# Effect of Endodontic Irrigation Protocols on Dentin Wettability and Tubule Penetration of Calcium Silicate Sealer

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## DEDICATION

This thesis is dedicated to my wife, Rachel. I can never sufficiently express my gratitude for the grace and unhesitating conviction with which you sacrificed so much to support me in this endeavor.

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# INTRODUCTION

Microbial irritants are understood to be the primary cause for the demise of the pulp and development of apical periodontitis. Host reaction to a variety of microbial factors results in local inflammation, breakdown of the attachment apparatus, and the development of pathologic entities described histologically as periapical cysts and granulomas. Elimination of these essential pro-inflammatory microbial factors is the overarching objective of endodontic therapy.

Chemomechanical preparation is considered the most important step in management of the infected root canal system, yet even with subsequent use of intracanal calcium hydroxide medicament, the canal cannot be predictably sterilized(1). Obturation of the root canal system is therefore indicated to eliminate avenues of leakage from the oral cavity or apical tissues into the root canal system and to seal within the system any irritants that cannot be fully removed during the canal cleaning and shaping procedures.

Endodontic sealers are employed to fill voids between the core obturation material and the canal wall. This interface has long been considered to be of critical importance to the outcome of endodontic procedures. Attempts have been made to optimize this interface through application of various chelating agents, surfactants, and alcohol-containing solutions(2) to root canal dentin prior to canal obturation(2–4).

The effect of these treatments on bond strength and dye leakage when used with legacy zinc oxide eugenol and epoxy-based materials have been well-characterized, and use of a so-called “final rinse protocol” is common practice in clinical endodontics(5, 6). Less is known about the effect of these regimens on penetration of sealer into the dentinal

tubules. The correlation between tubule penetration and in vitro leakage models has been controversial, however intercalation of the sealer into the microsurface of the dentin forms a physical barrier, improves retention, and entombs residual bacteria(7). The ability to extend the antimicrobial effects into infected dentin may likewise be especially beneficial to destroy bacterial located there(8). These properties are thought to be a particularly important parameter in the clinical efficacy of an emerging class of calcium silicate-based sealers due to lack of chemical adhesion to the canal wall.

It has been postulated that wettability of dentin can be increased through application of surfactant- or alcohol-containing solutions prior to obturation, facilitating deeper penetration of endodontic sealer into the tubules. The purpose of this investigation was to determine the effect of surfactants and ethanol on the wettability of dentin by a calcium silicate-based sealer, and to correlate those finding with penetration of sealer into dentinal tubules under simulated clinical conditions.

## REVIEW OF LITERATURE & BACKGROUND

Classically, the broad objectives of root canal therapy have been described as debridement, sterilization, and obturation of the root canal space(9). Debridement is accomplished through the use of specialized instruments which mechanically prepare the root canal space, removing the pulp and pulpal decomposition products, as well as a significant portion of the radicular bioburden. Yet anatomic constraints and the propensity of endodontic pathogens to colonize the radicular dentin to considerable distances prevent this mechanical approach from representing a comprehensive solution(1).

### Irrigation

The tenet of "sterilization," then, requires that adjunctive, and less destructive, antimicrobial measures be employed. The concept of chemomechanical preparation of the root canal system involves the use of endodontic irrigation solutions to remove inorganic and organic debris, remove microorganisms, and provide lubrication for instruments during mechanical preparation.

Many solutions have been used, but sodium hypochlorite has proved the most effective and widely used solution for irrigation of the root canal space. It is unmatched among commonly used irrigants in its capacity to dissolve organic tissue and has demonstrated antimicrobial efficacy against both bacteria and fungi(10–12). When applied in 5.25% concentration, sodium hypochlorite has been demonstrated to physically disrupt bacterial biofilms, and its use is correlated with increased clinical success when compared with chlorhexidine or hydrogen peroxide(13, 14). Yet even in combination

with calcium hydroxide intracanal medication, complete elimination of microbes and their associated irritants from the canal system remains impractical(15).

**Obturation**

Obturation of the root canal system is therefore indicated to seal within the system those irritants which cannot be fully removed, and to eliminate avenues of leakage from the oral cavity or apical tissues into the root canal system. Nearly all contemporary obturation techniques rely on a core of solid material which is adapted to the canal walls via mechanical compaction, thermoplasticization, or a combination thereof. Each has its limitations. Ideal adaptation of semisolid gutta-percha cones to canal wall irregularities cannot be predictably achieved via lateral compaction alone, and lateral forces imparted during the compaction process may contribute to radicular fracture(16–20). Thermoplasticized techniques more faithfully adapt to irregularities in the canal anatomy, however shrinkage upon cooling presents a practical barrier to the establishment of ideal adaptation to the canal wall(16, 21, 22). Use of vertical compaction force during cooling has been proposed to mitigate this limitation, yet even when apical pressure is applied extensive leakage has been demonstrated in canals filled solely with warm gutta-percha(23, 24).

**Endodontic Sealer**

Endodontic sealer is employed to fill voids within the core obturation material and improve adaptation to irregularities in the root canal system. An ideal sealer will adhere strongly to both dentin and the core material to minimize potential for leakage of microorganism, metabolites, or noxious bacterial byproducts into or out of the root canal

system. Most commonly used endodontic sealers also possess antimicrobial properties and support the tenet of disinfection of the root canal space.

Many materials have been proposed and studied, but sealers based on zinc oxide-eugenol or epoxide chemistry have been predominant in clinical use. These agents have excellent antimicrobial properties, have been shown to be acceptably biocompatible if extruded into the periapical region, and demonstrate flow, solubility, and radiopacity which is within the acceptable range recommended in the ISO 6876 standard(25–27).

More recently, a class of calcium silicate-based endodontic sealers have become the subject of considerable interest in research and clinical practice. EndoSequence BC Sealer™ is the most widely studied and used of this class of materials. It possesses flow, solubility, and radiopacity properties consistent with ISO 6878 recommendations, and demonstrates acceptable working time and viscosity for clinical use(28, 29). BC Sealer™ has demonstrated antimicrobial efficacy against E. faecalis monoinfections and biofilms, yet is not cytotoxic to human fibroblasts at any stage of setting(8, 30–32).

It is unique in demonstrating no shrinkage on setting, and has been shown to induce accretion of hydroxyapatite on its surface under simulated physiologic conditions(25, 33, 34). Due to the material's dimensional stability, a single cone technique has been recommended, in which the sealer comprises a larger proportion of the final obturation than is ideal with legacy materials(35, 36).

**Final Rinse**

Regardless of the properties of the specific sealer utilized for obturation, the extent to which it achieves the objectives of reducing leakage and exerting an

antimicrobial effect within the canal and root dentin depends on the intimacy of its contact with the dentin itself. Various combinations of solutions have been employed to alter the physical and chemical composition of the dentin in preparation for obturation. The two principal objectives of these so-called "final rinse" regimens are the removal of the smear layer formed during mechanical preparation and increasing the wettability of root dentin by endodontic sealer.

**Smear Layer**

McComb's identification of the "smear layer" of organic and inorganic debris following mechanical canal preparation introduced a new set of concerns with respect to irrigation(37). This plug of debris within the porous, tubular structure of pericanalar dentin was noted to be a barrier to irrigant exchange(38), bacterial migration(39), and penetration of sealer within the dentinal tubules(40). Equally concerning, Uitto demonstrated the potential for proteases produced by endodontic pathogens to degrade the collagenous component of the smear layer, resulting in the development of a gap between the obturation material and the canal wall which might contribute to leakage of metabolites and migration of pathogens(41).

McComb proposed the use of chelating agents following mechanical preparation, and found irrigation with a combination of cetrimide and ethylenediaminetetraacetic acid (EDTA) for a period of 24 hours following instrumentation to produce debris-free and patent dentinal tubules via scanning electron microscopic analysis(37). Yamada subsequently demonstrated that if irrigation with 5.25% NaOCl during mechanical preparation is followed by a flush of 10ml 17% EDTA and a further 10ml of 5.25%

NaOCl, near-complete elimination of the endodontic smear layer results(42). This final rinse regimen has been widely accepted in clinical practice since its introduction in 1983(5).

**Wettability**

Having exposed the tubular structure of the pericanalar dentin through removal of the smear layer, it is desirable that irrigation solutions, and ultimately obturation material, freely penetrate the structure of the dentin. The extent to which a liquid phase tends to minimize or maximize the surface area in contact with a solid substrate is referred to as the wettability of that solid(43). It is a function of the balance of adhesive (liquid to itself) and cohesive (liquid to the solid) forces(44). These forces are in turn dictated by the relative surface tension of the liquid and surface free energy of the solid phase. In practice, dentin tends to be incompletely wettable by either water or obturation materials. Therefore, one approach to optimizing the wettability of dentin by sodium hypochlorite has been to introduce agents which would decrease the surface tension of the irrigant(45).

Abou-Rass, *et al* demonstrated that the addition of 10% polysorbate 80 to sodium hypochlorite, EDTA, ethanol, or distilled water decreased the surface tension and increased the passive penetration of these solutions down the uninstrumented canals of extracted teeth by an average of 4-6mm(46). The addition of a fluorocarbon surfactant to sodium hypochlorite was likewise associated with a significant increase in tissue dissolution capacity(47). Finally, Cuningham observed that the addition of ethyl alcohol to sodium hypochlorite decreased surface tension and significantly increased the penetration of irrigation the solution into glass micropipettes(3).

A disadvantage to the addition of these agents to sodium hypochlorite was a decrease in stability(48). Free available chlorine was found to decline in as little as 15 minutes, limiting clinical utility(3). Stevens applied Cunningham's findings to the obturation phase of treatment and observed a significant decrease in dye penetration when a separate rinse of ethyl alcohol was introduced into the canal prior to drying and obturation using gutta-percha and Roth 801 zinc oxide eugenol-based sealer(2).

Commercial preparations have followed a similar approach, combining a chelating agent, surfactants, and in some cases antimicrobials to provide a single-step final rinse solution. SmearClear™ (Kerr Dental, Orange, CA), BioPure™ MTAD® (Dentsply, Tulsa Dental, Tulsa OK), and QMix® (Dentsply, Tulsa Dental, Tulsa OK) are the most commonly used. SmearClear™ consists of EDTA cetrimonium bromide and an additional proprietary surfactant(49). BioPure™ MTAD® (Dentsply, Tulsa Dental, Tulsa OK) consists of a mixture of antibiotic (doxycylcline hyclate: 150 mg/5 ml (3%), citric acid (4.25%), and a detergent (0.5% polysorbate 80 detergent)(50). QMix® (Dentsply, Tulsa Dental, Tulsa OK) consists of chlorhexidine, EDTA, and a proprietary detergent(51). Each of these agents has been demonstrated to be effective in removal of the smear layer, however less is known about their effects on the clinical performance of sealers subsequently used during obturation(49, 51–54). Specifically, no published data exists regarding the effects of these preparations on the wettability of dentin by calcium silicate-based materials.

One method of assessing the wettability of a solid by a liquid is to observe the physical conformation assumed by a droplet of the liquid when in contact with solid via

the sessile drop technique (Fig. 1). The angle formed by the solid and a line tangent to the surface of the liquid phase at the point of contact can be related to the solid-gas, solid-liquid, and solid-gas interfaces using Young's equation(44). Assuming the temperature and physical surface of the solid is homogenous and nonporous, and the liquid phase is controlled, changes in the contact angle formed will reflect changes in the surface free energy of the solid.

In practice, however, few substrates meet the assumptions listed above. On a non-ideal surface, the contact angle may vary locally based on heterogeneity in the composition, roughness, porosity or slope. These variations can "pin" the motion of the contact angle, and contribute the phenomenon described as hysteresis. Briefly, when the three interfacial tensions are in motion the angle formed on the advancing front of the droplet will have a higher observed contact angle than that on the receding front. Expansion of a sessile drop most closely approximates the advancing contact angle, or low wettability phase. Other techniques, notably the captive bubble and Wilhelmy Plate methods, have been devised to mitigate some of the foregoing shortcomings, however these techniques require relatively large solid phases of specific conformations and are generally not amenable to analysis of human dentin specimens.

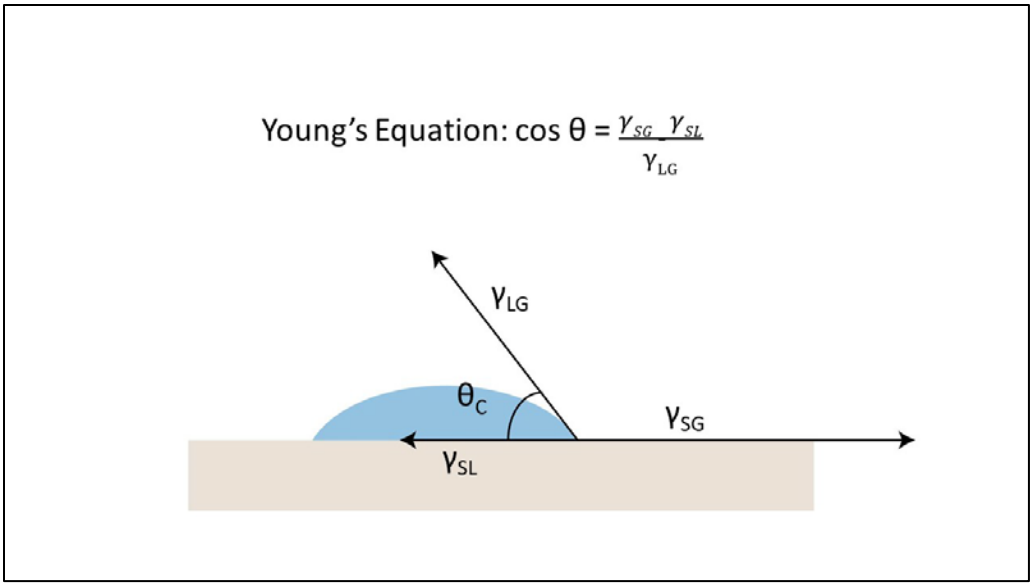



Figure 1. Young's equation and diagram of interfacial tensions affecting contact angle. γLG= liquid-gas tension. γSG= solid-gas tension. γSL= solid-liquid tension. θC = contact angle.

**Tubule Penetration**

A second means of quantifying wettability of a solid is by observing the behavior of a liquid in a capillary tube. If the adhesive force between the liquid and solid phase is sufficiently large, it will offset a portion of the liquid's mass as it advances up the tube. Dentin being a porous structure comprised of formations analogous to capillary tubes, it would be expected that penetration of sealer into the dentinal tubules would be determined in part by the extent to which the sealer wets the dentin(3, 43, 55).

Greater penetration of sealer into the dentinal tubules has classically been considered a positive indication of the seal and overall performance of endodontic sealer(40, 56, 57). DeDeus failed to demonstrate correlation between tubule penetration of AH Plus sealer with leakage of glucose, however leakage models themselves have been called into question as indicators of the quality of obturation(7, 58). Whether or not

a fluid-tight seal is established, the penetration of sealer into dentinal tubules entombs residual bacteria and presents a mechanical barrier to reinfection(59). Mechanical retention of the core obturation material is improved, and the antimicrobial activity of the sealer is extended into the root dentin(8).

In the case of EndoSequence BC Sealer™, tubule penetration might be of particular clinical relevance due to the unique properties of the material. The presence of a mineral infiltration zone at the sealer-dentin interface has been suggested to indicate a chemical cohesion with the root structure(60). Under these circumstances, increased surface area of dentin in contact with obturation material would be expected to result in more fluid-tight and contamination-resistant interface. BC Sealer™'s slight expansion on setting and accretion of hydroxyapatite on surfaces exposed to physiologic solutions would also indicate that any effects of this increased surface contact with dentin would be expected to improve, rather than degrade, with time(33, 61).

Scanning electron microscopy, light microscopy, and confocal laser scanning microscopy have been used in previous investigations to analyze the penetration of sealer into the dentinal tubules(7, 57, 62). Compared with the other techniques, confocal scanning laser microscopy has the distinct advantage of permitting evaluation of a subsurface section of the specimen. A laser is directed onto the specimen, and the characteristic fluorescence of marker is read in stereo, permitting focus on a specific plane in the z-axis. This eliminates the potential for the loss of sealer from exposed tubules, or the smearing of obturation material from the canal space into to previously unfilled tubules during sectioning.

One criticism of previous CLSM studies is the potential for the marker to dissociate from the substrate of interest and inaccurately depict its distribution(63). This can be mitigated through the use of a marker which preferentially fluoresces in the presence of the substrate of interest. An example of this is fluo-3 pentaamonium salt, which demonstrates a 100-fold increase in signal intensity when exposed to free calcium(64). Multiple investigators have confirmed that BC Sealer™ leaches calcium during setting, and the use of fluo-3 pentaamonium salt for evaluation of tubule penetration was previously validated by Jeong, *et al* using an analogous bioceramic material(28, 60, 65). The use of CLSM imaging with this marker should therefore be ideal for an investigation into the tubule penetration of BC Sealer™.

The depth and uniformity of tubule penetration has previously been shown to vary based on the physical properties of the sealer used, such as particle size, viscosity, and film thickness(59, 62, 66, 67). The effect of obturation technique and smear layer removal have been extensively reported, and can likely be generalized to a variety of materials. Attempts to modify the surface energy of dentin, by contrast, would be expected to have a material-specific effect on the clinical performance of a given material. Tuncer and Jardine have reported on the effect of surfactant-containing QMix® solution on tubule penetration of epoxy-based sealer, but these results may not be applicable to a substantially more hydrophilic calcium silicate-based material. To date no similar studies have been performed with BC Sealer™. Knowledge of the capacity of various irrigation solutions to affect the wettability and tubule penetration of dentin by

BC Sealer™ could guide clinicians in selecting a final rinse protocol which optimizes the clinical performance of this material.

## SPECIFIC AIMS

1. To quantify the effect of four final rinse protocols commonly used in endodontics on the wettability of dentin by calcium silicate-based sealer (Endosequence BC Sealer™).
2. To establish a relationship between sealer-dentin contact angle and the parameters of canal wall adaptation and tubule penetration in a simulated clinical model.

## HYPOTHESES

1. Treatment of dentin with surfactant- or ethanol-containing final rinse protocols will result in reduction of the contact angle between water and dentin, compared to a standard treatment of 17% EDTA.
2. Treatment of dentin with surfactant- or ethanol-containing final rinse protocols will result in increased penetration of BC Sealer™ into dentinal tubules, compared to a standard treatment of 17% EDTA.

## NULL HYPOTHESES

1. Treatment of dentin with surfactant- or ethanol-containing final rinse protocols will not result in a reduction of the contact angle between water and dentin, compared to a standard treatment of 17% EDTA and 5.25% NaOCl.
2. Treatment of dentin with surfactant- or ethanol-containing final rinse protocols will not result in increased penetration of BC Sealer™ into dentinal tubules, compared to a standard treatment of 17% EDTA.

## MATERIALS AND METHODS

The methods and use of extracted human teeth were granted Federal Category 4 exemption for records and tissue specimens by the University of Minnesota Institutional Review Board (**IRB HSC:** 1601E82161) because human tissues utilized were deidentified and previously collected. With the application approved the study was exempted from the full IRB review and further oversight.

### TUBULE PENETRATION STUDY

#### Tubule Penetration Pilot

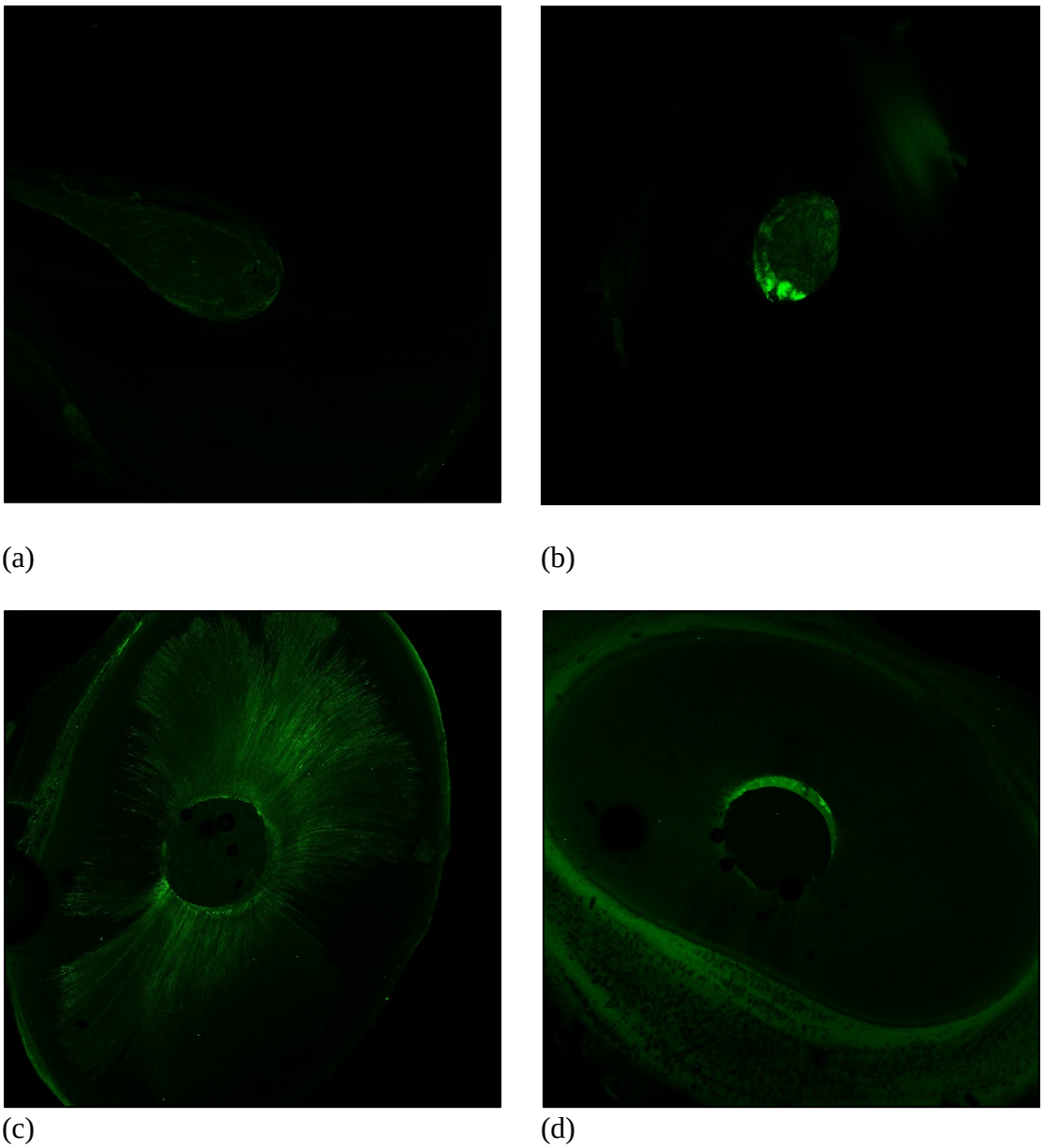
An initial pilot project was conducted to validate the use of fluo-3 pentaamonium salt for use as a marker with EndoSequence BC Sealer™ and to standardize the imaging and analysis parameters for the full-scale project. Endodontic access cavities were prepared in twelve extracted human maxillary molar teeth. A #10 stainless steel K-type hand file was introduced into the distobuccal canal and a working length was established 1mm short of visual patency at the major foramen. The canal was then prepared to this working length with ProTaper Gold™ (Dentsply Sirona, Tulsa, OK) rotary nickel-titanium files at 300rpm and 520g-cm of torque in sequence from S1 to F3. The canal was flooded with 5.25% sodium hypochlorite during preparation and recapitulation was performed with a #10 hand file between rotary instruments. Seven specimens underwent a 30 second flush of the canal system with 17% EDTA, followed by a 30 second flush of 5.25% sodium hypochlorite to remove the smear layer. The remaining five specimens underwent no further irrigation. All specimens were dried with paper points until 3 consecutive points were dry following insertion to WL.

Five EDTA-treated and five non-EDTA-treated specimens were immediately obturated using a single cone technique and a mixture of 2g EndoSequence BC Sealer™ and 1mg fluo-3 pentaamonium salt calcium indicator. One additional EDTA-treated specimen was obturated with gutta-percha and a mixture of 0.25ml deionized water and 0.5mg fluo-3 pentaamonium salt without sealer, while the final specimen was left unfilled. All specimens had their access cavities filled with Cavit™ (3M ESPE, St. Paul, MN) and were stored at 100% humidity and 20°C for a period of 2 weeks. Each root was sectioned at 1mm, 3mm, 5mm, and 7mm level using a diamond disc in a slow-speed laboratory handpiece, yielding three 2mm-thick samples of radicular dentin. The apical surface of each specimen was polished sequentially from 320 grit to 1000 grit Wetordry™ sandpaper (3M, St. Paul, MN), and mounted on glass slide. Silicone spacers were prepared, a 90% PBS/10% glycerol mounting medium introduced to each well, and a no.15 cover slip was applied. All specimens were imaged using a Fluoview 1000 BX2 Upright Confocal microscope (Nikon) at 488nm.

Autofluorescence of the unfilled root dentin specimen was evaluated and found to be acceptable (Fig. 2a). No significant fluorescence was observed in the marker-only specimen (Fig. 2b), validating that no signal would be expected if the marker dissociated and leached ahead of the sealer front. Adequate signal was observed from the BC Sealer™/fluo-3 pentaamonium mixture (Fig. 2c), and the presence of an intact smear was noted to prevent any penetration of labeled sealer into the dentinal tubules (Fig. 2d)

The primary investigator then experimented with laser intensity, high voltage, aspect ratio, scan speed, Kallman averaging, and offset to establish standardized imaging

parameters which would optimize the signal to noise ratio while preventing bleaching of the fluo-3 pentaamonium marker. Objectives of 2X, 4X, and 10X magnification were utilized to image 1mm, 3mm, and 5mm specimens. Resolution of the sealer penetration was inadequate with the 2X objective, and the field of view too narrow at 10X to observe the entirety of the penetrated area in a single image. 1mm sections could frequently be imaged in their entirety at 4x, however sealer penetration was generally poor across groups at that level. Tiling of 4X or 10X scans to image the entirety of 3mm and 5mm specimens proved unsatisfactory due to variations in brightness in the stitched images which precluded automated analysis via establishment of a signal threshold. At the 3mm level, a 4X objective typically captured the entire area of sealer penetration with adequate resolution and these parameters were adopted.



(a) (b)

(c) (d)

Figure 2. Confocal laser scanning micrographs of selected pilot specimens. (a) Autofluorescence. (b) Smear layer removed, marker-only. (c) Smear layer removed, marker plus BC Sealer™. (d) Smear layer intact, marker plus BC Sealer™.

TOOTH SAMPLE PREPARATION

One hundred twenty deidentified maxillary molars were obtained from previous waste tissue generated by the UMN Oral and Maxillofacial Surgery clinic and stored in a 10% formalin until use. The investigator discarded specimens with open apices, previous root canal filling, fractures, radicular resorption, severe dilacerations or fused distobuccal root anatomy as identified via visual inspection with a dental operating microscope (Global Surgical™ Corporation, St. Louis, MO, USA) at 10x.

Endodontic access was prepared in each specimen using a trans-metal crosscut fissure bur. A #10 K-file was introduced to the distobuccal canal and apical patency was visually confirmed. In the event that patency could not be achieved with a #10 file or a #30 file could be passively advanced past the major foramen, the specimen was discarded. Instrumentation was then completed with ProTaper Gold™ (Dentsply Sirona, York, PA) rotary nickel-titanium files at 300rpm and 520g-cm torque in sequence from S1 to F3 to 1mm short of the major foramen. Recapitulation using a #10 hand file and irrigation with 5.25% sodium hypochlorite via ProRinse® (Dentsply Sirona, York, PA) side-vented 30-gauge syringe was completed between rotary files. This process was repeated until eighty suitable specimens were prepared.

The teeth were then placed in vials individually numbered from one to eighty and randomly assigned to one of four groups using a computer aided random numbering service (www.random.org). The four experimental groups were divided according to the final rinse protocol to be completed as follows:

Group 1 (N=20): 5.25% NaOCl (30s), PUI, 17% EDTA (30s)

Group 2 (N=20) Smear Clear™ (30s)

Group 3 (N=20) 5.25% NaOCl (30s), PUI, 17% EDTA with 2% Tween 80 (30s)

Group 4 (N=20) 5.25% NaOCl (30s), PUI, 17% EDTA (30s), 95% EtOH (30s)

All irrigation solutions were introduced and constantly replenished with a ProRinse® side-vented 30-g irrigation syringe inserted to within 1mm of the working length. The duration of irrigation with each solution was precisely regulated with a digital stopwatch. Passive ultrasonic irrigation was performed with a Spartan MTS-1 piezo ultrasonic unit on low power using size 15 Zipper (Roydent, Johnson City, TN). Immediately following the final rinse in each respective regimen, the canal contents were aspirated with an endodontic aspirator tip attached to low volume suction (ROEKO Surgitip-endo, Coltene Whaledent, Cuyahoga Falls, OH). Paper points were then inserted to the full working length until three consecutive points were visually dry upon withdrawal.

Obturation of all specimens was completed using ProTaper Gold® gutta-percha molded cones (Dentsply Sirona, York, PA), and a sealer mixture consisting of EndoSequence BC Sealer™ (Brasseler USA, Savannah, GA), and Fluo-3 pentaamonium salt calcium indicator (Thermo Fisher, Waltham MA) in a ratio of 2g sealer to 1mg marker. A single cone technique was utilized, in which the apical 1/3 of the guta-percha cone was coated with the sealer mixture, inserted into the canal, and firmly seated to working length in a single motion. Gutta-percha extending coronal to the canal orifice was removed using a System B electronic heat source (Sybron Endodontics, Orange County, CA(check this)) set to 200°C. Firm apical pressure was applied to the gutta-percha via nickel-titanium pluggers. Cavit™ temporary filling material was placed in the

access cavity and the specimens were stored at 100% humidity and 20°C for a period of two weeks to ensure setting of the sealer.

The roots of the teeth in each of the five groups were then sectioned perpendicular to their long axis using a diamond disc in an IsoMet™ low speed cutting machine (Buehler, Lake Bluff, IL) 3mm and 5mm coronal to the root apex, resulting in a two mm section. The apical and coronal portions of the roots were discarded, and the apical surface of the retained segment was polished with Wetordry™ aluminum oxide abrasive sheets (3M, St. Paul, MN) sequentially from 400 to 1000 grit. Silicone spacers were arranged on glass slides to form wells into which the specimens were inserted. Specimens were immersed in mounting medium consisting of 90% phosphate buffered saline and 10% glycerol and a No. 1.5 coverslip was applied.

IMAGING

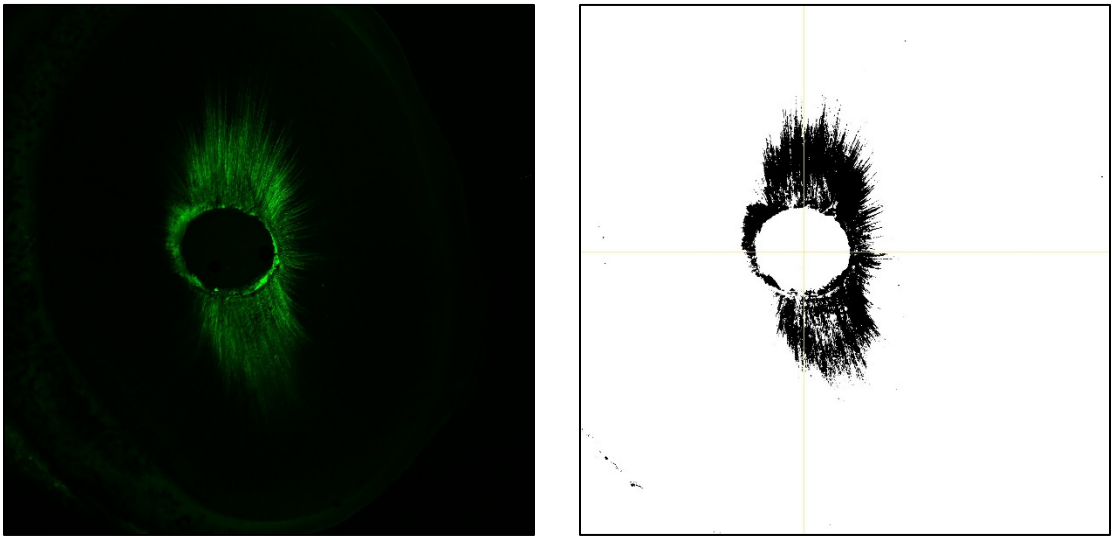
All specimens were examined using a Fluoview 1000 BX2 Upright Confocal microscope (Nikon, Tokyo, Japan). The laser source was set to 488nm and a standardized intensity of 44% was used for all specimens. The photomultiplier voltage was standardized at 465V. All specimens were examined using the 4x magnification objective.

The instrument's focus was adjusted inferiorly until the first evidence of fluorescence was noted and a z-axis reference was established to represent the specimen's surface. To minimize any artifacts which may result from smearing of sealer-containing debris during sectioning or polishing, an optical section 10um deep to this reference was

selected as the superficial boundary of the scan. Images were obtained by scanning five sections of 5um step size in a format of 1600x1600 pixels. A section 25um in thickness was imaged to ensure a representative section of the specimen was imaged, and to account for variation in dentinal tubule orientation.

Measurement and Analysis

All image analysis was performed using Image-J (NIH, Bethesda, MD) software. A composite of the five constituent images of each scan was prepared using the software's z-project feature (Fig. 3a). An automated binary mask function was then applied to each composite image, in which the background was subtracted and the signal from fluorescence was depicted in black (Fig. 3b).



(a) (b)

Figure 3. (a) Z-stack projection. (b) Binary mask with orthogonal reference planes.

Analysis of the tubule penetration was adapted from a protocol described by Zapata *et al*(62). To determine maximum depth of penetration, the point of deepest

penetration was measured from the canal wall to the point of maximum sealer penetration (Fig. 4). Sealer penetration depths were also measured at four points along the canal circumference correlating to the mesial, distal, buccal, and lingual surfaces. A cross-hair overlay was utilized to ensure consistency in defining the quadrants. Finally, the percentage of the canal lumen beyond which at least some sealer penetration is observed was calculated. The root canal wall was outlined and measured with the software measuring tool. The circumference along the canal walls into which sealer penetrated to any distance was then outlined and measured (Fig. 5a). The aggregate distance into which at least at least some penetration was observed was divided by the total canal circumference to calculate the percentage of sealer penetration (Fig. 5b).

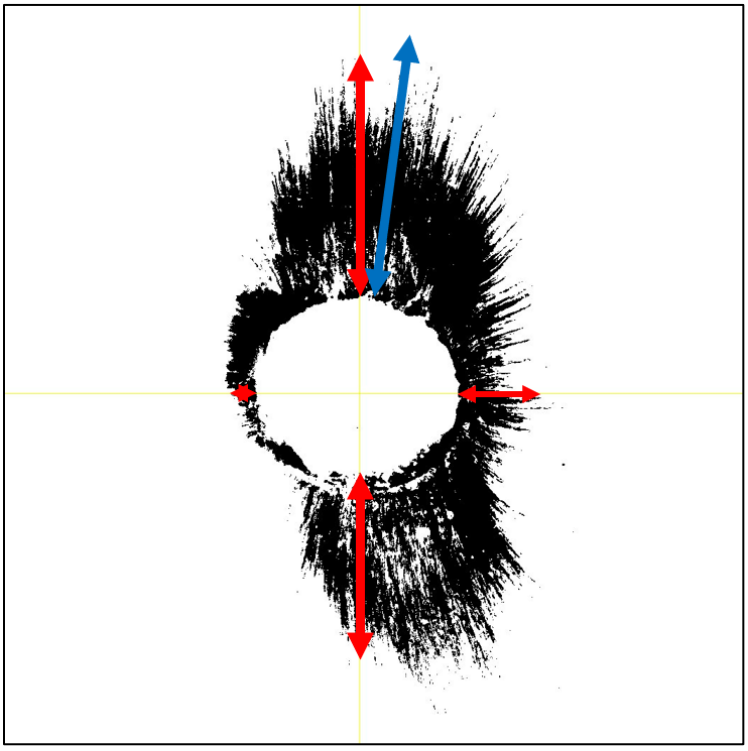


Figure 4. Example image with maximum tubule penetration (blue) and penetration at four cardinal directions (red) indicated by arrows.

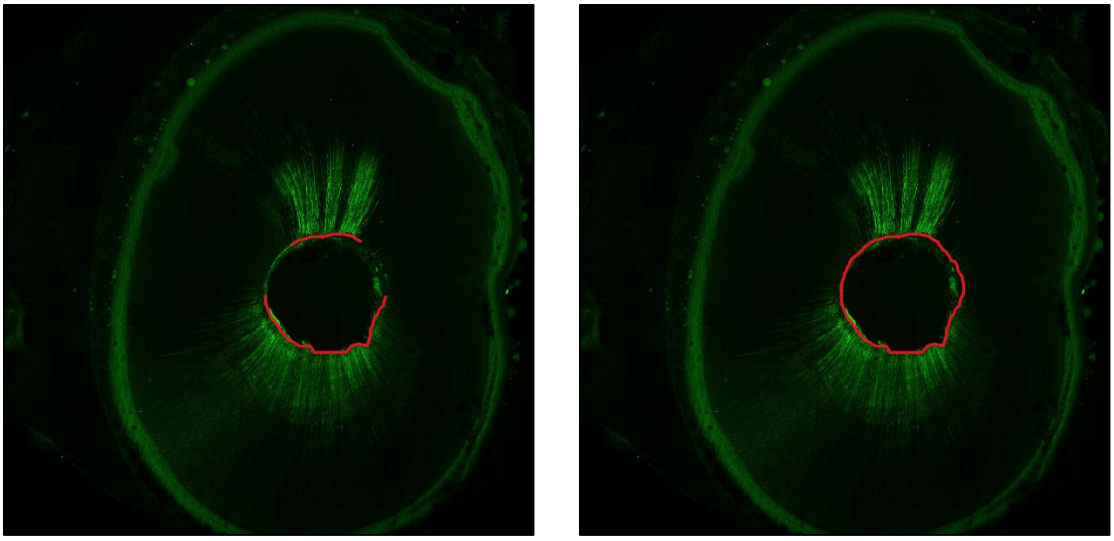


Figure 5. The circumference of the canal wall which has been penetrated by sealer (a) is divided by the total canal circumference (b) to determine the percentage of canal wall penetration

CONTACT ANGLE ANALYSIS

Sample Preparation

Twenty deidentified mandibular third molars were obtained from previous waste tissue generated by the UMN oral and Maxillofacial Surgery clinic and stored in a 10% formalin until use. The investigator discarded specimens with root restorations or fractures as identified via visual inspection with a dental operating microscope (Global Surgical Corporation, St. Louis, MO, USA) at 10x.

Flat surfaces were prepared in the teeth perpendicular to the buccal-lingual axis of the distal root using a diamond disk in a slow speed Ultimate XL laboratory handpiece (NSK America, Hoffman Estates, IL). The crown and both root apices were then prepared perpendicular to the mesial root long axis. So prepared, the tooth could be securely mounted to the IsoMet™ saw with the long axis of the distal root aligned with the diamond blade. An initial facing cut was made to the outer third of the distal root to

ensure that the upper and lower surfaces of the specimen would be parallel and sit level on the stage of the contact angle analyzer. A second cut was made at approximately the midline of the root, and a third cut prepared a parallel reference surface in the furcal third of the root. From the three cuts was derived two radicular dentin specimens, each with two parallel sides oriented along the root long axis. Each root half was abraded with 320 grit aluminum oxide paper under distilled water using a Buehler Ecomet 3™ polisher (Buehler, Lake Bluff, IL) until the irregularities resulting from the root canal space had been eliminated. This surface was then polished with 600, 800, and 1000 grit abrasive paper to provide a smooth and uniform surface for analysis.

The specimens were placed in vials individually numbered from one to forty and randomly assigned to one of four groups using a computer aided random numbering service (www.random.org). The four experimental groups were divided according to the final rinse protocol to be completed as follows:

Group 1 (N=10): 5.25% NaOCl (30s), PUI, 17% EDTA (30s)

Group 2 (N=10) 5.25% NaOCl (30s), PUI, Smear Clear™ (30s)

Group 3 (N=10) 5.25% NaOCl (30s), PUI, 17% EDTA with 2% Tween 80 (30s)

Group 4 (N=10) 5.25% NaOCl (30s), PUI, 17% EDTA (30s), 95% EtOH (30s)

For each group, specimens were sequentially immersed in vials of each the indicated solutions for precisely 30 seconds, as indicated by a digital stopwatch. Immediately following the final rinse in each respective regimen, each specimen was dried with a clean 2cm x 2cm gauze pad and returned to its numbered vial. All specimens were stored uncovered at room temperature for one week prior to contact angle analysis to minimize

variations in hydration.

All specimens were analyzed with a DMs-401 Contact Angle Meter (Kyowa Interface Science Co., Ltd, Niiza City, Japan). Distilled water was loaded into a glass syringe and 0.1ml drops were formed. The stage was raised into contact with the surface, and the spreading of the drop along the surface of the dentin specimen recorded by the instrument at 100ms intervals until no change was observed for three consecutive measurements. A duration of 6.2 seconds proved adequate to satisfy this criterion.

## STATISTICS

One-way ANOVA was used to compare the mean sealer penetration, maximum penetration, and percent penetration between groups. Pairwise comparisons with a Tukey adjustment for multiple comparisons were made if the overall ANOVA test is significant at the $p=0.05$ level. Similar analyses were conducted for the observations collected in the contact angle study.

# RESULTS

## Tubule Penetration

Mean Tubule Penetration

Figure 6 demonstrates the mean tubule penetration by labeled BC Sealer™ at four circumferential points of all specimens in each group. Values for individual specimens are given in Appendix I. Tubule penetration for the EDTA/Tween 80 group was significantly higher than that of the control (p=0.012). There was a trend towards increased tubule penetration in the SmearClear™ and EDTA/Ethanol groups, however this difference was not significant.

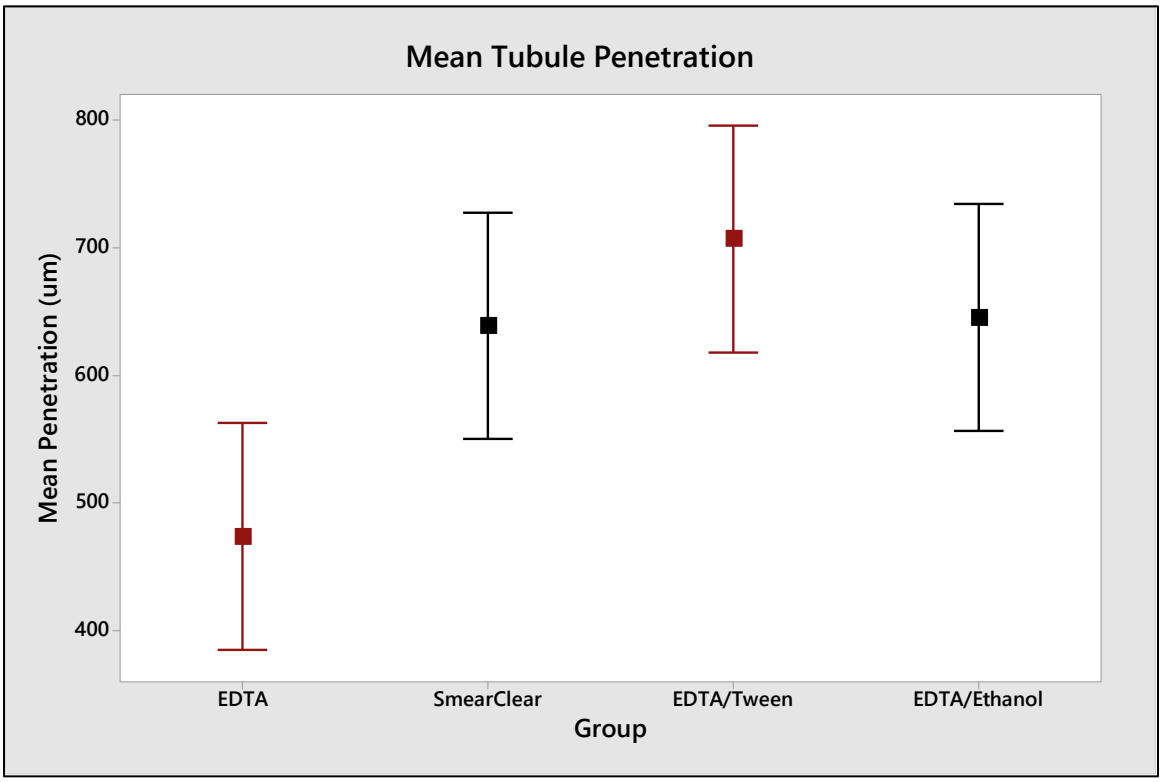



**Figure 6:** Mean tubule penetration by group. Pooled standard deviation was used to calculate intervals. Means in red indicate a significant difference (p<0.05).

Maximum Tubule Penetration

Maximum tubule penetration at any point along the canal circumference was recorded (Appendix I), and the mean value for all specimens in each group is depicted in figure 7. Consistent with the trend demonstrated by mean tubule penetration, the EDTA/Tween group demonstrated significantly greater maximum penetration than the control group ($p=0.003$).

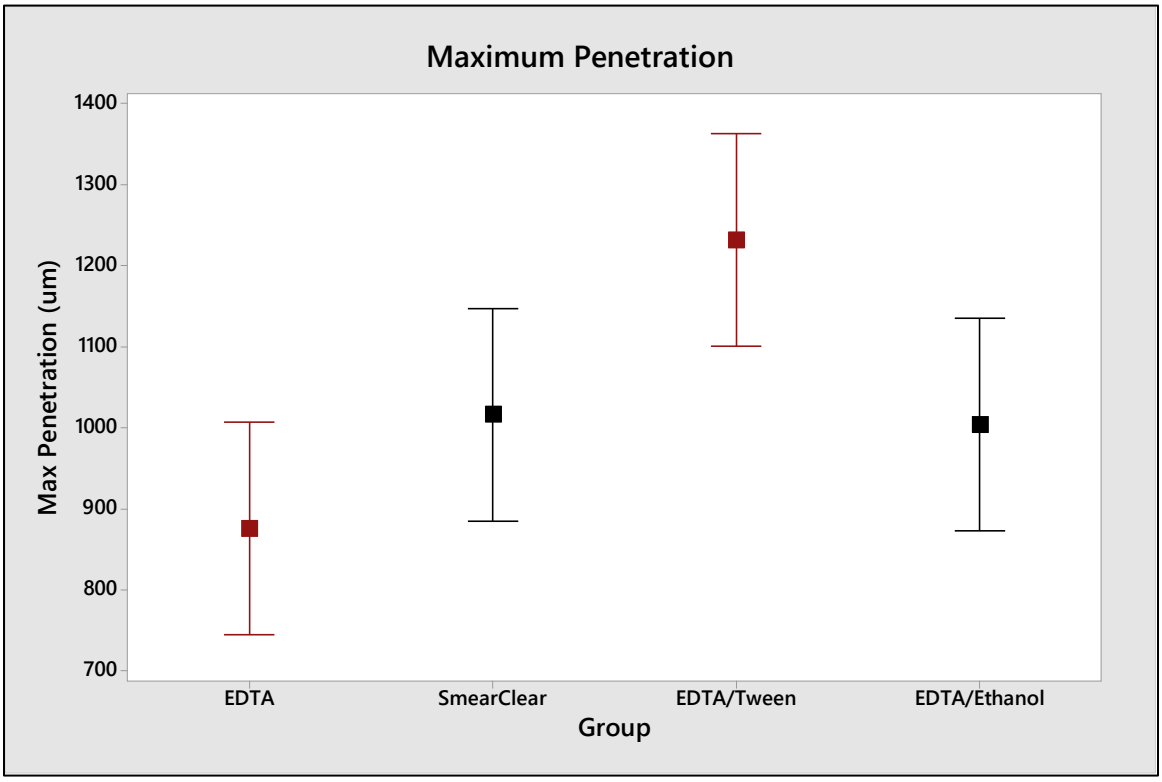



**Figure 7:** Interval plot of average maximum tubule penetration by group. Pooled standard deviation is used to calculate 95% confidence interval. Means in red indicate a significant difference ($p<0.05$).

Percent Canal Wall Penetration

Figure 8 demonstrates the proportion of the canal wall beyond which any penetration of labeled sealer was observed, expressed as a percentage. Raw data can be found in Appendix I. Mean percentage penetration for all groups was ranged from 89-93%, with no significant differenced noted between groups.

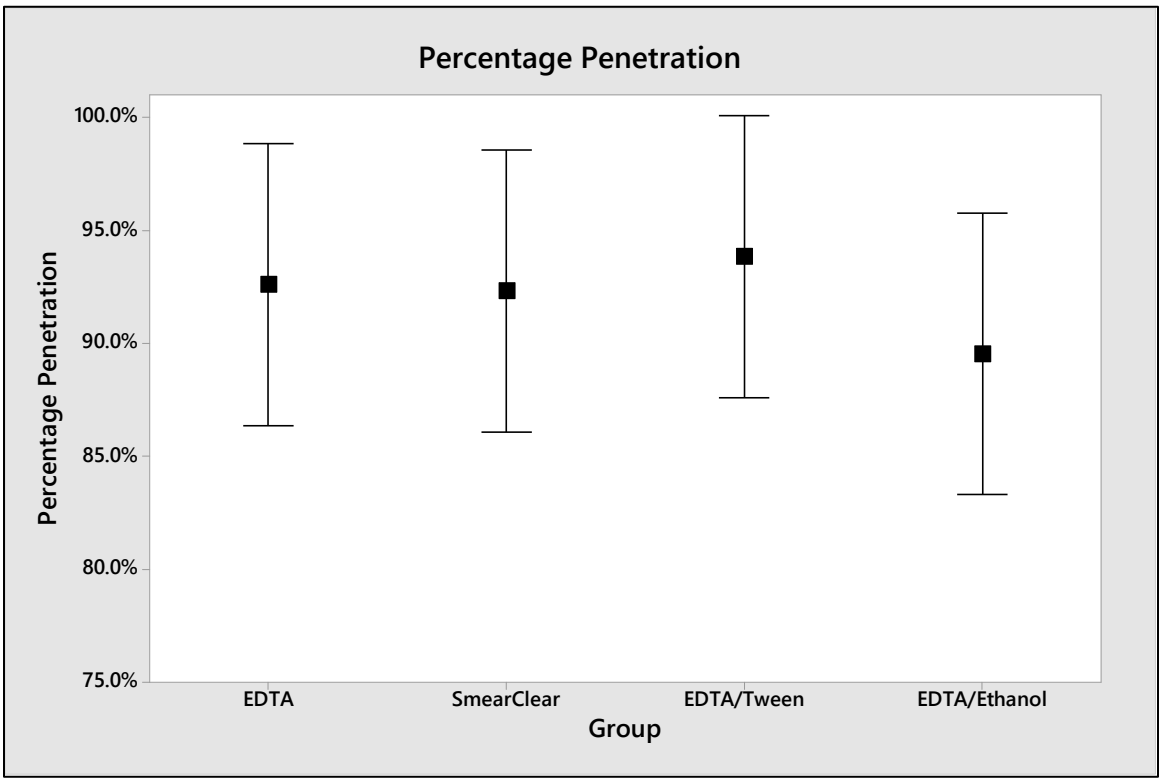



**Figure 8:** Interval plot of percentage of canal wall penetration by group. Pooled standard deviation is used to calculate 95% confidence interval.

## Sessile Drop Analysis

Static Contact Angle

Appendix 2 shows contact angle data. Table 1 shows the mean static contact angle, variability, absorbed volume and rate of absorption for each group. See figure 9 for a graphical representation of the mean static contact angle. SmearClear™, ethanol, and Tween-treated groups all demonstrated significant decreases in contact angle, compared with EDTA alone ($p<0.001$). The EDTA/Tween group demonstrated the greatest mean decrease in contact angle, which was significantly different from the ethanol group. No significant differences were observed between either EDTA/Tween and SmearClear™ groups.

| Group | Static Contact Angle (deg) | Variability (deg) | Absorbed Volume (ml) | Rate of Absorption (ml/ms) |
|---|---|---|---|---|
| EDTA | $37.8^{A}$ | $33.27^{B}$ | $0.25^{B}$ | $2.87E\text{-}5^{A}$ |
| SmearClear™ | $14.07^{BC}$ | $41.57^{AB}$ | $0.28^{B}$ | $9.40E\text{-}5^{B}$ |
| EDTA/Tween | $7.01^{C}$ | $51.8^{A}$ | $0.72^{A}$ | $9.00E\text{-}5^{B}$ |
| EDTA/Ethanol | $22.16^{B}$ | $37.32^{B}$ | $0.34^{AB}$ | $3.35E\text{-}5^{A}$ |

**Table 1:** Summary of contact angle data. Values that do not share a superscript letter are significantly different ($p<0.05$).

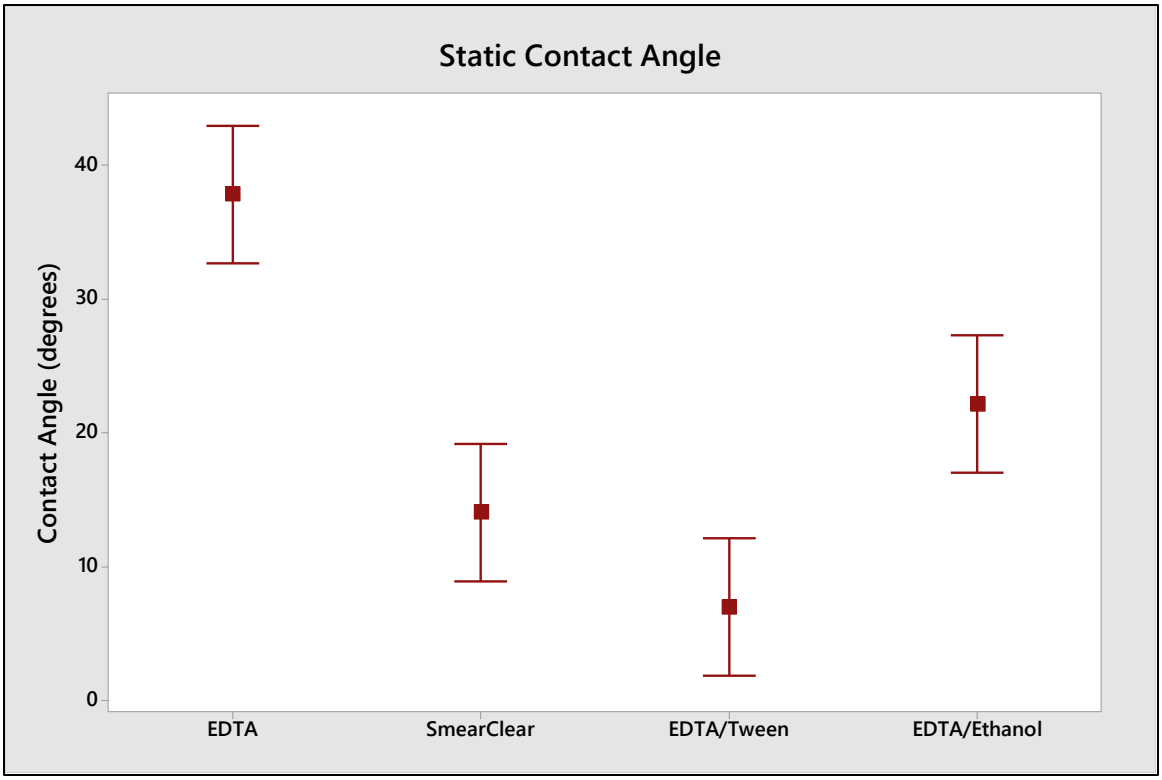



**Figure 9:** Interval plot of static contact angle by group. Pooled standard deviation is used to calculate 95% confidence interval. Groups significantly different than at least one other group are indicated in red ($p<0.05$).

Variability

Figure 10 depicts the mean variability between the initial contact angle formed by the droplet and the static contact angle at 6.2s. The mean variability of the EDTA/Tween group was significantly greater than that observed in the EDTA and EDTA/Ethanol groups ($p<0.001$). The variability observed in the SmearClear™ group was not significantly different from that observed in any other groups ($p>0.05$).

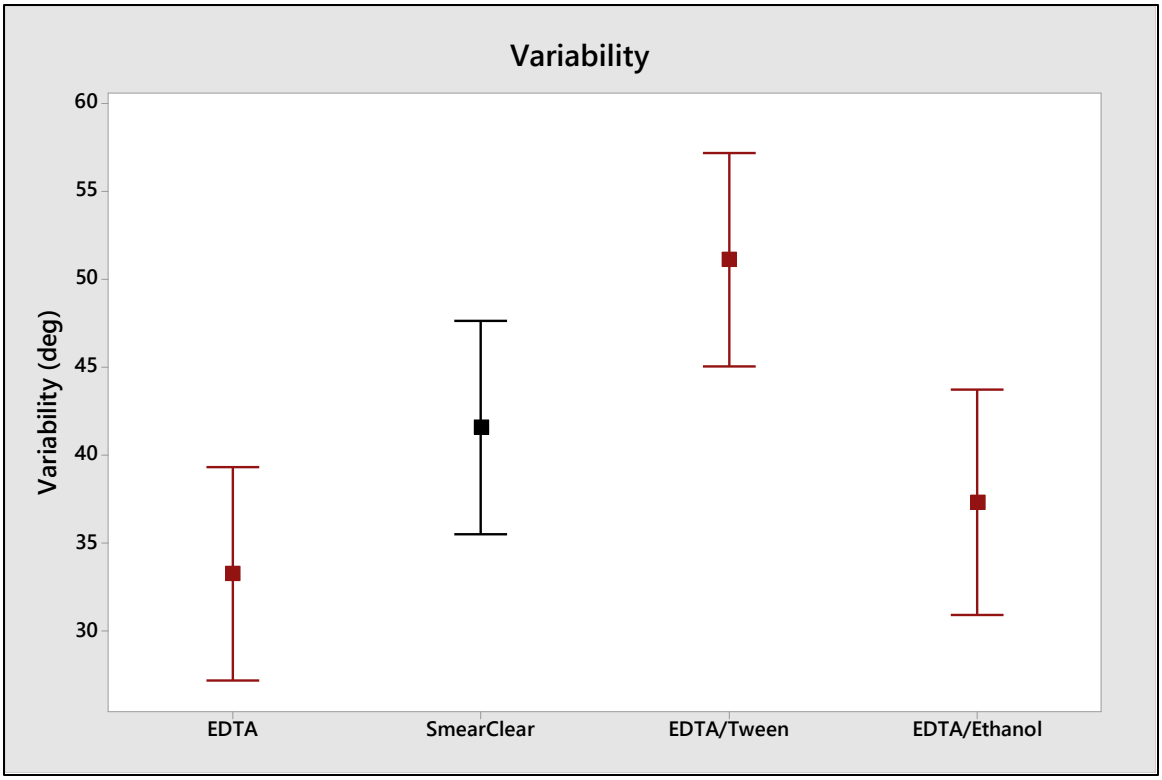



**Figure 10:** Interval plot of contact angle variability by group. Pooled standard deviation is used to calculate 95% confidence interval. Means in red indicate a significant difference ($p<0.05$).

Dynamic Contact Angle

Figure 11 presents a plot of mean contact angle vs. time. An exponential decay function has been fitted to the data, with the amplitude, offset, and decay constant presented in table 2.

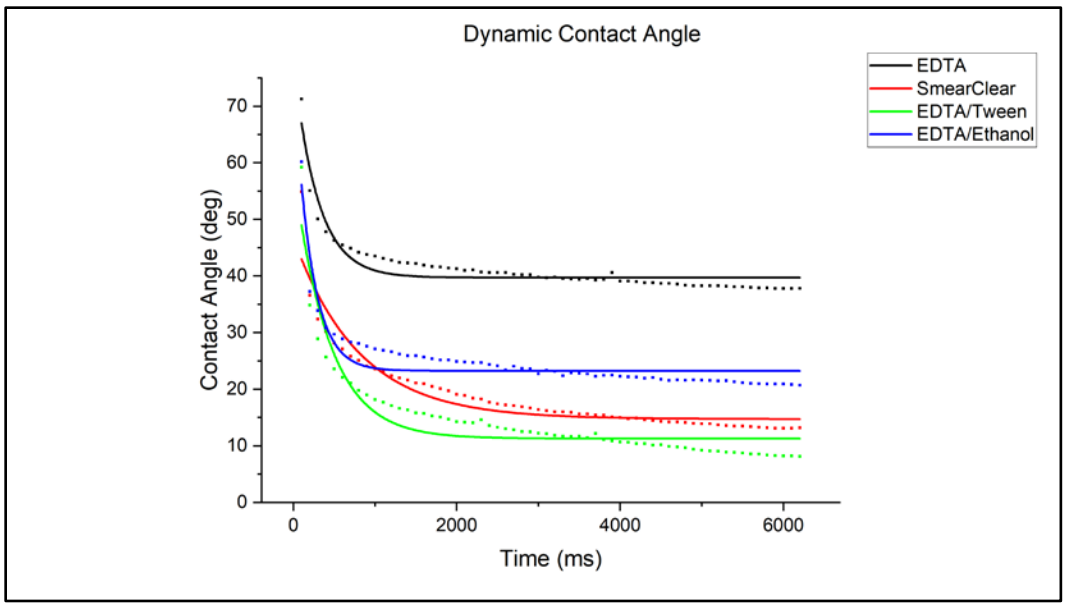



**Figure 11:** Mean contact angle vs. time by group. Solid lines represent an exponential decay function modeled by y=A1*exp(-x/t1)+y0, where A1= amplitude, y0 = offset, and t1= decay constant.

| Group | Decay Constant (t1) | Amplitude (A1) | Offset (y0) |
|---|---|---|---|
| EDTA | 293.79 ± 27.90 | 38.37 ± 2.88 | 39.716 ± 0.24 |
| SmearClear™ | 802.28 ± 72.91 | 32.03 ± 1.78 | 14.72 ± 0.40 |
| EDTA/Tween | 431.90 ± 42.72 | 47.54 ± 3.44 | 11.28 ± 0.42 |
| EDTA/EtOH | 213.00 ± 23.48 | 52.60 ± 5.09 | 23.23 ± 0.30 |

**Table 2:** Parameters of exponential decay function y=A1*exp(-x/t1)+y0 fitted to plots of contact angle vs. time.

Absorbed Volume

Figure 12 presents the mean total absorbed volume by experimental group. The EDTA/Tween group demonstrated significantly greater absorption compared to the control group ($p=0.036$), while differences between the SmearClear™ and EDTA/ethanol groups and others were not significant. A plot of absorption vs. time is depicted in Fig. 13, with a linear regression function modeling the average rate or absorption. The slope of the mean absorption function represents the rate of absorption of water in to the specimens. The rate of absorption for the SmearClear and EDTA/tween groups were 0.0094ml/s and 0.0090ml/s, respectively, which were significantly ($p<0.0001$) greater than the values for the EDTA and EDTA/Ethanol groups (0.0029ml/s and 0.0034ml/s, respectively). No significant differences were observed between EDTA and EDTA/Ethanol ($p=0.4081$) or between EDTA/Tween and SmearClear ($p=0.4081$).

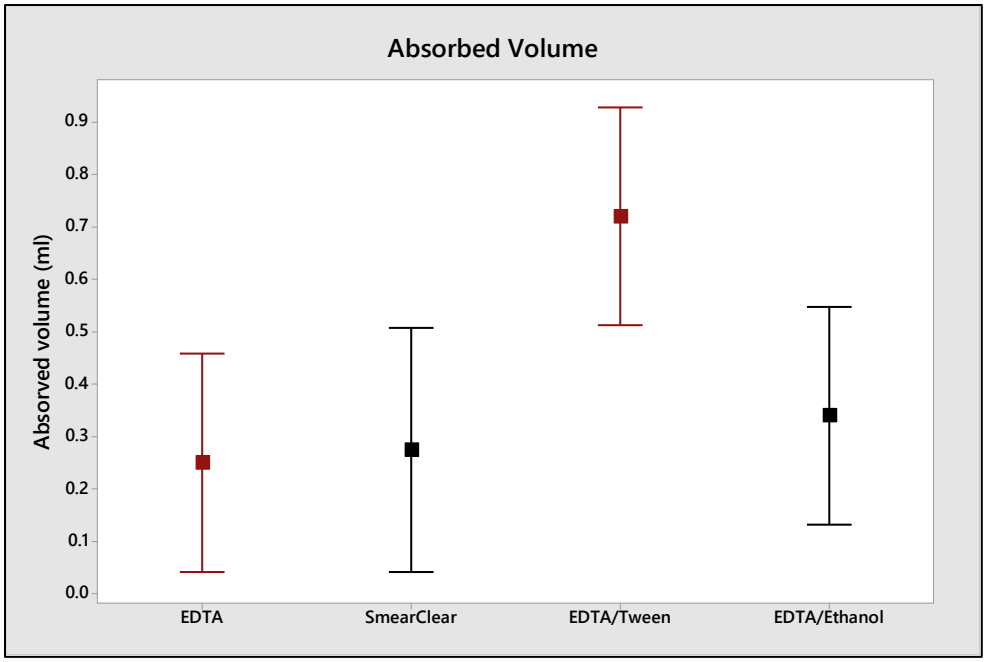



**Figure 12:** Interval plot of mean absorbed volume by group. Pooled standard deviation is used to calculate 95% confidence interval. Groups significantly different than at least one other group are indicated in red ($p<0.05$)

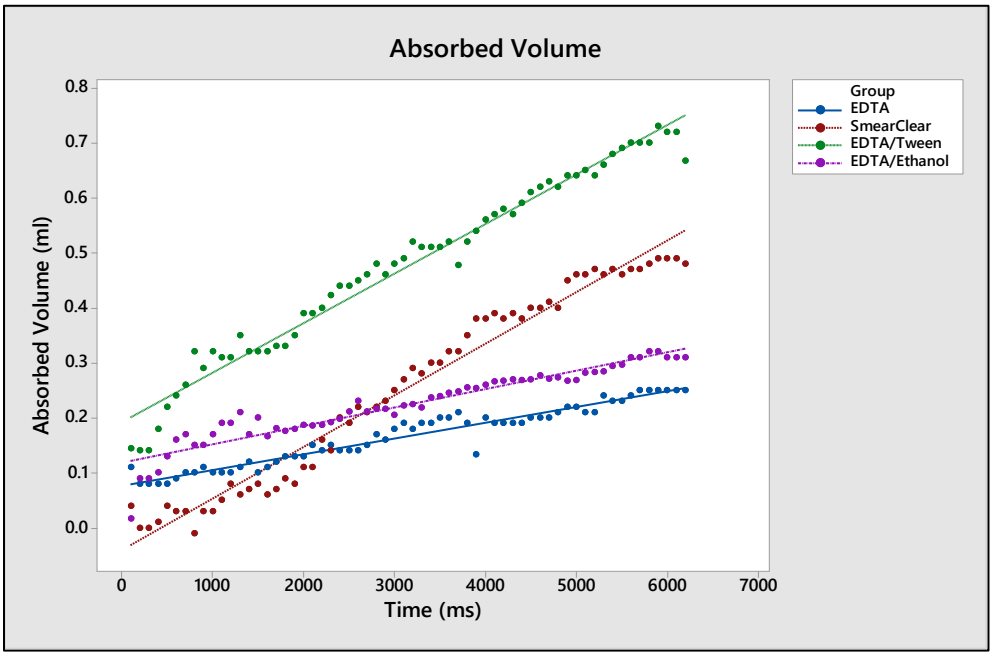



**Figure 13:** Plot of mean absorbed volume vs. time. Dashed lines indicate linear regression functions fitted to each group.

## DISCUSSION

The establishment of a fluid-tight seal within the root canal space has long been considered a critical component of successful endodontic therapy. Various obturation methods have been employed to optimize adaptation of gutta-percha obturation material to the irregularities of the canal wall, yet all techniques to date require the adjunctive use of a liquid sealer phase to produce an acceptable seal(24, 68–70). One factor which would be expected to affect the intimacy of contact made between the sealer and dentin is the relative surface free energy (wettability) of the dentin(71–73).

Several previous investigations have demonstrated the wettability of dentin to decrease following the removal of the smear layer with EDTA. Hu demonstrated that this effect is proportional to the change in surface roughness, which can be related to wettability via the Wenzel equation(44, 74). Since removal of the smear layer is generally indicated to improve disinfection of the dentin and achieve penetration of obturation material into the dentin substrate, this roughness-related decrease in wettability is unavoidable. In the present study, all specimens were polished to a uniform finish. Each experimental and control solution contained 17% EDTA, and the time of exposure was precisely regulated to control surface roughness. Previous scanning electron microscopic investigation has confirmed SmearClear™ to have efficacy equivalent to 17% EDTA alone in removal of the smear layer, and the contribution of differences in surface roughness to variations in contact angle is presumed to be negligible(49).

**Contact Angle**

The results of the present study agree with previous reports that the addition of surfactants to EDTA and a final rinse of 95% ethanol are both effective approaches to increasing the wettability of smear-free dentin(74–77). Compared to the control exposure of 17% EDTA alone, the use of SmearClear™, 2% tween 80, or a final rinse of 95% ethanol was associated with a significant decrease in static contact angle. These results are consistent with Ballal's observation of a reduction in contact angle between AH Plus sealer and dentin treated with an EDTA and tween 80-containing solution (QMix®) and root dentin, compared to EDTA alone(77).

When contact angle is plotted against time and fitted to an exponential decay function, the behavior of the droplet over time can be appreciated. The function's amplitude and decay constant can be used to describe the magnitude and rate of change of the contact angle as the droplet spreads on the solid. Both the SmearClear™ and EDTA/Tween groups demonstrate a more gradual, sustained, and higher amplitude transition in contact angle. A plot of absorbed volume vs. time indicates a similar trend, in which the SmearClear™ and EDTA/Tween groups demonstrate a marked increase in the rate of droplet absorption, compared to controls or the EDTA/Ethanol group. These functions are likely related. Because the sessile drop method incompletely accounts for hysteresis on a non-ideal solid, the recorded contact angle cannot be considered to be the only, or true, contact angle. As the droplet spreads against irregularities in the surface the advancing phase demonstrates an increased apparent contact angle, correlating to a lower wettability phase. Likewise, as a droplet retreats those irregularities tend to pin the

periphery of the droplet, increasing the apparent wettability of the specimen. In the case of absorption, if the diameter is maintained circumferentially due to these extraneous forces the droplet appears to be receding on both sides. For this reason, both contact angle and absorption must be considered when discussing the wettability of a solid. These factors complicate interpretation of the results, however the magnitude of the differences observed in both contact angle and absorbed volume leave little doubt as to the efficacy of the EDTA/tween regimen in increasing the wettability of dentin.

**Sealer Penetration**

To correlate the contact angle of surfactant-treated dentin to a clinically meaningful parameter, penetration of the sealer into dentinal tubules was evaluated. The results of the pilot study agreed with Jeong in validating use of Fluo-3 pentaamonium salt to trace penetration of calcium silicate-based endodontic sealer into dentinal tubules(60). Where a concentrated aqueous solution of fluo-3 pentaamonium marker was introduced to the canal in place of labeled BC Sealer™, no signal was observed within the dentinal tubules that can be attributed to the compound's nearly 100-fold increase in fluorescence when in the presence of free calcium. Since the marker's signal diminishes dramatically in the absence of dissociated calcium, concern that leaching of the marker ahead of the sealer itself is significantly reduced. Whereas the previous study used Smartpastebio, the current study is to our knowledge the first to apply this technique to the more widely used EndoSequence BC Sealer™ product.

The null hypothesis that there was no difference between groups exposed to a final rinse of EDTA alone and those in the EDTA/tween, SmearClear™, and EDTA/ethanol groups was rejected. Both the mean and maximum tubule penetration values observed in the EDTA/Tween group were significantly greater than those observed in the group exposed to EDTA alone.

The mean tubule penetration observed for the EDTA group was 473um, which was marginally greater than that observed by Jeong in the case of Smartpastebio (318um), but substantially less than the values reported by Chandra, et al for AH Plus epoxy sealer(60, 67). This can be attributed to differences in the particle size, viscosity, and surface tension of the various sealers, as well as potential methodological differences such as the duration of EDTA application and its concentration(36).

Percent sealer penetration was also assessed as a control for the relative efficacy of smear layer removal between groups. Several investigators have reported that intact smear layer is impenetrable to endodontic sealer and its removal is likely the principal determinate of the proportion of the canal surface area into which tubular penetration of sealer occurs(40, 78). In the present study, the exposure to EDTA, both in terms of concentration and duration, was closely regulated to minimize variability of the patency and diameter of exposed dentinal tubules. The results indicate that the proportion of the canal wall into which at least some penetration occurred was unaffected by the introduction of surface active agents. This is in agreement with the findings of Tuncer, who observed no significant difference in percent tubule penetration between specimens rinsed with QMix® (EDTA, chlorhexidine and polysorbate 80) or a mixture of EDTA

and chlorhexidine alone(79, 55). Consistency in percent penetration between groups confirms the uniformity of smear layer removal and eliminates inter-group differences in smear layer removal as a confounding factor in the absorption and contact angle analyses.

Taken in aggregate, the findings of the present study indicate that the introduction of surface active agents to the canal space prior to obturation increases tubule penetration by increasing wettability of dentin. Figures 14 and 15 demonstrate that both contact angle and absorption appear to be predictors of tubule penetration. These differences were significant in the case of the EDTA/Tween group, whereas SmearClear™ and EDTA/Ethanol group generally trended in the same direction as the EDTA/Tween group.

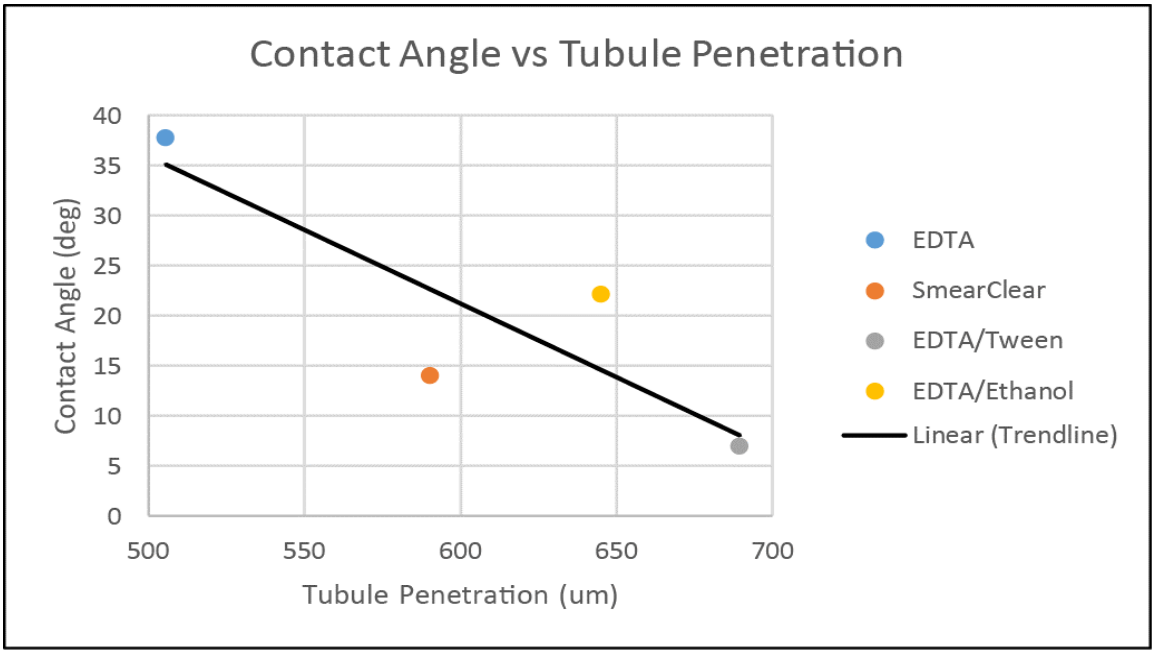



Figure 14: Plot of contact angle vs tubule penetration, with linear regression trend line.

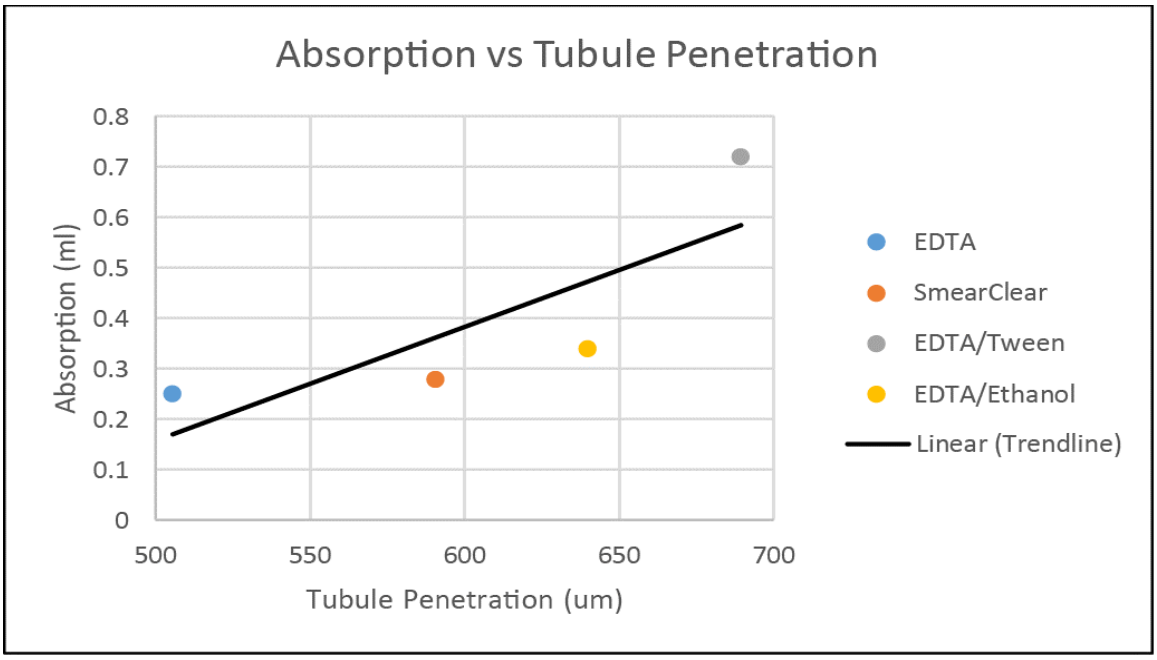



Figure 15: Plot of absorption vs tubule penetration, with linear regression trend line.

## Limitations

The use of deidentified human teeth as specimens in this study was both a strength and a significant liability. This research is intended to be translational and guide clinicians' practices on human patients. Using samples which are physically and anatomically representative of teeth encountered in clinical practice has obvious advantages. However, the pool of specimens must be assumed to have been somewhat heterogenous with respect to age and this is known to have an effect on the diameter of dentinal tubules, which may in turn have contributed to variability in tubule penetration.

A further limitation of the study was the potential for dissociation of the fluo-3 marker from BC Sealer™, resulting in overestimation of the extent of tubule penetration. Analysis of a group in which fluo-3 was introduced without sealer indicated that inadequate signal was produced by the marker alone, but leaching of both calcium and marker might yet be a source of error. Preparation of selected samples for SEM analysis would provide a more stringent control in this regard.

## Future Directions

The clinical advantages of increased penetration of root dentin by endodontic sealer are thought to be reduced leakage of metabolites, irritants, and microbial pathogens into and out of the root canal system. None of these clinically meaningful parameters could be directly evaluated in the present study. Further research is needed to more precisely define the clinical effects of the addition of 2% Tween to 17% EDTA prior to recommending its use in patient care.

## CONCLUSIONS

Within the limitations of this study, it can be concluded that:

1. Treatment of dentin with 17% EDTA/2% Tween, SmearClear™, or a final rinse of 95% ethanol significantly enhances wettability of human root dentin.
2. Application of 17% EDTA/2%Tween prior to obturation results in increased penetration of EndoSequence BC Sealer™ when used with a synchronized hydraulic condensation obturation technique.
3. Decreased contact angle is correlated with increased penetration of EndoSequence BC Sealer™ when used with a synchronized hydraulic condensation obturation technique.

## BIBLIOGRAPHY


1. Kvist T, Molander A, Dahlén G, Reit C. Microbiological Evaluation of One- and Two-Visit Endodontic Treatment of Teeth with Apical Periodontitis : A Randomized , Clinical Trial. 2004;(1):572–6.
2. Stevens RW, Strother JM, McClanahan SB. Leakage and Sealer Penetration in Smear-free Dentin After a Final Rinse With 95% Ethanol. J Endod. 2006;32(8):785–8.
3. Cunningham WT, Cole JS, Ph D. Effect of alcohol on the spreading ability of sodium hypochlorite endodontic irrigant. Oral Surg Oral Med Oral Pahol. 1980 54(3):333-335.
4. Cameron JA. The effect of a fluorocarbon surfactant on the surface tension of the endodontic irrigant , sodium hypochlorite . A preliminary report. Aus Den J. 1986;31(5):3-364-366
5. Dutner J, Mines P, Anderson A. Irrigation Trends among American Association of Endodontists Members : A Web-based Survey. J Endod. 2012;38(1):37–40.
6. Carvalho NK, Prado MC, Senna PM, Neves AA, Souza EM, Fidel SR, et al. Do smear-layer removal agents affect the push-out bond strength of calcium silicate-based endodontic sealers? Int Endod J. 2016;1–8.
7. De-Deus G, Brandao MC, Leal F, Reis C, Souza EM, Luna AS, et al. Lack of correlation between sealer penetration into dentinal tubules and sealability in

nonbonded root fillings. Int Endod J. 2012;45(7):642–51.

8. Zhang H, Shen Y, Ruse ND, Haapasalo M. Antibacterial Activity of Endodontic Sealers by Modified Direct Contact Test Against Enterococcus faecalis. J Endod. 2009;35(7):1051–5.

9. Schilder H. Cleaning and shaping the root canal. Dent Clin North Am [Internet]. 1974 Apr;18(2):269–96.

10. Du T, Wang Z, Shen Y, Ma J, Cao Y, Haapasalo M. Effect of long-term exposure to endodontic disinfecting solutions on young and old Enterococcus faecalis biofilms in dentin canals. J Endod. 2014;40(4):509–14.

11. Ruff ML, McClanahan SB, Babel BS. In vitro antifungal efficacy of four irrigants as a final rinse. J Endod. 2006;32(4):331–3.

12. Rôças IN, Siqueira JF. Comparison of the in vivo antimicrobial effectiveness of sodium hypochlorite and chlorhexidine used as root canal irrigants: A molecular microbiology study. J Endod. 2011;37(2):143–50.

13. Clegg MS, Vertucci FJ, Walker C, Belanger M, Britto LR. The Effect of Exposure to Irrigant Solutions on Apical Dentin Biofilms In Vitro. J Endod. 2006;32(5):434–7.

14. Ng YL, Mann V, Gulabivala K. A prospective study of the factors affecting outcomes of nonsurgical root canal treatment: Part 1: Periapical health. Int Endod J. 2011;44(7):583–609.

15. Haapasalo M, Shen Y. Irrigation in Endodontics Endodontics Irrigation Root canal Irrigant. Dent Clin NA. 2010;54(2):291–312.

16. Keles A, Alcin H, Kamalak A, Versiani MA. Micro-CT evaluation of root filling quality in oval-shaped canals. Int Endod J. 2014;47(12):1177–84.

17. Marciano MA, Ordinola-Zapata R, Cunha TVRN, Duarte MAH, Cavenago BC, Garcia RB, et al. Analysis of four gutta-percha techniques used to fill mesial root canals of mandibular molars. Int Endod J. 2011;44(4):321–9.

18. Saw LH, Messer HH. Root Strains Associated With Different Obturation Techniques. J Endod. 1995;21(6):314–20.

19. Zmener O, Pameijer CH, Serrano SA, Vidueira M, Macchi RL. Significance of Moist Root Canal Dentin with the Use of Methacrylate-based Endodontic Sealers: An In Vitro Coronal Dye Leakage Study. J Endod. 2008;34(1):76–9.

20. Lertchirakarn V, Palamara JEA, Messer HH. Load and strain during lateral condensation and vertical root fracture. J Endod. 1999;25(2):99–104.

21. Collins J, Walker MP, Kulild J, Lee C. A Comparison of Three Gutta-Percha Obturation Techniques to Replicate Canal Irregularities. J Endod. 2006;32(8):762–5.

22. Schilder H, Goodman A, Aldrich W. The Thermomechanical Properties of Gutta-percha. Part V. Volume Changes in Bulk Gutta-percha as a Function of Temperature and its Relationship to Molecular Phase Transformation. Oral Surg Oral Med Oral Pahol. 1985 Mar;59(3):285-96.

23. Buchanan S. Continuous Wave of Obturation Technique. Endodontic Practice. 1998;1:7-23

24. Pommel L, About I, Pashley D, Camps J. Apical Leakage of Four Endodontic

Sealers. J Endod. 2003;29(3):208–10.

25. Zhou H, Shen Y, Zheng W, Li L, Zheng Y. Physical Properties of 5 Root Canal Sealers. 2013;39(10):1281–6.

26. Siqueira JF, Bruno R. Antibacterial Activities of Root Canal Sealers Against Selected Anaerobic Bacteria. 1996;22:0–1.

27. Mcmichen FRS, Pearson G, Rahbaran S, Gulabivala K. A comparative study of selected physical properties of five root-canal sealers. 2003;629–35.

28. Zhou H, Shen Y, Zheng W, Li L, Zheng Y, Haapasalo M. Physical properties of 5 root canal sealers. J Endod. 2013;39(10):1281–6.

29. Camilleri J. Evaluation of Selected Properties of Mineral Trioxide Aggregate Sealer Cement. J Endod. 2009;35(10):1412–7.

30. Wang Z, Shen Y, Haapasalo M. Dentin Extends the Antibacterial Effect of Endodontic Sealers Against Enterococcus Faecalis Biofilms. J Endod. 2014;40(4):505–8.

31. Zhou H, Du T, Shen Y. In Vitro Cytotoxicity of Calcium Silicate – containing Endodontic Sealers. J Endod. 2015;41(1):1–6.

32. Chang SW, Lee SY, Kang SK, Kum KY, Kim EC. In Vitro Biocompatibility, Inflammatory Response, and Osteogenic Potential of 4 Root Canal Sealers: Sealapex, Sankin Apatite Root Sealer, MTA Fillapex, and iroot SP root canal sealer. J Endod. 2014;40(10):1642–8.

33. Salles LP, Gomes-Cornelio AL, Guimares FC, Herrera BS, Bao SN, Rossa-Junior C, et al. Mineral Trioxide Aggregate-based endodontic Sealer Stimulates

Hydroxyapatite Nucleation in Human Osteoblast-like Cell Culture. J Endod. 2012;38(7):971–6.

34. Bird DC, Komabayashi T, Guo L, Opperman LA, Spears R. In Vitro Evaluation of Dentinal Tubule Penetration and Biomineralization Ability of a New Root-end Filling Material. J Endod 2012;38(8):1093–6.

35. Samiei M, Aghazade M, Farhadi F, Shahveghar N, Torab A, Vahid Pakdel SM. Sealing Efficacy of Single-cone Obturation Technique with MTA and CEM Cement: An in Vitro Bacterial Leakage Study. J Dent Res Dent Clin Dent Prospects. 2014;8(2):77–83.

36. McMichael GE, Primus CM, Opperman LA. Dentinal Tubule Penetration of Tricalcium Silicate Sealers. J Endod. 2016;42(4):632–6.

37. Mccomb D, Smith DC. A Preliminary Scanning Electron Microscopic Study of Root Canals After Procedures. J Endod. 1975 Jul;1(7):238-42

38. Wang Z, Shen Y, Haapasalo M. Effect of Smear Layer against Disinfection Protocols on Enterococcus faecalis – infected Dentin. J Endod. 2013;39(11):1395–400.

39. Drake DR, Wiemann AH, Rivera EM, Walton RE. Bacterial Retention in Canal Walls In Vitro: Effect of Smear Layer. 1994;20(2):78–82.

40. White RR, Goldman M, Lin PS. The influence of the smeared layer upon dentinal tubule penetration by endodontic filling materials. Part II. J Endod. 1987;13(8):369–74.

41. Uitto V, Haapasalo M, Laakso T, Degradation ST. Degradation of Basement

Membrane Collagen by Proteases From Some Anaerobic Oral micro- Organisms. Oral Microbiol Immunol. 1988;97–102.

42. Yamada RS, Armas A, Goldman M, Lin PS. A Scanning Electron Microscopic Comparison of a High Volume Final Flush with Several Irrigating Solutions : Part 3. J Endod. 1983;9(4):137–42.

43. Hebbar RS, Isloor AM, Ismail AF. Chapter 12 - Contact Angle Measurements. Membrane Characterization. Elsevier B.V.; 2017. 219-255 p.

44. Rapp BE, Rapp BE. Surface Tension. Microfluid Model Mech Math. 2017 Jan 1;421–44.

45. Giardino L, Ambu E, Becce C, Rimondini L, Morra M. Surface Tension Comparison of Four Common Root Canal Irrigants and Two New Irrigants Containing Antibiotic. J Endod. 2006;32(11):1091–3.

46. Abou-Rass M, Patonai FJ. The Effects of Decreasing Surface Tension on the Flow of Irrigating Solutions in Narrow Root Canals. Oral Surgery, Oral Med Oral Pathol. 1982;53(5):524–6.

47. Cameron J a. The effect of a Fluorocarbon Surfactant on the Surface Tension of the Endodontic Irrigant, Sodium Hypochlorite. A Preliminary Report. Aust Dent J . 1986;31(5):364–8.

48. Guastalli AR, Clarkson RM. The Effect of Surfactants on the Stability of Sodium Hypochlorite Preparations. J Endod. 2015;41(8):1344–8.

49. Khedmat S, Shokouhinejad N. Comparison of the Efficacy of Three Chelating Agents in Smear Layer Removal. J Endod. 2008;34(5):599–602.

50. Yadav HK, Tikku AP, Chandra A, Yadav RK. Efficacy of Etidronic Acid , BioPure MTAD and SmearClear in Removing Calcium Ions From the Root Canal : An in Vitro Study. J Endod.2015;523–8.

51. Stojicic S, Shen Y, Qian W, Johnson B, Haapasalo M. Antibacterial and smear layer removal ability of a novel irrigant, QMix. Int Endod J. 2012;45(4):363–71.

52. Dai L, Khechen K, Khan S, Gillen B, Loushine BA, Wimmer CE, et al. The Effect of QMix, an Experimental Antibacterial Root Canal Irrigant, on Removal of Canal Wall Smear Layer and Debris. J Endod. 2011;37(1):80–4.

53. Tay FR, Gutmann JL, Pashley DH. Microporous, Demineralized Collagen Matrices in Intact Radicular Dentin Created by Commonly Used Calcium-depleting Endodontic Irrigants. J Endod. 2007;33(9):1086–90.

54. Jardine AP, Rosa RA da, Santini MF, Wagner M, Só MVR, Kuga MC, et al. The Effect of Final Irrigation on the Penetrability of an Epoxy Resin-based Sealer Into Dentinal Tubules: a Confocal Microscopy Study. Clin Oral Investig. 2016;20(1):117–23.

55. Tuncer AK. Effect of QMix 2in1 on Sealer Penetration into the Dentinal Tubules. J Endod. 2015;41(2):257–60.

56. Sen B, Wesselink P, Turkun M. The Smear Layer; a Phenomenon in Root Canal Therapy. Int Endod J. 1995;28(3):141-148

57. Okşan T, Aktener BO, Şen BH, Tezel H. The Penetration of Root Canal Sealers Into Dentinai Tubules. A Scanning Electron Microscopic Study. Int Endod J. 1993;26(5):301–5.

58. Wu M-K, Wesselink PR. Endodontic Leakage Studies Reconsidered. Part I. Methodology, Application and Relevance. Int Endod J. 1993 Jan;26(1):37–43.

59. Mamootil K, Messer HH. Penetration of Dentinal Tubules by Endodontic Sealer Cements in Extracted Teeth and in Vivo. Int Endod J. 2007;40(11):873–81.

60. Jeong JW, Degraft-johnson A, Dorn SO, Fiore PM Di. Dentinal Tubule Penetration of a Calcium Silicate – based Root Canal Sealer with Different Obturation Methods. J Endod. 2017;43(4):633–7.

61. Han L, Okiji T. Bioactivity Evaluation of Three Calcium Silicate-based Endodontic Materials. Int Endod J. 2013;46(9):808–14.

62. Ordinola-Zapata R, Bramante CM, Graeff MSZ, del Carpio Perochena A, Vivan RR, Camargo EJ, et al. Depth and Percentage of Penetration of Endodontic Sealers Into Dentinal Tubules After Root Canal Obturation Using a Lateral Compaction Technique: A Confocal Laser Scanning Microscopy Study. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology. 2009;108(3):450–7.

63. Patel D V, Sherriff M, Ford TRP, Watson TF, Mannocci F. The Penetration of RealSeal primer and Tubliseal into Root Canal Dentinal Tubules : a Confocal Microscopic Study. 2007;(Grossman 1982):67–71.

64. Paredes RM, Etzler JC, Watts LT, Zheng W, Lechleiter JD. Chemical Calcium Indicators. Methods. 2008;46(3):143–51.

65. Xuereb M, Vella P, Damidot D, Sammut C V., Camilleri J. In Situ Assessment of the Setting of Tricalcium Silicate-based Sealers Using a Dentin Pressure Model. J Endod. 2015;41(1):111–24.

66. Kokkas AB, Boutsioukis AC, Vassiliadis LP, Stavrianos CK. The Influence of the Smear Layer on Dentinal Tubule Penetration Depth by Three Different Root Canal Sealers: an in Vitro Study. J Endod. 2004;30(2):100–2.

67. Chandra SS, Shankar P, Indira R. Depth of penetration of four resin sealers into radicular dentinal tubules: A confocal microscopic study. J Endod. 2012;38(10):1412–6.

68. Wu M, Fan B, Wesselink PR. Diminished leakage along root canals filled with gutta-percha without sealer over time : a laboratory study. Int Endod J. 2000;(1):121–5.

69. Skinner R, Himmel V. The Sealing Ability of Injection-molded Thermoplasticized Gutta-Percha With and Without the Use of Sealers J Endod. 1987;13(7):315-7.

70. Pommel L, CACamps J. In Vitro Apical Leakage of System B Compared with Other Filling Techniques. J Endod. 2001;27(7):449–51.

71. Eick, Gwinnett, Pashley R. Current Concepts on Adhesion to Dentin. Crit Rev Oral Biol Med. 1997;8(3):306-35

72. Al-Omari WM, Mitchell CA, Cunningham JL. Surface Roughness and Wettability of Enamel and Dentine Surfaces Prepared with Different Dental Burs. J Oral Rehabil. 2001;28(7):645–50.

73. Erickson RL. Surface interactions of dentin adhesive materials. Oper Dent. 1992;Suppl 5:81–94.

74. Hu X, LIng J, Gao Y. Effects of Irrigation Solutions on Dentin Wettability and Roughness. J Endod. 2010;36(6):1064–7.

75. Phani Mohan R, Raghavendra Vivekananda Pai A, Vivekananda Pai A. The Comparison Between Two Irrigation Regimens on the Dentine Wettability for an Epoxy Resin Based Sealer by Measuring its Contact Angle Formed to the Irrigated Dentin. J Conserv Dent Jul-Aug. 2015;18(4):275–8.

76. Fahmy S, El Ashry S, El Gendy A. Dentin Wettability Enhancement For Three Irrigating Solutions and Their Effect on Push Out Bond Strength of Gutta-Percha / AH Plus. J Clin Exp Dent. 2015;7(2):e237–42.

77. Ballal NV, Tweeny A, Khechen K, Prabhu KN, Satyanarayan, Tay FR. Wettability of Root Canal Sealers on Intraradicular Dentine Treated With Different Irrigating Solutions. J Dent. 2013;41(6):556–60.

78. Persson CM, Persson M. Surfactants at Non-polar Surfaces Doctoral Thesis Stockholm 2002. 2002.

79. Kara Tuncer A, Tuncer S. Effect of Different Final Irrigation Solutions on Dentinal Tubule Penetration Depth and Percentage of Root Canal Sealer. J Endod. 2012;38(6):860–3.

# Appendix I: Sealer Penetration Measurements

## EDTA

| Specimen | Maximum Penetration | Maximum Penetration | Mean Penetration | 3:00 | 6:00 | 9:00 | 12:00 |
|---|---|---|---|---|---|---|---|
| Specimen | Max | % | AVG | N | S | E | W |
| **15** | 1131 | 100.0% | 797.25 | 740 | 488 | 830 | 1131 |
| **56** | 1198 | 57.5% | 445.5 | 932 | 0 | 201 | 649 |
| **35** | 1195 | 76.0% | 510.25 | 936 | 0 | 584 | 521 |
| **28** | 789 | 100.0% | 543.5 | 471 | 387 | 599 | 717 |
| **41** | 1273 | 100.0% | 450.75 | 1258 | 446 | 35 | 64 |
| **69** | 632 | 74.7% | 173.25 | 406 | 97 | 0 | 190 |
| **23** | 353 | 100.0% | 80.75 | 134 | 47 | 83 | 59 |
| **3** | 1137 | 100.0% | 433.75 | 495 | 662 | 477 | 101 |
| **63** | 468 | 70.1% | 324.5 | 255 | 254 | 429 | 360 |
| **58** | 792 | 85.1% | 394.5 | 792 | 584 | 75 | 127 |
| **46** | 993 | 100.0% | 738.75 | 528 | 753 | 993 | 681 |
| **48** | 846 | 100.0% | 350.25 | 473 | 130 | 283 | 515 |
| **47** | 392 | 100.0% | 183 | 392 | 113 | 143 | 84 |
| **24** | 933 | 100.0% | 541.5 | 699 | 148 | 355 | 964 |
| **31** | 1078 | 100.0% | 677 | 396 | 759 | 491 | 1062 |
| **17** | 146 | 100.0% | 94.5 | 71 | 206 | 47 | 54 |
| **14** | 1322 | 100.0% | 759.75 | 807 | 55 | 954 | 1223 |
| **53** | 1043 | 100.0% | 680 | 831 | 324 | 763 | 802 |
| **75** | 1011 | 100.0% | 768 | 806 | 590 | 881 | 795 |
| **68** | 804 | 88.3% | 408.5 | 0 | 513 | 317 | 804 |
| **Mean** | **876.8** | **0.925819** | **467.7625** | | | | |
| **Standard Deviation** | **339.9979** | **0.132487** | **230.64978** | | | | |

## SmearClear™

| Specimen | Maximum Penetration | Maximum Penetration | Mean Penetration | 3:00 | 6:00 | 9:00 | 12:00 |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| **Specimen** | **Max** | **%** | **AVG** | N | S | E | W |
| **16** | 836 | 61.8% | 484.75 | 0 | 559 | 544 | 836 |
| **36** | 924 | 100.0% | 487 | 503 | 613 | 170 | 662 |
| **2** | 883 | 76.9% | 365.75 | 0 | 380 | 357 | 726 |
| **54** | 1335 | 69.6% | 909.75 | 849 | 1092 | 612 | 1086 |
| **1** | 952 | 100.0% | 620.25 | 761 | 841 | 185 | 694 |
| **63** | 738 | 95.6% | 544 | 738 | 545 | 733 | 160 |
| **21** | 950 | 100.0% | 759 | 901 | 950 | 637 | 548 |
| **25** | 897 | 100.0% | 470.75 | 126 | 295 | 565 | 897 |
| **70** | 708 | 100.0% | 388.25 | 708 | 618 | 145 | 82 |
| **34** | 1196 | 100.0% | 1092.5 | 1196 | 1102 | 1065 | 1007 |
| **26** | 1275 | 100.0% | 694 | 414 | 514 | 814 | 1034 |
| **7** | 1474 | 65.5% | 619.25 | 508 | 0 | 1282 | 687 |
| **27** | 1022 | 100.0% | 632.5 | 750 | 101 | 664 | 1015 |
| **60** | 865 | 86.6% | 625 | 865 | 0 | 729 | 906 |
| **37** | 1204 | 100.0% | 641 | 968 | 832 | 569 | 195 |
| **74** | 1349 | 100.0% | 1014.75 | 1349 | 1265 | 960 | 485 |
| **49** | 989 | 100.0% | 723.5 | 633 | 501 | 771 | 989 |
| **67** | 1219 | 89.9% | 608.75 | 147 | 328 | 1219 | 741 |
| **42** | 580 | 100.0% | 457.5 | 580 | 421 | 503 | 326 |
| **72** | 914 | 100.0% | 631.75 | 885 | 602 | 696 | 344 |
| **Average** | **1010.417** | **0.91442** | **648.93056** | | | | |
| **Standard Deviation** | **232.8001** | **0.114608** | **190.58166** | | | | |

## EDTA/Tween

| Specimen | Maximum Penetration | Maximum Penetration | Mean Penetration | 3:00 | 6:00 | 9:00 | 12:00 |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| **Specimen** | **Max** | **%** | **AVG** | N | S | E | W |
| **39** | 1379 | 100.0% | 949 | 1379 | 1102 | 675 | 640 |
| **19** | 894 | 100.0% | 549 | 783 | 516 | 533 | 364 |
| **10** | 1216 | 100.0% | 857.5 | 1216 | 954 | 414 | 846 |
| **65** | 1678 | 100.0% | 959.75 | 1056 | 591 | 1678 | 514 |
| **78** | 1080 | 100.0% | 819.5 | 870 | 566 | 1063 | 779 |
| **5** | 1137 | 100.0% | 562.5 | 364 | 30 | 719 | 1137 |
| **79** | 1341 | 100.0% | 725.25 | 307 | 1341 | 182 | 1071 |
| **20** | 1327 | 100.0% | 1063.75 | 809 | 1107 | 1327 | 1012 |
| **43** | 1651 | 100.0% | 603.5 | 1651 | 763 | 0 | 0 |
| **11** | 1199 | 100.0% | 482.5 | 501 | 1199 | 0 | 230 |
| **13** | 1047 | 100.0% | 758.75 | 1047 | 975 | 413 | 600 |
| **33** | 1292 | 100.0% | 645.5 | 655 | 593 | 904 | 430 |
| **40** | 1437 | 55.7% | 891.25 | 1437 | 967 | 537 | 624 |
| **52** | 929 | 100.0% | 516 | 308 | 27 | 929 | 800 |
| **29** | 910 | 84.0% | 465 | 493 | 894 | 98 | 375 |
| **71** | 1003 | 100.0% | 485.25 | 113 | 306 | 643 | 879 |
| **4** | 1465 | 60.4% | 663.5 | 1311 | 1278 | 65 | 0 |
| **73** | 1612 | 79.5% | 816.25 | 1612 | 1399 | 189 | 65 |
| **76** | 1128 | 100.0% | 667 | 1042 | 562 | 328 | 736 |
| **50** | 894 | 97.0% | 652.75 | 617 | 814 | 454 | 726 |
| **Average** | 1230.95 | 0.962283022 | 708.375 | | | | |
| **Standard Deviation** | 243.6103446 | 0.115316312 | 196.1138487 | | | | |

## EDTA/Ethanol

| Specimen | Maximum Penetration | Maximum Penetration | Mean Penetration | 3:00 | 6:00 | 9:00 | 12:00 |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| **Specimen** | **Max** | **%** | **AVG** | N | S | E | W |
| **12** | 733 | 100.00% | 545.3 | 733 | 584 | 226 | 638 |
| **38** | 1027 | 100.00% | 759.0 | 807 | 431 | 1027 | 771 |
| **8** | 699 | 63.92% | 346.0 | 699 | 0 | 0 | 685 |
| **6** | 1231 | 88.41% | 737.3 | 1231 | 803 | 807 | 108 |
| **32** | 668 | 100.00% | 449.8 | 146 | 802 | 183 | 668 |
| **64** | 1008 | 100.00% | 678.3 | 715 | 1008 | 560 | 430 |
| **66** | 1179 | 46.06% | 450.3 | 455 | 1179 | 167 | 0 |
| **30** | 747 | 100.00% | 616.8 | 591 | 747 | 527 | 602 |
| **77** | 1227 | 100.00% | 765.8 | 657 | 807 | 1227 | 372 |
| **61** | 780 | 100.00% | 424.3 | 780 | 573 | 261 | 83 |
| **18** | 1066 | 84.35% | 306.8 | 1066 | 76 | 85 | 0 |
| **22** | 1140 | 100.00% | 912.5 | 1140 | 1044 | 708 | 758 |
| **80** | 756 | 75.57% | 463.8 | 475 | 0 | 756 | 624 |
| **9** | 1194 | 100.00% | 999.3 | 477 | 918 | 1408 | 1194 |
| **51** | 129 | 90.00% | 717.0 | 1090 | 1229 | 474 | 75 |
| **45** | 1769 | 100.00% | 798.3 | 1769 | 809 | 355 | 260 |
| **55** | 1303 | 58.67% | 680.0 | 0 | 311 | 1106 | 1303 |
| **59** | 1300 | 83.42% | 788.5 | 1300 | 64 | 839 | 951 |
| **44** | 1206 | 100.00% | 805.8 | 1090 | 1206 | 337 | 590 |
| **57** | 904 | 100.00% | 657.8 | 886 | 904 | 345 | 496 |
| **Average** | 1003.3 | 0.9 | 645.1 | | | | |
| **Standard Deviation** | 342.33 | 0.16 | 189.59 | | | | |

## Appendix II: Sessile Drop Measurements

**EDTA**

| Specimen | Static Contact Angle | Variability | Absorbed Volume |
|---|---|---|---|
| **5** | 32.2 | 29.9 | 0.2 |
| **22** | 50.3 | 36.5 | 0.4 |
| **11** | 34.4 | 43.6 | 0.0 |
| **30** | 25.5 | 47.2 | 0.8 |
| **37** | 32 | 42.1 | 0.3 |
| **15** | 41.2 | 15.6 | 0.4 |
| **6** | 30.7 | 34.7 | 0.3 |
| **27** | 42.2 | 27.1 | 0.1 |
| **8** | 42.2 | 27.1 | -0.1 |
| **21** | 47.5 | 28.9 | 0.1 |

**Smear Clear**

| Specimen | Static Contact Angle | Variability | Absorbed Volume |
|---|---|---|---|
| 40 | 7.9 | 47.7 | 0.6 |
| 14 | 10.1 | 41.7 | -0.5 |
| 39 | 20.2 | 42.5 | 0.2 |
| 17 | 21.9 | 26.8 | 0.4 |
| 10 | 11.3 | 38.2 | 0.5 |
| 32 | 8.3 | 55.2 | 0.3 |
| 20 | 3.9 | 50.6 | 1.5 |
| 18 | 36.2 | 27.9 | -0.1 |
| 23 | 7.7 | 43.4 | 0.8 |
| 1 | 13.2 | 41.7 | 0.2 |

**EDTA/Tween**

| Specimen | Static Contact Angle | Variability | Absorbed Volume |
|---|---|---|---|
| 24 | 1.8 | 58.4 | 1.2 |
| 19 | 7.9 | 52.9 | 0.6 |
| 33 | 10.1 | 53.7 | 0.5 |
| 31 | 4 | 43.3 | 1.3 |
| 36 | 6.9 | 48.9 | 0.9 |
| 12 | 7.7 | 55.8 | 0.6 |
| 38 | 9.9 | 54.3 | 1.1 |
| 3 | 8 | 45.5 | 0.3 |
| 16 | 12.2 | 46.4 | 0.4 |
| 29 | 12.6 | 51.8 | 0.3 |

**EDTA/Ethanol**

| Specimen | Static Contact Angle | Variability | Absorbed Volume |
|---|---|---|---|
| 26 | 12.6 | 30.9 | -0.1 |
| 7 | 27.6 | 39.5 | 0.4 |
| 4 | 37.7 | 20.2 | 0.1 |
| 9 | 14.9 | 46.2 | 0.6 |
| 34 | 17.7 | 60.9 | 0.7 |
| 13 | 37.6 | 50.9 | 0.3 |
| 28 | 15.8 | 31.5 | 0.5 |
| 25 | 16.1 | 29.3 | 0.2 |
| 2 | 23.5 | 26.6 | 0.4 |
| 35 | 18.1 | 26.6 | 0.3 |