. B. ProRinse® Side-Vented Needle (with attached tubing)
Needles continue to be the most utilized method of irrigant delivery due to low cost and easy handling. One can easily determine the volume of irrigant delivered and the depth of needle placement (van der Sluis et al., 2006). ProRinse® side vented needles were used in this study (Fig. 1.B.)

1.3.1.2. Limitations

Despite the advantages of irrigant delivery via needles, needles are generally known to have a weak mechanical flushing action. The likelihood of needle delivered irrigants reaching isthmi, fins or deltas is slim making thorough debridement improbable (Nair et al., 2005; Susin et al., 2010). Studies have shown that irrigant is only delivered 1mm deeper than the tip of the needle (Boutsoukius et al., 2009)(Fig. 2), that the depth of needle placement is limited by the taper of the canal (Ram, 1977; Chow, 1983)(Fig. 3) and that needle irrigation is unable to overcome the vapor-lock phenomena at the apical part of the canal (de Gregorio et al., 2010)(Fig. 4 E).

Figure 2. Irrigant Flow Trajectories According to Velocity Magnitude. Boutsoukius et al. (2009) IEJ Vol. 42.
<table>
<thead>
<tr>
<th>Size of Canal</th>
<th>Size of Largest Needle that Could Reach the Apex</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>21 gauge</td>
</tr>
<tr>
<td>70</td>
<td>23 gauge</td>
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<tr>
<td>60</td>
<td>23 gauge</td>
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<td>30 gauge</td>
</tr>
<tr>
<td>20</td>
<td>30 gauge</td>
</tr>
<tr>
<td>15</td>
<td>Cannot be reached for any commonly available needle</td>
</tr>
</tbody>
</table>

Figure 3. Needle Placement Limited by Canal Taper. Chow (1983) JOE Vol. 9 (11).

Figure 4. Apical Vapor Lock Prevents Irrigant Flow Apically in Positive-Pressure Needle Irrigation (E). De Gregorio et al. (2010) JOE Vol. 36 (7).
Factors shown to improve syringe irrigant efficacy include: use of smaller gauge needles, large irrigant volume and closer approximation to the apex with placement (Sedgley et al., 2005; van der Sluis et al., 2006).

The degree of canal curvature is another constraint of needle irrigation. Canals with a high degree of curvature tend to be instrumented to a smaller size, limiting the apical placement of the syringe and the volume of irrigant reaching the apical third of the canal (Sedgley et al., 2005). As a result, efforts to find better methods of irrigant delivery have been explored and there are now numerous irrigant delivery systems on the market (Townsend et al., 2009) in addition to standard needle irrigation.

1.3.2. **EndoActivator**

1.3.2.1. **Properties**

The EA is a cordless, battery-powered handpiece with a sonic motor. The sonic motor provides options of 2,000, 6,000 and 10,000 cycles per minute (cpm) and there are activator tips in three sizes (15/.02, 25/.04, and 35/.04) (Figs. 5 & 6). The manufacturer recommends that the EA be used after the canal has been instrumented and irrigated by manual syringe irrigation. Tronstadt et al. (1985) was the first to report the use of a sonic instrument for endodontics. The tips of the EA are activated via sonic energy (6 kHz) in an irrigant filled canal for 30-60 seconds/canal (Jensen et al., 1999; Sabins et al., 2003).
The cleaning efficacy of the EA has been shown promising by research where it was shown to be equally effective as the ultrasonically activated irrigant system (Jensen et al., 1999). Other research has, however, shown it to be only marginally better than needle irrigation in canal debridement (Johnson et al., 2012). Regardless of its comparison to other irrigant agitation systems, sonically powered agitation devices are mainly regarded as an improvement over needle irrigation (Nair et al., 2010).

1.3.2.2. Limitations

Sonic instruments operate at a lower frequency (1-8 kHz) than ultrasonic instruments resulting in a reduced number of nodes travelling down the instrument Therefore; they produce smaller shear stresses (Ahmad et al., 1987) than ultrasonics. Additionally, the micro-acoustic streaming and cavitation seen with ultrasonic irrigant systems, is limited in sonic instruments (Huffaker et al., 2010). Studies of canals with a
higher degree of curvature restrict the movement and vibratory motion of the tips leading to less efficient cleaning (Walmsley et al., 1989).

1.3.3. ProUltra® PiezoFlow™

1.3.3.1. Ultrasonic Properties

Ultrasonics can be described as acoustic energy with a frequency that is greater than the upper limit of human hearing (> 20 kHz). This high frequency energy is used in endodontic treatment, after mechanical instrumentation, to transmit the energy from a file to an irrigant in the canal. The file oscillates within the irrigant creating cavitation and microacoustic streaming of the irrigant, which improves canal debridement (Gu et al., 2009)(Fig. 7).

Figure 7. Acoustic Streaming Around Ultrasonically Activated File In Water (left) and Schematic Drawing (right). Ahmad et al. (1987) JOE Vol. 13(10).
Richman first introduced the concept of utilizing ultrasonics in endodontics in 1957 (Richman, 1957), but it was Martin that first discovered a ‘sonosynergism’ of cleaning and disinfecting the canal system when irrigants were used in conjunction with ultrasonics (Martin, 1976). Files can be ultrasonically activated within the canal and can be active (cut dentin) or passive (no dentinal cutting), which is also known as passive ultrasonic irrigation (PUI). The files operate with transverse vibration and have a characteristic pattern of nodes and antinodes along their length (Walmsley, 1987; Walmsley et al., 1989). Currently, ultrasonics is only utilized to agitate irrigant as their use to cut dentin proved impossible to control (Lumley et al., 1993). Furthermore, PUI works best when it does not contact the canal wall at all as doing so results in a reduction of acoustic streaming and cavitation (Ahmad et al., 1987).

The beneficial effects of ultrasonics are primarily related to the large hydrodynamic shear stresses generated by the acoustic microstreaming that occurs around the oscillating file (Ahmad et al., 1987; Plotino et al., 2007). Transient cavitation also occurs with ultrasonics where high-speed flow gradients cause the creation of bubbles, which expand and then collapse. It is thought that such cavitation may contribute to canal debridement much like the benefits of cavitation seen in industrial ultrasound cleaning (Mohalkar et al., 2004). Sabins et al. (2003) found that ultrasonic agitation produced significantly cleaner canals than did sonic agitation. The enhanced flushing action by using ultrasound is well documented (Cunningham et al., 1982; Stock, 1991; Lumley et al., 1993) where more bacterial spores and dentin debris were removed during ultrasonic irrigation than hand irrigation (Gutarts et al., 2005; Burleson et al., 2007; Carver et al., 2007).
1.3.3.2. ProUltra® PiezoFlow™ Properties

PUS uses continuous ultrasonic irrigation (CUI) at 40 kHz for simultaneous continuous irrigant delivery and ultrasonic activation unlike passive ultrasonic irrigation (PUI), which requires intermittent replenishment of irrigant between ultrasonic file activations. The CUI device has a rigid irrigant delivery needle with an outside diameter of 0.5mm (equivalent to a size 50 file)(van der Sluis et al., 2007)(Fig. 9). Due to the large needle size, enlargement of the canal to a size 40 is required to prevent canal wall contact so that ultrasonic acoustic streaming can occur (Ahmad et al., 1987).

![Figure 8. ProUltra® PiezoFlow™ Ultrasonic System](image)

The benefits of continuous irrigant flow are to prevent root overheating which can be detrimental to paradental tissues, improved cleaning of the canal and enhanced tissue dissolution as the irrigant temperature increases (Cameron, 1988; Carrasco et al., 2004).

1.3.3.3. ProUltra® PiezoFlow™ Limitations

The reduced efficiency of ultrasonic irrigation has been documented in narrow, curved and less tapered canals (Krell et al., 1988; Stock, 1991); more remaining debris is
seen in curved canals versus straight canals after irrigation (Lee et al., 2004; van der Sluis et al., 2005). This remaining debris is the result of the rigid ultrasonic needle that is prevented from penetrating the canal more than 1 to 2 mm into the orifice before canal wall contact (Adcock et al., 2011; Malentacca et al., 2012). As a result, several studies have demonstrated the limited ability of the PUS to reduce canal bacteria and debris better than conventional needle irrigation. The restricted canal debridement can be attributed to needle rigidity, the frequency of curved canals and the inability to determine when the needle is contacting the canal wall (Heard et al., 1997; Mayer et al., 2002; Harrison et al., 2010).

Additionally, the debridement and cleansing effects of microacoustic streaming and cavitation only occur in liquid. The reaction between NaOCl and tissue results in the formation of gas bubbles, which become trapped in the apical third of the canal forming a fluid barrier known as apical vapor lock. This fluid barrier prevents cavitation and microacoustic streaming from taking place (Schoeffel, 2008). Only apical negative pressure systems are able to overcome the vapor lock phenomenon.

1.3.4. EndoVac®

1.3.4.1. Properties of Apical Negative Pressure

Until 2006 all irrigant systems on the market extruded irrigant outward, otherwise known as positive pressure irrigation. Up to this point, research was clear that close approximation of the cannula/needle to the working length would be critical for apical debridement of tissue, debris and bacteria. Naturally, this close positioning of the needle to the apical foramen was a concern and continues to be a concern to this day. The risk
of irrigant extrusion is high and the subsequent complications (pain, tissue edema, trismus, ecchymosis, tissue necrosis, hyperesthesia, paresthesia and nerve anesthesia) can be serious and in some cases permanent (Hülsmann et al., 2000).

In 2006, a novel irrigant system, called EndoVac® (EV) was introduced which utilized apical negative pressure (Fukumoto et al., 2006). This system utilizes a suction tip placed at the end of an irrigant filled canal, drawing irrigant down the canal to the suction tip, overcoming the vapor lock phenomenon and eliminating the risk of irrigant extrusion (Parente et al., 2010; Mitchell et al., 2010).

1.3.4.2. EndoVac® Properties

The EV consists of a combined delivery/evacuation tip also called the master delivery tip (MDT) that is attached to a syringe containing the irrigant and high-speed suction. Additionally, there are also two suctioning cannulas that can be placed to working length to suction fluid from the deepest part of the canal. Initially the macrocannula (ISO size 55) is used to suction large bits of debris remaining in the canal, which is then followed by the microcannula (ISO size 32). The cannula suction encourages the flow of the irrigant to travel down the canal where it is then suctioned in and then up the cannula. The canals must be shaped to a minimum size of 35 to accommodate the microcannula (Miller et al., 2010; Abarajithan et al., 2011). The microcannula contains 12 microscopic holes of 0.1mm diameter (Fig. 10).
Figure 9. EndoVac® Components
Macrocannula (Upper Left), Microcannula (Upper Right), Master Delivery Tip (Lower Left), and Microcannula (Lower Right). Haapasalo (2010) Dent Clin N Am., Vol. 54 (2).

Whether or not this new type of negative pressure irrigation system is superior to positive pressure devices is controversial. There is research indicating that the antimicrobial efficiency of EV is not any better than conventional needle irrigation (Brito et al., 2009; Miller et al., 2010; Pawar et al., 2012); yet other research states that EV clearly leads to greater canal cleaning (Nielsen et al., 2007; Shin et al., 2010; Siu et al., 2010). Some research simply states that at the very least EV improves the antimicrobial efficacy of irrigants (Hockett et al., 2008; Cohenca et al., 2010).

1.3.4.3. EndoVac® Limitations

The EV System has numerous components, which can be somewhat challenging to set up and includes various tubes and different cannulas. All components must be properly connected prior to usage.

There are also specific requirements with using the EV System prior to use or system fluid mechanics will not work appropriately and there is increased risk of irrigant
extrusion. These requirements include a minimum canal shape of 35/.04 to working length and the user must ensure the following is performed:

- The user must ensure there is an intact clinical crown with an access opening of at least 6-8mm from cavo-surface angle to pulp floor for proper fluid mechanics.
- The irrigant stream must be directed at an axial wall 45° from canals axial plane in molars, 60° in premolars and 90° in anterior teeth or positive pressure can be created in the pulp canal increasing risk of extrusion.
- The master delivery tip must never be placed closer than 5mm from the coronal opening of the pulp canal.
- The master delivery tip and macrocannula must be used before using the microcannula as the microcannula pores can be easily plugged with debris. If the microcannula becomes plugged a new one must be placed.

(Schoeffel, 2007; Schoeffel, 2008; Schoeffel, 2009)

1.4. Irrigation Dynamics

1.4.1. Apical Preparation Size/Taper

Over the years research has established that more bacteria can be removed from the canal when the irrigant (NaOCl) is delivered closer to the working length (Sedgley et al., 2005). Delivery in close proximity to the apical constriction can only be accomplished when the size or taper of the canal can accommodate the delivery device being utilized. Specifically, irrigation has been shown to be more efficacious with increased size and taper of the canal (Card et al., 2002; Falk et al., 2005).

Irrigant flow is limited by increased canal curvature. One study of canals with a 24 to 28 degree of curvature instrumented to a size 27/.04 showed remaining bacteria of
approximately 50% after irrigation (with NaOCl). When these canals were then instrumented to a size 46/.04 there was a significant improvement in irrigant efficacy (Nguy et al., 2006). It was discovered that increased canal curvatures cause irrigant flow to be impeded resulting in reducing flushing ability and decreased mechanical efficacy (Boutsioukis et al., 2010).

The relationship between apical canal taper and irrigant volume is clear. When there is a larger canal taper, more irrigant can be flushed apically (Baugh et al., 2005; Brunson et al., 2010). This allows more debris to be flushed from the canal, allows more irrigant exchange and faster tissue dissolution (Stojicic et al., 2010). Although an increased apical size allows for more effective irrigation, this is not always practical in a curved canal. Such canal enlargement increases the risk of root perforation, root fracture and results in weakened root structure (Albrecht et al., 2004; Lim et al., 1985). The canals in the teeth used in this research were instrumented with ProTaper® rotary NiTi files to a final size of F4 equivalent to an iso size 40/.06.

1.4.2. Irrigant Volume

NaOCl molecules that are involved in reactions to break down pulp tissue and bacteria are quickly consumed, resulting in a decline in activity. Therefore, continuous replenishment with fresh NaOCl is required to continue the tissue dissolution and to also assist with removal of dissolved tissue remnants (Clarkson et al., 2006; Stojicic et al., 2010).

The solvent action of NaOCl affects exposed pulp tissue through available chlorine molecules. Deeper tissues are protected from the effects of chlorine and are only dissolved once the tissues surrounding them are gone. As a result, new available
chlorine molecules are required through replenishment of NaOCl (Senia et al., 1971; Spanó et al., 2001) to reach successively deeper tissue layers.

1.4.3. Needle Size and Depth of Placement

The size of the irrigant delivery device is limited to the size of the canal at working length. Prior examination of the interrelationship between irrigant device and canal size has shown that insertion depth of the irrigation needle closer to its substrate is most favorable for canal irrigation (Shen et al., 2010). There is better fluid exchange that occurs with usage of smaller needles. The deeper penetration into the canal that is possible with smaller size needles results in better cleaning (Ram, 1977: Kahn et al., 1995).

There has been a shift of thought regarding mechanical instrumentation from its main role as one of primarily a debriding function, to one regarded more as a radicular access for irrigation (Huang et al., 2008; Bronnec et al., 2010). Irrigation devices relevant to this study and their size are shown in Figure 10.
1.4.4. Irrigant Flow Dynamics

The design of the needle tip influences flow pattern, flow velocity and apical wall pressure. These parameters are important for irrigation effectiveness and safety (Shen et al. 2010). Recent developments in computational fluid dynamics (CFD) technology had enabled these basic parameters to be simulated. This technology enables complex numerical simulation of canal irrigation so the effects of needle tip design can be studied (Hsieh et al., 2007; Boutsioukis et al., 2009).

CFD analysis has shown that the presence of a needle side vent results in a 17-19% reduction in apical pressure. Closure of the end in a side-vented needle further reduces apical pressure by a 2.5 to 3.0-fold decrease (Shen et al., 2010). Clearly, there is less risk of apical irrigant extrusion with closed-ended endodontic needles but there is a compromise that comes with increased safety. The irrigant only extends a short distance
(between 1-3mm) apically from the needle tip, due to the diminished apical pressure, when placed 3mm from the apex in a straight canal (Boutsoukis et al., 2009; Shen et al., 2010). This means that irrigants need to be delivered closer to working length (to be effective), which may abrogate the safety benefits of reduced apical pressure.

Irrigant velocity, alongside the needle, on canal walls shows variable patterns dependent on the needle design. The flow observed in side-vented needles on the opposing wall to the vent/opening was observed as very low, approaching zero. As a result of this reduced flow, the side opposing the vent was significantly less clean than the side facing the vent (Huang et al., 2008; Shen et al., 2010). The side adjacent to the opening, however, benefited from a higher Reynolds number (indicative of a more turbulent fluid flow) and a higher shear stress, which is conducive in removal of surface-adherent bacterial biofilm (Gulabivala et al., 2010).

1.4.5. Apical Patency

Studies have shown that maintaining apical patency with a size 10 file 1mm beyond the WL results in more canals having improved irrigant delivery to the apical third of the canal. It has been postulated that the patency file facilitates removal of the air bubble or vapor lock effect (Vera et al., 2011; Vera et al., 2012) thus allowing more irrigant to reach the full working length of the canal.

1.5 Dentinal Tubules

1.5.1. Dentinal Tubule Properties

With increasing age the number of patent tubules decreases at a statistically significant rate due to an increase in peritubular dentin that occurs over time (Bang and
The number of tubules also varies with location; there are statistically less tubules in the apical dentin compared with midroot, cervical or coronal dentin of the root (Carrigan et al., 1984)(Fig.12).

In the apical dentin advanced sclerotic changes are seen in the tubules where the peritubular dentin becomes more mineralized with increasing age (Love, 2004). This results in the ultimate obliteration of dentinal tubules. The relatively few dentinal tubules present in the apical dentin may explain the high success rate of endodontic therapy; there are fewer tubules in which microbes can live (Carrigan et al., 1984; Orstavik et al., 2004; Love, 2004; Kakoli et al., 2009).

1.5.2. Dye Penetration

Penetration of dye within the canal is better in the coronal and middle thirds due to a decrease in dentinal tubules from coronal dentin to apical dentin (near the pulp), ranging from 40,000/mm² in the coronal to 14,400/mm² in the apical. Additionally, the irregular structure of the secondary dentin and the presence of cementum-like tissue in
the apical root dentin (Mjör et al., 2001) may also contribute to the decreased dye penetration in apical region of the canal.

In older individuals, whom have more peritubular dentin (sclerosis), Thaler et al. found there to be decreased dentine permeability with age that did not follow a uniform pattern. As a result, dye penetration into the dentinal tubules was not equally distributed but found deeper in the bucco-lingual direction compared with the mesio-distal direction (Thaler et al., 2008)(Fig.12).

Figure 12. Irregular Dye Penetration Around the Canal
Light Microscope Pictures Showing Two Samples of Middle Root Sections
(a) Maximum Penetration bucco-lingually as sclerosis primarily occurs in the mesio-distal direction (b) Irregular Dye Penetration. Thaler et al. (2008) IEJ, Vol. 41(12).

1.6 Objectives

The aims of this study are to:

1. To compare the efficacy of irrigant agitation systems to conventional needle irrigation in the penetration depth of NaOCl into dentinal tubules at specific locations within the canal.
2. To understand how these agitation systems function.

3. To gain an understanding of the benefits and limitation of these agitation systems compared to standard needle irrigation.

4. To assess the clinical implications in usage of these devices.

1.7 Hypothesis

The use of irrigant agitation methods will be more effective in facilitating the deeper penetration of NaOCl into dentinal tubules than can occur with a conventional side-vented needle.
Chapter 2: Material and Methods

2.1 Experimental Design

This in vitro study was designed to compare the penetration depth of NaOCl into dentinal tubules using the EV, PUS, and the EA irrigation agitation systems and to compare them with the conventional needle irrigation. Two parameters were measured and analyzed: (1) the maximum penetration depth of NaOCl on the basis of technique and location within the canal, and (2) the number of surfaces of the canal wall affected by NaOCl penetration compared with the number of surfaces penetrated by dye.

2.2 Tooth Selection and Preparation

This study was conducted using non-endodontically treated extracted human teeth (maxillary 1st premolars, canines, lateral incisors, central incisors and mandibular canines) that had mature apices and intact crowns. Sixty teeth were selected. The root curvatures were measured externally against a protractor and teeth with curvatures less than 15 degrees were chosen. The root lengths ranged between 14 to 24mm and the width of the roots at the CEJ was between 4-8mm; both were measured with a ruler. Only canals where apical patency could be achieved were selected. The teeth were stored in a 0.01% NaOCl solution at room temperature.

A standard access cavity preparation was made in each tooth and the working length determined by insertion of a size 10 stainless-steel K file (Dentsply Maillefer, Tulsa, OK) into the canal until the instrument tip was just visible at the apical foramen. A barbed broach (Dentsply Tulsa Dental) was used to remove the bulk of the pulp tissue. Canal patency was verified by a squirt of sterile water from the apical foramen. The outer root
surface was then wrapped with latex rubber (to create a seal between the root and the suction device) and then placed over a high-speed suction and crystal violet dye (BD Diagnostic Systems, Sparks, MD) was syringed into the canal with a needle. This was done until the dye was seen flowing from the apical end of each root for 5 seconds. The teeth were then immersed in dye for five days. After 5 days, the teeth were removed from the dye and rinsed in tap water for 10 minutes. The teeth were wiped dry, the foramen sealed with laboratory wax and the roots were painted with varnish to create a closed system simulating the clinical situation.

The canals were then instrumented using ProTaper® rotary niti files (S1-S2-F1-F2-F3-F4) (Dentsply Tulsa Dental) using a crown-down technique. Each file was used for 30 seconds. Prior to each instrument change, 1ml of NaOCl was placed in the canal using a 30 gauge side-vented needle (ProRinse®, Dentsply Tulsa Dental). The total time of instrumentation was 3 minutes and the total volume of 6% NaOCl was 6 ml. The final apical size was F4 (40/.06).

2.3 Experimental Groups

The teeth were randomly assigned to four experimental groups. Each experimental group was irrigated with one of four irrigant methods using 6% NaOCl. A digital peristaltic pump (Reglo Digital MS-2/8, Ismatec®, Wertheim-Mondfelt, Germany) was utilized to deliver the irrigant.

- Group 1: (n=15): EndoActivator®. The teeth were irrigated with a side vented needle at a rate 2.5ml/15 sec placed 1mm from working length and then sonically agitated for 30 seconds with the EA placed 1 mm from working length. The size 25/.04
polymer tip was used at 10,000 cpm. This was performed two times. Volume of NaOCl: 5ml.

• Group 2: (n=15): ProUltra® PiezoFlow™ System. The teeth were irrigated with the PUS at a rate 15ml/min for 1 min, inserted 75% to working length. The ultrasonic energy and the irrigant dispensing occurred simultaneously; continuous ultrasonic irrigation (CUI). Volume of NaOCl: 15ml.

• Group 3: (n=15): EndoVac®. The teeth were irrigated with apical negative pressure using the MDT and the macrocannula for 5ml/min for 1 min, slight up and down motion, placed just short of binding. Volume of NaOCl: 5ml.

• Group 4: (n=15): ProRinse® side-vented needle. The teeth were irrigated with positive pressure at a rate of 5ml of 6% NaOCl/min for 1 min. The needle was placed 1 mm from working length. Volume of NaOCl: 5ml.

The volume of 6% NaOCl used with irrigant method was not standardized but instead followed manufacturers recommendations for flow rate and irrigant volume. Therefore, the total volume of NaOCl used with the EA, EV & N experimental groups was 11ml while the total volume of NaOCl used with PUS experimental group was 21ml.

The teeth were then rinsed in tap water and dried with paper towel. The coronal portion of each tooth was mounted in acrylic resin to facilitate sectioning.


2.4 Sectioning of the Teeth

The acrylic end of each sample was mounted in the Isomet® 5000 Linear Precision Saw (Buehler, Illinois, USA)(Fig. 13) and sectioned in 1 mm thick slices perpendicular to the long axis of the tooth. The first sample was the apical most sample and the last sample was the most coronal sample. Each section was placed in a plastic sample vial and labeled by tooth number and section such that the tooth number and section were easily identifiable.

Figure 13. Isomet® 5000 Linear Precision Saw

2.5 Microscopic Evaluation

Each section was positioned on a microscope slide and evaluated with the Nikon® Eclipse® Microscope (Nikon Instruments, Tokyo, Japan)(Fig.14) at 40 times magnification. The apical side of each sample was evaluated and photographed with the NIS Elements™ Software (Nikon Instruments, Tokyo, Japan).
The penetration of NaOCl was measured with the NIS-Elements™ software. The measurement was made in micrometer (µm) units for all four groups at levels 3, 5 and 7mm from working length (WL) for the apical surface of each sample (Fig. 15). The samples were identified by location in the canal; samples 3, 5, and 7mm from WL were called apical, middle and coronal, respectively.

Figure 15. NIS Elements™ Photographs at Magnification (40x) at 3mm (A), 5mm(B) and 7mm (C) from working length.
2.6 Quadrant Evaluation

Quadrants were defined as mesial, distal, buccal and lingual quadrants; each quadrant was measured as a sector equal to one quarter of a circle (Fig. 16). The quadrants that were affected by NaOCl were defined as the quadrants out of four that were visibly bleached white. The quadrants that were penetrated by dye were defined as quadrants out of four that were visibly dyed purple.

Percentage of quadrants affected by bleach was calculated as:
The number of quadrants affected by NaOCl / number of quadrants penetrated by dye.
Figure 17. Experimental Flowchart

60 extracted teeth
Canals accessed, pulp tissue removed & dye placed in canals for 5 days

Teeth removed from dye & instrumented to 40:04 with 8ml of 6% NaOCl

Teeth randomly assigned to one of four experimental groups

EndoActivator
5ml of 6% NaOCl

PiezoFlow
15ml of 6% NaOCl

EndoVac
5ml of 6% NaOCl

Needle
5ml of 6% NaOCl

Teeth sectioned in 1mm thick sections perpendicular to long axis

Sections evaluated with microscope at 40x magnification and NaOCl penetration measured

Sections visually analyzed for number of quadrants affected by NaOCl penetration out of quadrants dyed
2.7 Statistical Analysis

Statistical analysis was performed in SPSS 16.0 (SPSS Inc. Chicago, IL, USA) for Windows.

The experimental groups were statistically compared for dye penetration to determine if there were significant differences between them. The Pearson Chi-Square test was performed for each experimental group based on location (coronal, middle or apical) in the canal.

Means and standard deviations of maximum NaOCl penetration depth were calculated and recorded. Homogeneity of variance was determined using Levene’s test. Two-way ANOVA was applied to evaluate the maximum penetration depth at each location in the canal with location and technique as factors. A significance level of 0.05 was used. One-Way ANOVA with post hoc analysis with LSD adjustment were made within each group to obtain further sub-group comparisons (e.g. EV coronal versus EV apical).

For the affected quadrant analysis, the penetration areas of NaOCl (quadrants bleached white/quadrants stained by dye) were scored from 0 (no penetration) to 4 (four areas penetration) as ordinal data. The Pearson Chi-square test with Cochran-Mantel-Haenszel (CMH) method was used to test for significant differences among treatment groups separately at each location of the canal and for all different treatments. A significance level of 0.05 was used.
Chapter 3: Results

3.1 Comparison of Dyed Quadrants for Experimental Groups

The four experimental groups were compared for the number of quadrants that exhibited dye penetration. This step confirmed there were no statistically significant differences in dye penetration between the groups, so that comparisons could be made between them. The chi-square test was performed comparing each group by location in the canal (coronal, middle, and apical). The p values for each location were >0.05 indicating that there was no statistically significant difference in dye penetration between the groups based on location (coronal, middle or apical) in the canal. The Chi-Square Test results by location:

- Coronal (comparing all four experimental groups) (p=0.764)
- Middle (comparing all four experimental groups) (p=0.291)
- Apical (comparing all four experimental groups) (p=0.535)

A comparison of the quadrant dye penetration for each location (coronal, middle and apical) as well as surface, which presented dye penetration, is shown in Tables 1,2, 3 & 4 and Figure 18.

Table 1. Experimental Groups-Dyed Quadrants by Location & Surface.
Table 2. Experimental Groups-Dyed Quadrants in Coronal Location by Location & Surface

<table>
<thead>
<tr>
<th>CORONAL LOCATION</th>
<th>Total Surfaces (out of 60)</th>
<th>Dyed Surfaces (out of 15 surfaces)</th>
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<td>Group</td>
<td>m</td>
<td>d</td>
</tr>
<tr>
<td>N</td>
<td>59</td>
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</tr>
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<td>EA</td>
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<td>EV</td>
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<tr>
<td>PUS</td>
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<td>13</td>
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Table 3. Experimental Groups-Dyed Quadrants in Middle Location by Surface

<table>
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<th>MIDDLE LOCATION</th>
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<th>Dyed Surfaces (out of 15 surfaces)</th>
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<td>43</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>PUS</td>
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Table 4. Experimental Groups-Dyed Quadrants in Apical Location by Surface

<table>
<thead>
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<th>EV</th>
<th>PUS</th>
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</table>

Figure 18. Number of Dyed Quadrants (out of 60) by Location in the Canal
3.2 Maximum Penetration Depth of NaOCl by Location and Group

The penetration depth was only calculated for the quadrants which had dye penetration. The means and standard deviations of maximum NaOCl penetration depth were calculated (Table 2). The groups were tested for homogeneity of variances using the Levene’s Test (p=0.210), which indicated that the variances were homogenously distributed. Since the data was normally distributed, parametric tests were used to compare the groups.

The Two-Way ANOVA was performed to compare the experimental groups considering location (coronal, middle, apical) and experimental groups (N, EA, PUS, EV) as factors.

Table 5. Maximum Mean Penetration Depth by Group and Location

<table>
<thead>
<tr>
<th>PENETRATION DEPTH MEANS &amp; STANDARD DEVIATION</th>
<th>N</th>
<th>EA</th>
<th>EV</th>
<th>PUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORONAL</td>
<td>341.7± 209.16</td>
<td>340.2± 232.77</td>
<td>298.8± 225.60*</td>
<td>179.1± 85.86</td>
</tr>
<tr>
<td>MIDDLE</td>
<td>302.5± 146.07</td>
<td>246.9± 145.93</td>
<td>234.3± 134.76</td>
<td>264.5± 135.63</td>
</tr>
<tr>
<td>APICAL</td>
<td>197.9± 98.47</td>
<td>215.6± 150.38</td>
<td>143.5± 126.07*</td>
<td>201.4± 124.24</td>
</tr>
</tbody>
</table>

* Post hoc tests (p<0.05) - significant difference between maximum NaOCl penetration depth for EV at coronal and apical locations
3.2.1. Differences by Location

The maximum mean penetration depth of NaOCl for each group and location is shown in Figure 19. When comparing the penetration by location, only the EV group showed statistically significant differences. Post hoc test were performed (p<0.05) and the results indicated statistically significant differences in the maximum penetration depth of NaOCl for the EV group between the coronal and apical locations (indicated by * in Table 2). Despite the non-significance of the differences between locations, data shows that in general, there is a higher penetration at the coronal level, followed by middle and then apical. This phenomenon was only different for the PUS group, where the middle area was the one with the highest penetration followed by the apical and then coronal, as seen in Figure 18.

![Figure 19. Maximum Mean Penetration Depth by Group and Location](image)
3.2.2. Differences by Technique

The maximum mean penetration depth by group shows that the highest mean penetration was found for the N, followed by EA, then EV and finally the PUS. When comparing these groups by location, we found a p-value greater than 0.05 (p=0.215) showing that the difference between the experimental groups was not statistically significant.

The One-Way ANOVA (p > 0.05) was performed to evaluate subgroup comparisons. The median penetration depth was calculated for each group (N, EA, EV, PUS), all locations combined. Although all locations were combined there was still no statistically significant difference between groups. These results may be due to the higher variability within each group as seen by the large confidence intervals indicating greater levels of variance within the penetration depth for each group (Figure 20).

![Figure 20. Median NaOCl penetration by Technique](image-url)
3.3. NaOCl Affected Quadrants by Group Compared

Overall, the technique that resulted in the most NaOCl affected (bleached white) quadrants by location was determined by counting the number of NaOCl affected quadrants and comparing them to the number of dyed quadrants (stained purple). The highest number of quadrants bleached white (compared to dyed quadrants) occurred in the coronal with the EA, in the middle third with the EV and in the apical with the EA. These findings can be seen in Table 3 as percentage penetration calculated as percentage of bleached /dyed quadrants and is shown in Figure 21.

Table 6. Percentage NaOCl Penetration by Group & Location

<table>
<thead>
<tr>
<th>Percentage NaOCl Penetration (# NaOCl quadrants/#Dyed Quadrants)</th>
<th>NaOCl Quadrants</th>
<th>Dyed Quadrants</th>
<th>Percentage Penetration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>EA</td>
<td>EV</td>
</tr>
<tr>
<td>CORONAL</td>
<td>48</td>
<td>50</td>
<td>46</td>
</tr>
<tr>
<td>MIDDLE</td>
<td>44</td>
<td>44</td>
<td>40</td>
</tr>
<tr>
<td>APICAL</td>
<td>20</td>
<td>30</td>
<td>15</td>
</tr>
</tbody>
</table>

Figure 21. NaOCl Affected Quadrants by Group & Location
The Pearson Chi-square test was used to compare experimental groups by location within the canal (coronal, middle, apical) (p=0.872) and then by technique (N, EA, PUS, EV) (p=0.675). This test shows that there was no statistically significant difference in percentage penetration (NaOCl affected quadrants/dyed quadrants) between groups or by location within the canal.
Chapter 4: Discussion

The present study was conducted to compare the effect of agitation on the penetration depth of NaOCl into dentinal tubules at a microscopic level. To date there have been very few studies that have evaluated the penetration of NaOCl into dentinal tubules at a microscopic level. In 2010, Zou et al. did a study examining the penetration of irrigants (NaOCl) into dentinal tubules without the use of agitation. A microscopic examination was performed and the maximum penetration depth of NaOCl into dentinal tubules was 300 μm. Prior research has shown that E. faecalis can, however, penetrate dentinal tubules to up to a depth of 1000 μm (Haapasalo et al., 1987). Therefore, adjuncts to standard needle irrigation have been sought. Agitation of the irrigant has been shown to enhance the removal of canal debris and to reduce irrigant saturation (Nadalin et al., 2009; Ribeiro et al., 2011). Therefore, the current microscopic study was performed to ascertain if agitation would improve the penetration depth of NaOCl in dentinal tubules. Our study was created to simulate the clinical use of commonly utilized irrigant agitation systems and to compare them with standard needle irrigation in terms of NaOCl penetration depth. The access openings made in the teeth simulated the size and shape of the access openings made in vivo; the access cavities were able to accommodate instrumentation files without binding along canal walls and provided space to act as a reservoir for canal irrigant. This reservoir allowed a volume of irrigant to be available for replenishment, which is clinically relevant for proper tissue dissolution (Druttman and Stock, 1989). Many studies remove the coronal and/or apical sections of the extracted tooth, which would have an impact on the replenishments of irrigants in the canal. To avoid these problems, in the current study, the apical end of each root was covered with wax to create
a closed environment to simulate a typical clinical setting. In vivo there are tissues surrounding the root and as a result the canal behaves as a closed-end channel; this results in the formation of a vapor lock in the apical end of the canal during irrigation (Tay et al., 2010). By closing the end of each canal, as we did perform in our study, all of the teeth had limited irrigant replacement in the apical part of the canal as occurs with a closed system and the canals were as earlier stated standardized and like in vivo parameters.

In the present study, the deepest mean NaOCl penetration in the coronal and middle locations occurred with N; the deepest mean NaOCl penetration in the apical location occurred with the EA. With all irrigant methods, the best mean penetration depth occurred coronally, followed by the middle, then the apical location. Despite these differences, none of this data showed a statistically significant difference. A study with the same methodology and a larger sample size may be required to identify some changes as the data showed a tendency to this similar pattern. In a study by Nair et al. (2010), similar results were found, where all methods showed the best debris removal in the coronal third, followed by the middle third, then finally the apical third. Nair et al. examined debris removal in the canal after using the EA, F-files (plastic polymer rotary file) and passive ultrasonic activation (PUS). The EA cleaned the apical, middle and coronal thirds of the canal better than the other irrigant methods. The EA was significantly better in the apical and middle thirds for debris removal than the F-files. It should be noted that the EA is only utilized after standard needle irrigation. Another study by Pawar et al. (2012) demonstrated that antimicrobial efficacy of EV comparable to that of standard needle irrigation. Further, Johnson et al. (2012) showed that sonic irrigation to be no better for
debris debridement than standard needle irrigation. The result of this study, which supports the use of standard needle irrigation, is clearly supported by prior research studies.

The finding of our current study showed that N delivery of irrigant is comparable to that of EV, EA and PUS in terms of irrigant penetration in dentinal tubules may be explained by limitations that occur with irrigation of teeth in general. Retrospectively, we hypothesize that the age of the teeth were an important factor limiting dye penetration. Previous research has shown that with increasing age there is increased peritubular dentin that can impede dye and irrigant penetration within dentinal tubules (Bang et al., 1970; Love, 2004). Further, research has also shown that there is a higher level of sclerosis in some racial groups (Malaysian), which would also lead to less NaOCl penetration (Whittaker et al., 1996). The inability to select teeth of similar age (and tubule sclerosis) may have biased our groups in term of irrigant penetration, which is a limitation of the analysis of the penetration depth results of this research. We hypothesize that a smaller particle size other than crystal violet dye could be used to improve the current methodology.

The second consideration is that it is not possible to standardize the solutions extracted teeth have been stored in prior to being donated. Teeth left in solutions with a pH that is not neutral for long periods of time will have variable dentin permeability (Correr et al., 2006), which could have affected the penetration of both dye and NaOCl. This may have nullified the results of this study. Further, the variation in canal morphology (Vertucci, 2005) found within teeth also makes it difficult to standardize teeth; after instrumenting ovoid and round canals there is differing amounts of remaining
debris in the canal (Paqué et al., 2010) which can impede irrigant penetration. Therefore, teeth instrumented with more ovoid shaped canals may have had less irrigant penetration due to higher debris levels in the canal. This is also a limitation of our study and results should be analyzed carefully. Still, we believe that studies using natural teeth have a high value and are likely closer to clinical practice.

An interesting finding for the PUS was the deepest penetration occurring in the middle location unlike other studies that have shown comparative penetration in the coronal and middle third for the PUS (Castelo-Baz et al., 2012). In the research performed by Castelo-Baz et al. (2012) the PUS was moved in an up and down motion throughout the irrigation procedure which is most likely why the penetration of irrigant was similar 6 mm and 4 mm from working length. In our research study the PUS was held at one position throughout irrigation, which is likely why the irrigant penetration at 7 mm was not as deep as was observed at 5 mm from working length. In addition the PUS was the only group to utilize 21 mm of NaOCl (as per manufacturer’s recommendations) while the other groups only received 11 mm of NaOCl. This larger volume of irrigant, held at one position, was likely the reason that the PUS showed the deepest penetration in the middle location.

When we analyzed the dye and NaOCl penetration by quadrant the results were interesting. The NaOCl affected quadrant calculation was determined by comparing the number of irrigant-penetrated quadrants/number of quadrants dyed. The quadrants were defined as mesial, distal, buccal and lingual quadrants; each quadrant was measured as a sector equal to one quarter of a circle. In this study, there was no significant difference in percentage penetration depth between irrigant methods. This non-
significant finding may be due to the way in which the quadrants were examined where the placement of the circle over the microscopic photos was subjective in that the quadrants may have been shifted slightly to the right or left for each tooth (from the line angles joining the four surfaces-mesial, buccal, distal, lingual). This would have resulted in the percentage penetration score calculation that less accurate. Another limitation was the irregular penetration of crystal violet dye and the deeper penetration found in the bucco-lingual direction compared with the mesio-distal direction, which was also seen in a previous study (Paqué et al., 2006). This lack of equal penetration in the apical root sections has been previously correlated to advanced age (Thaler et al., 2008), race (Whittaker et al., 1996) and less numerous and smaller dentinal tubules in this region. Irrigants in the canal are able to penetrate dentinal tubules, which are filled with liquid, by passive diffusion (Spreter von Kreudenstein & Stüben, 1955). Diffusion through the dentinal tubules is controlled by physical factors including dentinal diffusional surface area, dentin thickness, temperature, widening of tubules and by the size, charge, concentration and water or lipid solubility of the diffusing substance (Galvan et al., 1994). As a result, it was not possible to measure the NaOCl in the apical 3mm of each root and in some of the mesial and distal quadrants more coronally located where sclerosis prevented dye penetration. Future studies using dye penetration may not be suitable for evaluating irrigant penetration in the apical third of the root unless the donor age is known so that the apical sclerosis/dye penetration can be standardized. Although the apical part of the canal did not allow dye penetration, the lack of penetrability in this area may also suggest less penetration of microorganisms in this region as well. It has been reasoned by some research that the increasing dentinal sclerosis that occurs with time
may be one reason for better clinical outcomes of root canal treatment in older patients (Ørstavik et al., 2004). Perhaps irrigation in the apical third of the canal is not as critical as once presumed (Vasiliadis et al., 1983; Mjor et al., 2001). Our results would agree, since there was a smaller number of quadrants penetrated with dye, which would also mean sclerotic and less penetrated by bacteria.

In addition, research has shown decreased ultimate tensile strength (UTS) of dentin in areas of increased peritubular dentin (as occurs with aging), leading to increased brittleness (Arola et al., 2009). The increased mineralization seen with aging results in diminished crack growth resistance (Koester et al., 2008). This may be the reason vertical root fractures (VRF) start in the apical part of the root. We hypothesize, based on the various mesial and distal quadrants, which were not penetrated by dye, that this could have an impact on fracture direction. It would be interesting to see future studies comparing dentinal properties of buccal/lingual dentin to mesial/distal dentin to see why VRFs are more common in the buccolinguinal direction where there is less sclerosis and brittleness than occurs mesiodistally. One would speculate that there would be more strength in the buccal and lingual dentin where there is less brittleness, but this is not the case.

The results of this study conflicts with the findings of other studies (Townsend et al., 2009; Shin et al., 2010; Jiang et al., 2012), where one irrigant method has been demonstrated to be more effective than the other methods in that study. Such results, indicating one irrigant method to be the most efficacious, may be caused by the challenges encountered with irrigation studies. The evaluation of irrigant methods is difficult and studies are problematic to compare since research methodologies determining irrigant
efficacy have not been established. As a result, irrigation studies measure a plethora of irrigant parameters; where each parameter is identified as an indicator for irrigant effectiveness. These measured parameters include debris removal (Ribeiro et al., 2012), microbial reduction (Pawar et al., 2012), tissue dissolution (Malentacca et al., 2012), fluid dynamics (Boutsioukis et al., 2010) and irrigant penetration (Zou et al., 2009) within the canal. In addition to these various parameters, studies utilize varying volumes of irrigant, different irrigant types and variable irrigation/agitation times. As a result, every study must be examined closely to see what parameters are being measured to identify the study’s clinical relevance. For example, the microcannula of the EV can become easily plugged. Since the microcannula is used at working length to aspirate irrigant, it is important to ensure the small pores (0.1mm diameter) remain free of debris. Some studies have reported the EV as an effective method of irrigation (Nielsen et al., 2007) in terms of facilitating the best debris removal compared to other irrigant methods while other studies report the EV to be no better than standard N irrigation (Brito et al., 2009) in the reduction of intracanal bacteria. Although each of these studies had three cycles of microirrigation, neither mentioned checking the pores for clogging. It can be speculated that the EV system may have performed better in one study than another simply due to the amount of debris remaining in the canal, which may have caused pore clogging, during microirrigation. As a result, it is hard to ascertain if the microcannula is free of debris during microirrigation between research studies.
Chapter 5: Conclusion

This study supports the use of needle irrigation with the use of the EA (sonic agitation method) to enhance irrigation in the apical third. The study of endodontic irrigation is evolving with newer methods being developed with the intent of reaching the fins, apical ramifications and deltas that are difficult to reach by standard needle irrigation alone. The effectiveness of these new systems is difficult to ascertain as the standardization of research methods is challenging and our reliance on in vitro testing, where we can never be completely confident that the results will be conveyed to the clinical setting. It can be said however, that it is clear that the simpler the irrigation system the better. A recent survey by the American Association of Endodontists (AAE) found that only forty five percent of members said they used agitation adjuncts to enhance irrigation efficacy (Dutner et al., 2012). Clearly, simpler agitation methods, rather than more costly and complex ones, may have a chance of reaching the hands of the remaining fifty five percent of AAE members who do not currently use agitation with irrigation at all. The EV system is not only cumbersome to use, requiring at least two people to hold all the devices, it is costly since the microcannula can become easily clogged. The ProUltra Piezoflow is good in theory, as the cavitation and microacoustic streaming have been shown effective in cleaning the canal system (especially in the isthmuses and canal ramifications). The benefits of acoustic streaming and cavitation, however, quickly become diminished or prevented when the needle contacts the canal wall. It is well known that the most commonly treated teeth in endodontics are the posterior teeth and few of these have straight canals. Therefore, if you cannot feel the needle touching the wall you are really using a needle at only 75% to working length. As for the
EndoActivator, this system goes hand in hand with needle delivery of the irrigant. The flexible polymer tips of the EA easily follow the canal curvature without instrumenting the canal and sends sonic energy into the canal for further debris removal in the apical third. Clearly the EA and N go together and are affordable, easy to use and efficacious in cleaning the canal.

The field of endodontic irrigation continues to expand with novel irrigation methods including the use of nanoparticles. Chitosan nanoparticulates and zinc oxide nanoparticulates have demonstrated significant antibiofilm properties lasting up to three months (Shrestha et al., 2010) in duration. These nanoparticles can be delivered to dental hard tissue by high intensity focused ultrasound (HIFU)(Ohl et al., 2010) and recent research has reported that nanoparticles can also reinforce dentin collagen (Shrestha et al., 2012). Since nanoparticles can be delivered by ultrasonic energy it would be worthwhile to evaluate the delivery of nanoparticles by sonic energy (such as the EA). Still, these methods have to go through various studies with a variety of methodologies. As shown in our current study, small changes in the methodology and the use of real teeth may show that new technologies are sometimes only more cumbersome and expensive, with the same final efficiency. As mentioned before, the easier the irrigant delivery system is to use, the more likely it is to be used.
References


Ohl, S. W., Khoo, B. C., Kishen, A. (2010). Characterizing bubble dynamics created by high intensity focused ultrasound for the delivery of antibacterial nanoparticles into a dental hard tissue. Journal of Engineering in Medicine, 224(11), 1285-1296.


