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Comparison of an Ultrasonic and a Non-Ultrasonic Endodontic Irrigation Protocol: A Clinical Study

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Comparison of an Ultrasonic and a Non-Ultrasonic Endodontic Irrigation Protocol: A Clinical Study

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Master of Dental Science Thesis

Comparison of an Ultrasonic and a Non-Ultrasonic Endodontic Irrigation Protocol: A Clinical Study

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Introduction

History Background of Endodontics

The study of oral and odontogenic disease has come quite a distance from the first discovery of oral bacteria or "animalcules" in the late 1600's[1]. As early as 1894 the scientific community has associated pulpal disease with bacteria due to the discovery by Miller that bacteria could infect and persist in the pulpal tissues causing pulpal changes[2]. While this study changed the way we looked at bacterial involvement in the pulpal symptoms and pulpal changes of patients it was not until the 1960's that it was truly associated with endodontic pathology. The seminal paper by Kakehashi et al. was the first to definitively show that the presence of bacteria in the pulpal tissues leads to pulpal pathology and periapical breakdown[3]. In this study Kakehashi illustrated this finding by using an animal study involving one group of germ-free rats that had pulpal exposure and a second group of standard rats with normal oral bacteria and a similar pulpal exposure. In the germ-free rats the exposed pulpal tissue and periapical tissues showed no signs of pathology or injury while those of the germ-present rats showed signs of pulpal necrosis and periapical periodontitis. It was to give the fledgling field of Endodontics the direction it needed to direct treatment and scientific investigation aimed at more definitive treatment.

The thought that treatment of the pulpal chamber and root canal systems was a viable treatment had been challenged by a multitude of factors during the first half of the 20th century. The work of Hess and others had shown the root canal system to be a maze of interconnecting lateral canals, ramifications, fins and

isthmi [4,5,6,7,8]. It is this complexity that led L. Grossman to remark "One may well ask at this point if root canal work is justified in view of the complexity of the canals, since by no method can all the minute ramifications be filled"[9]. This underlying conclusion had even gained popularity in the early 1900's due to the broad acceptance of the theory of "focal infection". It was Hunter in 1910 who introduced the concept of "oral sepsis" and that this condition would lead to a wide array of systemic disease, such as gastritis, anemia, ulcers, colitis and nephritis[10]. This idea of the teeth being a reservoir of infection leading to systemic disease would lead to widespread extraction of teeth as a supposed superior treatment rather than restorative dentistry. Proponents of this theory would use invalid scientific means in determining causes of systemic bacteria by culturing extracted teeth and then correlating these findings with distant organ pathology[11,12]. This adoption of a flawed theory resulted in excessive and unnecessary tooth extraction as it was felt that not only pulp-less teeth but rather any tooth that could be compromised by inflammation or periodontal pathology was deemed a risk for systemic infection. The widespread medical sentiment was that teeth were simply reservoirs for infection and were related to such far-reaching diseases as tonsillitis, eye disease, arthritis, cholecystitis and diabetes [13,14,15].

It was by defending and improving the treatment of endodontics with sound research and valid discussion that endodontic treatment began again to be seen as an appropriate treatment. With growing quality and success of non-surgical and surgical endodontic treatment and an awareness of the

microbial populations and their eradication the theory of focal infection was largely dismissed. A growing number of dental professionals dedicated to the development and study of root canal treatment began the American Association of Endodontics in 1943. This specialization in the treatment of endodontic pathology was further recognized by the American Dental Association in 1963 as an official specialty area of dentistry.

It is only with progressive understanding of the biological systems involved in endodontic pathology that we have been able to overcome these barriers to treatment. By identifying bacteria as the underlying cause of periapical periodontitis and clearly showing the therapeutic benefit of removing these bacteria and their byproducts the field of endodontics has developed and continues to improve the standards of treatment.

Microbiology of Endodontics

Microbiology of Endodontics- Introduction

It is through the work of Kakehashi that we are able to directly identify bacteria as the causative agent in periapical periodontitis. The oral cavity is a veritable ocean of bacteria with over 700 bacterial species as possible residents[16]. In fact the nature of endodontic infection is one of polymicrobial interactions and this heterogeneity of the bacterial population is part of the pathogenicity in periapical periodontitis[17,18]. While the oral cavity is populated by a wide array of bacteria the endodontic microbiological community consists of a consistent family of bacteria.

The bacteria are able to invade the pulpal space and tissues by means of caries, dental surgical procedures, trauma-induced cracks and fractures of the coronal and radicular tooth structure. While bacteria can be found in necrotic pulpal tissue with an intact crown this mode of invasion is likely related to trauma to the periodontium or through exposed dentinal tubules[19]. The theory that bacteria could seed the necrotic pulpal tissue by route of the blood stream, known as anachoresis, has been largely dismissed. In the study by Moller et al. the devitalized pulps of the entire sample (n=26) of monkey teeth were sterile and necrotic for more than 6 months suggesting that bacterial infection by anachoresis is not likely [20].

The endodontic infection is a dynamic process with different bacteria playing different roles in the progression of the disease. The progression from a carious crown to pulpal infection involves bacteria that are facultative anaerobes due to the higher oxygen content in the coronal tooth structure. This oxygen level relationship changes along the length of the root canal and apically the obligate anaerobic bacteria dominate the microbial landscape[21, 22, 23]

The primary endodontic infection is characterized by Gram-negative anaerobic rods[24]. The most common species recognized in these primary endodontic infections come from the *Prevotella*, *Porphyromonas* and *Tannerella* genera[25,26,27]. Other commonly found species include the Fusobacterium genus represented by *Fusobacterium nucleatum* and spirochetes from the *Treponema* genus[28,29]. While it has been suggested that Gram-negative bacteria are associated with symptomatic infections it has also been shown that these types

of bacteria are present in asymptomatic infections as well[30,31,32].

While the primary endodontic infection is characterized by the presence of obligate anaerobic Gram-negative bacteria, the secondary endodontic infection unresponsive to treatment is characterized by the presence of facultative anaerobic Gram-positive bacteria [33,34]. The predominant bacteria from the secondary infection include lactobacilli, staphylococci, *E. faecalis*, and *Propionibacterium* [35,36,37].

The true pathogenicity of these bacterial infections is a product of the polymicrobial community. In a study by Fabricius et al. it was shown that when individual bacterial strains were inoculated into the root canals of monkeys only mild apical periodontitis resulted [38]. When bacterial strains were combined in the inoculation a severe periodontitis was seen. These polymicrobial communities create a host response and apical periodontitis by releasing factors such as lipopolysaccharide, a component of Gram-negative bacterial cell walls, leukotoxins and enzymes related to cellular breakdown such as collagenase, hyaluronidase and protease [39,40,41,42,43]. It is the removal of the source of these pathogenic factors that is the focus of endodontic treatment.

Microbiology of Endodontics- Identification and Culturing

Determining the cause and mechanisms of endodontic infection is a constantly developing investigation. With so many bacteria present in the oral cavity identifying the type most likely associated with endodontic infection can direct therapeutic treatment and research. There are several methods most

commonly associated with microbial identification.

The standard method for sampling these bacteria has long been the method of culturing[44,45]. The widespread use of culturing as a means of bacterial analysis is due to several factors. Culture analysis allows for pathogenicity and physiological studies, is broad-range in nature, allows for quantification and susceptibility tests and finally it is easily available in a clinical and laboratory setting [46]. The uncultivated bacteria that represent a growing proportion of endodontic microbiology unfortunately are very resistant to the culture method technique. This resistance to culture is likely due to a multitude of factors including a lack of the nutrients or growth factors, overfeeding conditions, toxicity of the culture medium, inhibition by other cultured species and bacterial dormancy [33,47,48].

One of the difficulties in defining the bacteria associated with endodontic infection is the large proportion of uncultivated bacteria that represent a significant portion of the bacteriologic population[49]. Another more recent addition to the methods of identification of bacteria is the use of molecular biology. By investigating the DNA and RNA components of bacteria found in the root canal system rather than the culturability of these organisms the investigator is able to identify a significantly greater amount of these un-cultivated bacteria [50,51,52] . The most common types of molecular biological techniques are PCR, DNA-DNA hybridization and FISH. Each method relies on the presence of genomic components and high-sensitivity inherent in these methods to identify the bacteria present. It is this high sensitivity and ability to identify any and all

genomic remnants of microbial infection that can be considered a limitation of molecular biological techniques. The identification of dead cells that were not viable at the time of sampling is an obvious result of these techniques as well as the detection of relatively insignificant bacteria in the root canal system in relation to their proportion of the overall bacterial population[53,54]. While there are questions as to the direct/indirect relationship between apical periodontitis and the ever increasing number of bacteria identified by molecular techniques it remains a vital and rapidly developing method of microbial identification.

Despite the advent of molecular biological techniques and the known limitations of culturing, the use of culture status in clinical trial analysis has led to significant findings regarding the outcome of treatment. In a study done by Sjögren et al. , fifty-five teeth presenting with apical periodontitis (endodontic microbial infection) were cleaned and obturated [55]. Each tooth was cultured just after treatment and irrigation with sodium hypochlorite and before obturation. Using anaerobic bacteriologic techniques the results of the culture found a 40% positive culture rate and a 60% negative culture rate. The status of the teeth and periapical tissues were then followed for five years and evaluated for healing. Amazingly, of the 60% of teeth with no cultivable bacteria at the time of obturation, 94% showed complete periapical healing. In contrast, the 40% of teeth with cultivable bacteria at the time of obturation had a markedly lower healing rate of only 68%. Clearly the culture status of the root canal system at the time of obturation had a significant effect on the outcome. This result and the implications were followed up by several studies showing that the culture status

at the time of obturation had a significant effect on treatment outcome[56,57]. The information obtained by culture outcome studies then becomes relevant to the clinical practice of endodontics and can still be used to evaluate the comparative benefits of new therapeutic techniques.

Microbiology of Endodontics- Biofilms

The formation of a biofilm by bacteria results in a polymicrobial community characterized by bacteria, a fluid medium and a solid surface for adhesion[58,59]. This community of bacteria has a higher resistance to antimicrobial agents due to several factors such as (1) resistance due to the extracellular matrix, (2) resistance due to a lower growth rate and nutrient availability, and (3) antimicrobial resistant phenotype conversion [60,61]. The resistance of bacteria in a biofilm community to antimicrobial agents has been shown to be as much as a thousand times higher than bacteria that are simply in planktonic form[62]. The root canal is an ideal candidate for biofilm formation with the needed solid surface, fluid medium and bacterial presence. The root canal also presents an environment that promotes biofilm formation in both primary and secondary endodontic infection because of the unfavorable and low nutritional conditions. It then becomes readily apparent that a relationship between apical periodontitis and biofilm formation and presence is likely. In fact, the relationship of biofilms to apical periodontitis has been demonstrated in a recent study by Ricucci et al. 2010 in which 106 teeth of both untreated and treated root canals were evaluated after extraction for the prevalence of biofilms. In 77% of the sampled teeth

biofilms were found in the apical root canal system. In addition a significant relationship was identified with larger apical lesion sizes and biofilm presence[63]. The presence of biofilms can also lead to persistent endodontic infection causing failure following root canal treatment[64]. These organisms found in biofilms are more resistant to treatment than those found in a planktonic state. Therapeutic treatment modalities then should be considered which address the removal and disinfection of the biofilm community in the root canal system.

Treatment and Therapeutic Modalities

Treatment and Therapeutic Modalities – Mechanical

To clean and sterilize the root canal system the canal and its wide variation in anatomy must be negotiated to its length. It was this realization that led Fauchard in the 18th century to perform pulp extirpation with a roughened needle[65]. Fauchard referred to his treatment as trepanation in the sense that any instrument placed into the pulpal chamber would relieve the patient's symptoms. Since that time the method of canal negotiation and instrumentation has seen many advances. Instrumentation of the root canal can be broken down into two main divisions, that of hand instrumentation and of rotary-engine driven instrumentation. Each type of instrumentation has its unique abilities and limitations in endodontics. The first of these two types to advance was hand instrumentation.

It was not until 1935 that G.V. Skillen recognized that the walls of the intra-radicular space should be instrumented to remove debris and necrotic tissue [66].

Before this time more focus was spent on the material placed in the canal at obturaton than the mechanism of treatment.

Early endodontic instruments consisted mainly of wires and needles and they were simply used to relieve pain or make space for the obturating material. The Kerr company began producing endodontic instruments in 1904 and called them K instruments and they have become the most widely used and copied instruments in endodontics. In the early 1900's there was a large increase in the use of endodontic instruments consisting of metal files, points and broaches that were not-standardized as to taper and size. Obviously this led to difficulty in achieving consistent results in treatment. In 1959 Ingle finally introduced a standardized system of instrumentation that allowed for consistent and reproducible results between operators, instrumentation and obturation[67]. This standardization dictated that the diameter and taper of each instrument type would be consistent. The diameter would be related to the width of the instrument at the beginning of the cutting blades, a point called D1 or diameter 1mm. They also set forth a standard increment in size from one instrument to the next and the numbering of this system would be related to the instrument metric diameter. The types of hand instruments used for cleaning and shaping of the canal today include files, reamers, Hedstrom files and broaches.

The hand file is the single most used instrument in endodontic treatment. The K file, introduced by Kerr in 1904, uses a rasping or reaming motion to enlarge and negotiate the canal. The file is made by twisting a metal blank that is either triangular, square or more recently, diamond shaped in cross section

and this shape when twisted creates cutting blades along the length of the file. Excessive force or rotation in these instruments can cause failure and separation and care should be taken to not use the instrument past its limitations [68, 69, 70].

The reamer instrument is very closely related to the file. In contrast to the file which can be used in both a reaming and rasping motion, the reamer should only be used in reaming the canal walls. The reamer is made by putting less helical flutes on the metal blank with less twists and is used by penetrating, rotating one quarter to one half turn, and retracting the file with the cut of dentin occurring on retraction similar to the use of a file.

The Hedstrom file is different in fabrication and geometry from both the files and reamers. The Hedstrom file is made by cutting spiraling flutes into a stainless steel blank. With this configuration the Hedstrom cuts only on the outstroke or retraction of the file and can be threaded into the canal creating a high likelihood of separation and breakage of the file[71, 72].

The barbed broach is the oldest hand instrument to remove material from the intraradicular spaces. The broach is made by cutting or notching barbs into round wire and is not intended for use in machining dentin. Binding or engagement with root surface can result in the inability to withdraw the instrument. A broach is only to be used for removal of pulpal tissue or other necrotic debris in the canal space.

While hand instruments continue to be a major part of the endodontic armamentarium the introduction of nickel-titanium to endodontics forever changed the methods of instrumentation. The first presentation of nickel-titanium

in endodontic use was by Walia et al. in 1987, who correctly theorized that the low elastic modulus would permit the engine-driven negotiation of curved canals that had not been possible with the stainless steel instruments available[73 ,74]. The ability to use rotary instrumentation safely to negotiate and instrument the canal space was finally possible. Many types of rotary instrumentation files are now available with different geometries and methods of cutting and machining the dentinal wall. Despite the desirable properties of nickel-titanium and ease of use in endodontics, the canal cleanliness and instrumentation has not been shown to be significantly better than hand instrumentation with bacterial removal not improving and transportation or zipping of the canal [75 ,76 , 77]. No amount of instrumentation will completely reduce the intracanal bacteria and thus it must be combined with chemical treatment of the canal space.

Treatment and Therapeutic Modalities – Chemical

In spite of the advances in mechanical preparation with the introduction of rotary instrumentation, much of the canal space remains un-instrumented and can harbor bacteria. Through the use of micro-computed tomography the effect of instrumentation on the canal walls can be quantified. It has been shown that even after complete mechanical preparation 35-53% of the root canal surface has not been instrumented[78,79,80]. If mechanical instrumentation alone is not capable of debriding and removing bacterial pathogens then other treatment must be added to the protocol. The most widely used method employed to address this gap in instrumentation is the use of irrigants.

Material that remains untouched or compacted into the root canal anatomy consists of both organic and inorganic components. In addition, as previously discussed, the presence of biofilms in the uninstrumented canal anatomy provides more material that can cause treatment failure[81, 82]. Any irrigants used for removal of these materials must address both these organic and inorganic components. The use of an inactive or neutral irrigant such as saline or water will only result in manual flushing of freely movable debris and does not provide an efficient means of bacterial reduction in the canal. In studies using culturing techniques the use of water or saline was shown to be the least effective in achieving a negative bacterial culture[83,84,85]. The use then of an active irrigant that causes a chemical change of the components in the root canal system is ideal. Many different types of irrigants are employed in the practice of endodontics with different indications and uses. The use of anti-bacterial irrigants such as sodium hypochlorite (NaOCl) or chlorhexidine gluconate are able to substantially reduce the bacterial load when compared to a neutral irrigant [86]. The limitation of antibacterial irrigants is their lack of effect on the inorganic components found in the debris of the root canal system. For the removal of inorganic components the use of chelating agents such as EDTA aid in removing dentinal debris and the smear layer created in the instrumentation steps. A review of the various irrigants and their individual properties will illustrate both their advantages in use and the possibilities from combination.

The most widely used endodontic irrigating solution is sodium hypochlorite (NaOCl). The antibacterial effect of NaOCl comes from the HOCl, hypochlorous

acid. This acid disrupts oxidative phosphorylation and DNA synthesis in bacteria but the canal must have a pH of 4-7 for the acidic form to be present. Sodium hypochlorite was first introduced as an antiseptic by Dakin in 1915 and was used in the 0.5% concentration and buffered to a pH of 9. As an endodontic irrigant NaOCl has been shown to be highly effective in eliminating bacteria in concentrations ranging from 5.25% to 0.5% [87,88,89]. Conversely, NaOCl is highly cytotoxic and can be very damaging to vital tissue in endodontic treatment. This can result in a "sodium hypochlorite accident" where irrigant escapes from the apical foramen of the canal from improper irrigation technique, i.e. excessive force, binding in the canal or placing the needle beyond the apical foramen. A balance between cytotoxicity and antibacterial effectiveness has been studied by Spangberg et al. and at 0.5% concentration level there is an optimal level of cell compatibility while dissolving necrotic tissue along with the retained antimicrobial effect[90]. Additionally, studies have found that 1% and 0.5% have significantly less cytotoxic effects than 5% NaOCl[91,92]. To prevent NaOCl damage to vital tissues outside the root canal anatomy the use of 0.5% to1% seems to be a safe protocol.

Another popular antimicrobial irrigant is chlorhexidine. Chlorhexidine is a cationic molecule that disrupts cytoplasmic membrane. It has been found to be effective as an antimicrobial and against C. albicans[93,94,95]. One of the unique properties of chlorhexidine is its substantivity in the canal after use as an irrigant. In studies by White et al. the substantivity of chlorhexidine was evaluated by sampling irrigated canals up to 72 hours after treatment and measuring

antimicrobial activity and they found that in concentrations of 0.12% to 2% the antimicrobial activity remained for 24 and 72 hours respectively[96,97]. Chlorhexidine is less cytotoxic than sodium hypochlorite and can be used with less negative outcomes, i.e., chlorhexidine is used as an antibacterial periodontal mouth rinse[98]. The largest shortcoming of chlorhexidine is its inability to dissolve tissue and thus it cannot be used as the sole irrigant in endodontic treatment[99]. To take advantage of the substantivity and antimicrobial effects of chlorhexidine in endodontic treatment efficiently it must be used with other irrigants. While it would seem to make sense to combine sodium hypochlorite and chlorhexidine as an irrigant they are not compatible. The combination of the two irrigants forms a precipitate that impedes the instrumentation and cleaning of the root canal and there are conflicting data that it may contain para-chloroalinine [100,101,102]. It becomes necessary to separate the two irrigants during root canal irrigation by drying or alternative irrigant.

The removal of the inorganic component in the root canal is of primary importance because it allows penetration of the antimicrobial irrigants to areas of the dentin that may harbor bacteria. The most common irrigant used for removal of the inorganic material is ethylenediaminetetraacetic acid or EDTA. As a chelating agent, EDTA can remove not only the inorganic components blocking antimicrobial irrigants but also removes the “smear layer” which is a machined surface created during instrumentation. Anatomically the radicular smear layer consists of two components. The organic component of the smear layer is comprised of odontoblastic processes, microorganisms and necrotic material

while the inorganic component is comprised of dentinal debris[103]. Physically the smear layer is actually made of two separate layers, the first superficial layer that is 1-2 microns thick and a thicker layer that extends up to 40 microns into the dentinal tubules creating debris plugs[104]. As mentioned, the smear layer prevents irrigant penetration to dentinal tubules and contains bacterial debris and byproducts and removal should be a part of irrigation. EDTA is the most common irrigant used for removal of inorganic material and the smear layer [105,106]. The use of EDTA has been shown to increase the antimicrobial effect of irrigants on the intracanal bacteria [107,108]. Additionally, it has been shown that sodium hypochlorite irrigation should be followed by EDTA and not repeated due to excessive erosion of the exposed dentinal surface [109].

The combination of multiple irrigants then can be an effective means of microbial reduction after mechanical shaping and cleaning. The use of sodium hypochlorite removes the organic tissue components and has antimicrobial properties. Following sodium hypochlorite, EDTA is used as an irrigant to removal the created smear layer and remaining inorganic components. The final rinse of chlorhexidine gives the canal another exposure to an antimicrobial irrigant and has substantivity that lasts beyond treatment.

Treatment and Therapeutic Modalities – Activated Irrigant

Irrigation has become a critical component of adequate root canal treatment. In an effort to further improve the effectiveness of irrigation various methods have been devised to augment the irrigation process.

Applying mechanical agitation, or activating the irrigants has been shown to improve the cleanliness of the canal [110]. Various modalities of activation of irrigant are available. Mechanically agitating an irrigant can be done by sonic or ultrasonic activation. Using sonic or ultrasonic activation converts electric energy into waves with certain frequencies. This energy produces a rapid movement of fluid in a circular motion around the vibrating instrument. This rapid movement is called acoustic streaming and occurs inside the canal when activating irrigant [111,112,113]. The effect of acoustic streaming is to cause a directional flow of the irrigant to the coronal part of the root canal and is propagated by the node formation along the length of the instrument being activated[114]. In contrast to ultrasonic irrigation which operates at a much higher frequency, sonic activation uses a lower frequency and results in only one node formation at the tip of the activated instrument while ultrasonically activated instruments have multinodal formation along the length of the instrument[115]. This uninodal formation in sonic activation prevents the type of acoustic streaming seen in ultrasonic irrigation. A second contrast between sonic and ultrasonic activation is ultrasonically activating an instrument can result in cavitation of the irrigant while sonic cannot due to its lower energy. Cavitation is the formation and collapse of bubbles in a liquid medium and the subsequent release of energy and has been used in industrial ultrasound cleaning, megasonic chip cleaning and lithotripsy [116,117,118]. Ultrasonic irrigation has been used since 1980 when it was first described by Weller who termed it “passive ultrasonic irrigation” meaning that the file did not instrument the canal but only activated the irrigant[119]. Since that time many

studies have noted the positive increase in irrigating ability of NaOCl when paired with ultrasonic activation finding that the dissolving ability of NaOCl increases and that more smear layer is removed with NaOCl with this combination [120,121,122]. A recent study by Al-Jadaa et al. showed that passive ultrasonic irrigation with NaOCl dissolved significantly more tissue in simulated curved canals than sonic activation NaOCl [123] In another recent study ultrasonic irrigation was shown to be more effect in an in-vitro analysis of manual, sonic and ultrasonic irrigation protocols in the apical third of the canal[124]. In addition, passive ultrasonic irrigation has been shown to reduce the amount of bacteria in in-vitro testing [125] [126,127,128] . The study by Weber et al. (127) showed that in in-vitro testing the use of Chlorhexidine as the irrigant when combined with ultrasonic irrigation was superior in antimicrobial effectiveness to NaOCl and again illustrated the substantivity of Chlorhexidine with continued bacterial inhibition up to 168 hours after treatment. In one in-vivo study by Carver et al. a final ultrasonic irrigation regimen with the MiniEndo ultrasonic system (Spartan EIE Inc, San Diego, CA) which ultrasonically activates the irrigating needle while irrigating was described. The study found that the addition of a final ultrasonic irrigation regiment reduced the bacterial colony forming units and was found to be 7 times more likely to yield a negative culture.

Clearly ultrasonic irrigation has changed the way we view the disinfection of the root canal system. While there have been many studies evaluating the abilities of ultrasonic irrigation to augment the irrigant properties during irrigation in-vitro, there is a paucity of literature investigating the correlation of these finding

in the clinical patient.

Treatment and Therapeutic Modalities – Calcium Hydroxide and two-visit treatment

The complete eradication of bacteria, the goal in all mechanical and chemical treatment of the root canal, is a difficult end-point. Despite the advances in mechanical instrumentation and chemical irrigants and modes of irrigation the canal can continue to harbor bacteria that may lower prognosis. When treating apical periodontitis anything that can increase the ability to remove bacteria should be evaluated and incorporated into treatment. The use of calcium hydroxide as an interappointment dressing has been recommended for use in endodontic treatment[129]. Calcium hydroxide is ideal because of its high pH of 12.5 and is a strong bactericidal agent. It is placed in the canal as a powder or as a paste mixed with sterile water. The use of an intracanal medicament in a multi-visit approach has been strongly indicated since the classical Swedish studies of the 1980's and 90's. In the study by Sjögren et al. the use of calcium hydroxide as a 7-day interappointment dressing was able to eliminate completely all culturable bacteria from the root canal[130]. Bystrom et al. reported that the use of calcium hydroxide as an intracanal medicament for four weeks resulted in 34 of 35 canals free of cultivable bacteria [131]. Not only has the culture status and ability to obtain a negative culture been investigated, but it has been shown that a two-visit treatment with calcium hydroxide results in a higher healing rate as compared to a single visit[132,133] .

There is some controversy still in the endodontic community as to whether treatment should be completed in one visit or in two-visits with the placement of

an intracanal medicament when treating apical periodontitis. Those who subscribe to the concept of one-visit treatment list the reduction in clinical time, the patient convenience and lower risk of bacterial leakage from the temporary filling as their reasons for selecting treatment [45]. In addition, the literature has conflicting results as to the overall success of two-visit treatment compared to one-visit treatment with some studies showing no difference in success rates or microbiological sampling [134,135,136].

The aim of treatment should not be based on operator or patient convenience but rather the removal of the etiologic factors of the disease. It is possible that the addition of a second visit of instrumentation and irrigation could play a role in the increased success of a multi-visit treatment approach. The use of intracanal medicament in a two-visit approach is a well accepted method for lowering the bacterial content of the canal and would therefore increase the success of treatment.

Rationale

When treating or preventing apical periodontitis the primary purpose of treatment must be kept in mind which is to remove and prevent the contamination of the intracanal space by microorganisms. Whether this is done with new techniques of rotary instrumentation, alternative irrigants or instrumentation protocols the end-result must be the same. When root canal treatment is completed with obturation in the presence of bacteria the prognosis is diminished[137]. As irrigation and ultrasonic activation have increased in use

there has been a shift toward combining the irrigants: sodium hypochlorite, EDTA and chlorhexidine for the reasons previously cited. To date there are no clinical studies evaluating the use of ultrasonic activation and a combined irrigant approach on the culture status of teeth receiving root canal treatment. The purposes of this study were: (1) to evaluate the effect a Passive Ultrasonic Irrigation protocol would have on the bacterial culture status of teeth when compared to a Non-Ultrasonic Irrigation protocol in a single visit, (2) to compare the effect of an intracanal medicament (calcium hydroxide) on the bacterial culture status of teeth with the initial culture following instrumentation and irrigation, (3) to compare the effect of a second exposure to both instrumentation and irrigation on bacterial culture status of the sampled teeth, and (4) to compare the overall negative bacterial culture rate from one-visit and two-visit treatment. The hypothesis is that the introduction of an irrigation protocol that utilizes the sequential combination of commonly used endodontic irrigants that are each ultrasonically activated will result in a clinically measureable difference in the presence of intracanal bacteria when compared to an irrigation protocol utilizing only sodium hypochlorite without ultrasonic activation. The null hypothesis of this study is that there will be no statistical difference between the microbiologic culture rates between a new irrigation protocol utilizing a Passive Ultrasonic Irrigation protocol (PUI) and a Non-Ultrasonic Irrigation protocol (NUI).

Materials and Methods

The study protocol was approved by the Institutional Review Board of the University of Connecticut Health Center.

A sample size determination was calculated with power analysis. Using the outcome of comparable studies and research done previously at the University of Connecticut Department of Endodontology an effect size of 0.8 was used. The power analysis showed a sample size of 25 for each group with a total sample size of 50 as desirable to demonstrate significance. A total sample size of 50 was used and we obtained written consent from each participating patient.

Participant selection

Any patient presenting to the endodontic clinic for root canal treatment of a posterior tooth was considered for inclusion into the study. Inclusion criteria were the following:

- Presence of radiographic signs of apical periodontitis i.e. periapical radiolucency
- Negative pulp response to cold testing
- Patient consent to participate in the study

The variables of age, gender, tooth type, pre-operative pain and presence or absence of a sinus tract was also recorded for analysis.

Treatment protocol

All treatment and culture collection was done by a single operator for this study. A diagram outlining the treatment method and individual steps can be found in figure 1. Following initial routine preoperative radiograph and pulp testing, each tooth was isolated with a rubber dam. The tooth, rubber dam retainer, and area of the rubber dam surrounding the tooth were disinfected following the endodontic clinic standard disinfection protocol [138]. This disinfection protocol as described by Moller includes swabbing the tooth with 30% hydrogen peroxide followed by 5% iodine tincture. After the initial disinfection all caries and restorative material were removed and the remaining tooth structure was subsequently disinfected a second time using the initial method. Any remaining iodine was then inactivated using 5% sodium thiosulphate and a bacteriologic sample was taken to confirm the elimination of all cultivable bacteria from the surface of the tooth.

As noted, all cultures were taken by a single operator and were done with sterile paper cones sized medium and placed into 8mm culture tubes containing thioglycollate medium, vitamin K-1 and hemin (BBL ™ Becton, Dickinson and Company, Sparks MD). Immediately following sampling all cultures were placed in an incubator for a total of 7 days at 37^0 C and 100% humidity and were observed each day of the observation time for any signs of turbidity.

After the primary bacteriologic culture (identified as C1) was taken the pulp chamber was entered and pulp vitality/necrosis was assessed visually. At this time canals were located and lightly instrumented with stainless steel hand files

to create space for paper point placement. In addition, a Gates-Glidden drill size 2 was used if the orifice showed constriction significant to the point of paper point obstruction. No irrigation was used up until this point. Following this, each canal was filled with sterile saline and a second bacterial culture was taken (identified as C2). This culture was for confirmation of microbial infection of the canals.

Standard clinical instrumentation protocol followed the second bacterial culture. This involves the preflaring of canals and obtaining working length approximately 1 mm short of the radiographic apex confirmed by electronic apex locator (Root ZX, Morita, Irvine CA). This is followed by full instrumentation with rotary and hand instruments used in a crown down fashion under copious irrigation with 1% sodium hypochlorite (NaOCl). Protaper rotary instruments size S1-F2 and Vortex rotary instruments size 25-50 were used on all teeth with the master apical file (MAF) being determined by the clinician (DentSply, Tulsa OK). If needed, hand files were used to instrument the apical third of canals larger than a size 50. The size of the MAF was recorded for statistical analysis.

After the full chemo-mechanical preparation was determined to be complete by the operator a card was removed from an envelope that would indicate the final irrigation protocol to be used, either passive ultrasonic irrigation or non-ultrasonic irrigation. A diagram outlining the protocol developed for this study can be found in figure 2. These cards indicating irrigation modality had been pre-randomized by randomization software and packaged by the research assistant, not the operator, so as to blind the operator to irrigation method. The following information was recorded on each treatment card: the tooth being treated, MAF,

presence of pre-operative pain and presence/absence of sinus tract.

The final irrigation method in both groups was performed using a NaviTip 31g 27mm Sideport needle (Ultradent, South Jordan UT). This needle size allowed for all final irrigation to be performed 1mm from working length as the minimum master apical file size was a 0.25mm (ProTaper F2) and the size of a 31 gauge needle is 0.26mm. All ultrasonic activation was done with the NSK Varios 750 ultrasonic unit with a frequency of 30KHz and a maximum power of 8W set at ¾ power setting (NSK, Kanuma Japan). The activated file was a Varios u-file, size 15, stainless steel ultrasonic file.

If the card indicated passive ultrasonic irrigation (PUI), the following protocol was followed:

1. The canals were filled with 1ml 1%NaOCl

2. PUI of the canals was done with a #15 Varios ultrasonic file for 30 seconds

3. Canals were refilled with a fresh 1ml of 1%NaOCl and PUI was resumed with the #15 Varios ultrasonic file for 30 additional seconds.

4. Following 1%NaOCl irrigation, the canals were dried by paper point and canals were filled with 1ml 17%EDTA following which PUI was

done for 30 seconds.

5. Canals were refilled with a fresh 1ml of 17%EDTA and PUI was resumed with the #15 Varios ultrasonic file for 30 additional seconds.

6. Following 17%EDTA irrigation, the canals were dried with paper points and canals were filled with 1ml 2%Chlorhexidine following which PUI was done for 30 seconds.

7. Canals were refilled with a fresh 1ml of 2%Chlorhexidine and PUI was resumed with the #15 Varios ultrasonic file for 30 additional seconds.

8. Following 2% Chlorhexidine activation, canals were flushed with sterile saline and dried with paper points.

9. Prior to bacterial culture the canals were filled with a mixture of 0.3%L-α-lecithin in 3%Tween 80 to inactivate any antimicrobial effect of chlorhexidine and then flushed with sterile saline. A hand file equal in size to the MAF was then inserted into the canals and lightly reamed against the walls to remove any debris/bacteria from the dentin walls of the canal. After debris suspension into medium a third bacterial sample was taken (identified as C3).

If the card indicated non-ultrasonic irrigation (NUI), the following protocol was followed:

1. The canals were filled with 1% NaOCl

2. Irrigation of the canals was continued for a total of 3 minutes and used a total of 6mL of 1% NaOCl with a flow rate of 2mL/min.

3. Following 1% NaOCl irrigation the canals were flushed with sterile saline and dried with paper points.

4. Prior to bacterial culture the canals were filled with 5% Sodium Thiosulphate to inactivate the NaOCl and then flushed with saline. A hand file equal in apical size to the MAF was then inserted into the canal and lightly reamed against the canal walls to remove any debris/bacteria from the dentin walls of the canal. After debris suspension into medium a third bacterial sample was taken (identified as C3).

Following this third bacterial sample all clinical data had been collected from

the first visit as pertains to this investigation. The canals were then temporized by first placing calcium hydroxide (Henry Schein, Melville NY) into the canals by use of lentulo-spiral. After placement of medicament a 3mm layer of Cavit (3M ESPE, St. Paul MN) and superficial layer of FUJI IX (GC Corporation, Tokyo Japan) was placed and the patient was scheduled to return for completion of root canal treatment and obturation no sooner than 7 days.

At the second visit the tooth was isolated as before with rubber dam and disinfection. The Fuji IX temporary restoration was then removed to expose 2-3mm of remaining Cavit in the access cavity. The tooth surface, surrounding rubber dam and clamp, and access cavity were scrubbed with 30% hydrogen peroxide. This was followed by 5% iodine tincture for disinfection. The iodine was then inactivated using sodium thiosulphate and a bacterial sample was taken (denoted as C4) to confirm surface decontamination. Then the canals were accessed through the remaining Cavit with slow speed and the canals were flushed with sterile saline to remove any remaining calcium hydroxide or temporary material. At this time a bacterial sample was taken (denoted as C5) to evaluate canal status after intracanal medicament therapy. The operator then completed any additional instrumentation and irrigation with 1%NaOCl prior to assessment of canal preparation. When all additional treatment was completed and the tooth was ready for obturation any remaining NaOCl was inactivated with sodium thiosulphate and the canals were flushed with sterile saline. A final bacterial culture was taken (denoted as C6) and the canals were obturated using cold lateral condensation of gutta percha and AH 26 sealer. The tooth was then

temporized with Cavit and Fuji IX as before and referred to the primary dentist for completion of coronal restoration. All pertinent clinical data had been collected at this time.

All bacterial culture samples were observed by the same operator for signs of turbidity. If turbidity was noted it indicated presence of sampled bacteria and was a positive culture and the day of turbidity was recorded for statistical analysis.

As negative controls five teeth were included in the investigation. These teeth exhibited no signs of apical periodontitis or periapical lucency and tested positive to cold testing. Three teeth were assigned to the PUI group and two were assigned to the NUI group. The same treatment protocol as outlined previously for the corresponding groups was applied to the control teeth and bacterial cultures were evaluated for turbidity in the same method previously discussed.

Post-operative (PO) pain was recorded for each patient. Post–operative pain was defined as any unscheduled contact with the patient that required additional interventional therapy including; (1) pharmacological prescription or (2) palliative treatment in an unscheduled visit.

Statistical analysis was performed on all recorded data. Chi-square and Fishers Exact tests were performed on treatment group (PUI vs. NUI) and outcome of culture testing (culture 3) to evaluate for significance. Fisher's exact test was also performed to test for significance of post-operative pain and treatment modality. Multivariate linear regression was performed on all independent variables to test for significanc.

Results

All positive surface cultures (C1 and C4) and associated data were excluded from statistical evaluation.

All negative control patients showed no signs of bacterial contamination or positive culture results throughout treatment.

A total of 50 teeth in 49 patients were included for evaluation in this prospective clinical study. The age of participants ranged from 12-72 with a mean age of 38 and was evenly distributed (see Figure 3). There were a total of 26 females and 23 males included in the sample population (see Table 1). A total of 4 patients did not return for the second visit for obturation. The remaining 46 teeth were evaluated for bacterial culture results after intracanal medicament therapy with calcium hydroxide and a second visit of instrumentation and irrigation.

Comparing PUI versus NUI showed that in the PUI group 21 of 25 teeth (84%) had a negative C3 culture at the end of the irrigation protocol (Table 2 and Figure 4). The NUI group resulted in 20 of 25 teeth (80%) that had a negative C3 culture at the end of the irrigation protocol. This difference (PUI 84%: NUI 80%) was not statistically significant ($p>.05$).

After intracanal medication with calcium hydroxide 40 of 46 remaining teeth (87%) in the total sample had a negative C5 culture (Table 3 and Figure 5). This number increased to 42 of 46 teeth (91%) having a negative bacterial culture after the second instrumentation and irrigation was completed (Table 4 and Figure 6). There was no statistically significant difference between the C5 post-

medicament culture (87%) and the C6 post-instrumentation/irrigation culture (91%), ($p>.05$).

For purposes of evaluation and statistical analysis the individual group culture results were combined and the total culture result of the entire sample was representative of the one-visit culture status. The overall negative culture rate of the entire sample was 82% for one-visit. The comparison of one-visit (82%) to the culture result immediately following intracanal medicament (87%) and to the second instrumentation and irrigation (91%) does show an improved negative culture rate (Figure 7), although this change was not statistically significant ($p>.05$).

In evaluating pre- and post-operative pain there was an incidence of 15 treated teeth (30%) with reported pre-operative pain. There was an even lower incidence of post-operative pain with only 4 of 50 treated teeth (8%) having recorded post-operative pain as previously defined. With such a low sample size no statistical analysis was performed.

Multivariate linear regression was performed on the recorded independent variables including: age, gender, master apical file size, treatment modality, pre-operative pain, post-operative pain and presence or absence of sinus tract. None of the investigated variables had a significant effect on the dependent variable of culture status.

Discussion

The most challenging yet most vital aspect of root canal treatment is the removal of bacteria from the root canal system. This difficulty can be attributed to a host of factors. The complexity of the root canal system and the presence of inaccessible and uninstrumented surface area following standard endodontic treatment make bacterial removal increasingly complex[139,140,141] This coupled with the presence of bacterial biofilms in the canal make mechanical removal of bacteria alone not feasible with current technology [142]. Therefore, adjunctive chemical disinfection must be used to obtain superior results.

All teeth included in this study had evidence of apical periodontitis and tested culture positive for bacteria. The use of either protocol resulted in a highly efficient removal of bacteria from the root canal system with 82% having a negative culture after the first visit of treatment. After a minimum of 7 days of an inter-appointment dressing of calcium hydroxide 87% of teeth had a negative culture. The addition of additional instrumentation and irrigation at the second visit yielded an even higher percentage of culture negative teeth. Ninety-one percent were found free of cultivable bacteria; however, this was not statistically different from the percentage of bacteria free canals achieved at the end of the first visit. This finding is notable in that the majority of previous studies evaluating the bacterial status of teeth following chemo-mechanical preparation in the first visit have a negative culture rate of 40-60% [143,144,145,146,147,148,149].

In this investigation we used the intermittent flush method (Int FM) for irrigation and activation with ultrasonics. It has been shown in a study by van der

Sluis et al. (2010) that refreshing the irrigant during passive ultrasonic activation over a period of 3 activation cycles resulted in a cumulative effect on debris removal from the root canal[150]. In the van der Sluis study a cycle of 40 seconds was used, 20 seconds for activation and 20 seconds for refreshment repeated three times for a total of 2 minutes. The protocol outlined for the current study used the IntFM for refreshment with the following parameters, 30 seconds activation, 15 seconds flushing, repeated twice for each irrigant component used. The refreshment of irrigant is necessary to facilitate removal of intracanal debris and has been shown to be equally or more effective than refreshment with a continuous flow of irrigant in the pulp chamber [151]

Huffaker et al. evaluated the bacterial reduction following a sonic irrigation protocol and a standard irrigation protocol (146). In this prospective clinical study 84 patients were randomly assigned to either of the two protocols after complete chemo-mechanical instrumentation and cleaning had been completed. The canals were sampled after the completion of the irrigation protocol and cultured for 1 week and analyzed for turbidity. During this evaluation, irrigation was completed with a 27g open-ended irrigating syringe with 2mL of irrigating solution. In the final evaluation the authors found that the sonic irrigation protocol resulted in a 41% negative culture rate while the standard irrigation protocol resulted in a 48% negative culture rate at the end of the first visit. The addition of a second visit including an interappointment dressing of calcium hydroxide resulted in an overall increase in negative culture rate from the first-visit mean of 44% to 73%. This study was performed at the same teaching institution with

similar conditions and treatment philosophy as the present study. There were three differentiating factors between the standard irrigation group of the Huffaker et al. study and the non-ultrasonic group in this investigation. First, the current study used a 31g irrigating side-vented needle that was used to length. Second our study used 1% NaOCl as irrigating solution. Lastly, we had an increase in total irrigation volume from 2mL to 6mL.

The change in irrigant needle depth is a likely factor in the divergent results of culture status. In a study by Sedgley et al. the effect of needle depth during irrigation was evaluated along with the amount of irrigant used[152]. In this study 30 permanent cuspids were instrumented to an apical size 60 and a bioluminescent bacterial strain was inoculated in the canals. Evaluation of bioluminescent marker removal was evaluated after irrigation at either 1mm or 5mm from working length and with 3mL or 6mL of irrigant. The authors found that irrigation with 6mL of irrigant at 1mm from working length was significantly more effective in removing bioluminescent marker than at 5mm. The effect of needle irrigation penetration depth was also analyzed in recent studies by Bronnec et al. [153,154]. In these studies 30 extracted mandibular molars with moderate to severe curvature were instrumented to a size F3 Protaper file and flushed with .5mL of sodium diatrizoate for radiographic evaluation. The parameters of apical taper, volume of irrigant, needle tip insertion depth and needle tip design were evaluated throughout treatment by recording the amount of radiographic flushing irrigant that was replaced with standard sodium hypochlorite. The authors found that in a standard syringe irrigation model, the

most dominant factor for irrigant replacement in apical areas was depth of insertion for the irrigating needle.

Several studies have now shown through Computational Fluid Dynamic modeling that needle tip design and needle insertion depth have significant effects on the flow of irrigant in the apical region and the dynamic of that flow [155,156,157,158,159]. Boutsioukis et al. have shown through dynamic modeling of the canal and irrigation needle that within a closed system, needle depth of 1mm from working length allowed for irrigant replacement even when using a side-vented needle tip [155]. In addition, a side-vented needle tip design is recommended as it protects against apical extrusion of irrigant by reducing the mean apical pressure as compared to open ended or beveled needle tip designs.

In comparing the present study and the Huffaker et al. study, the NaOCl concentration (0.5% in Huffaker et al. and 1% in this investigation) likely played only a minor role. In a clinical study Shuping et al. used 1.5% NaOCl irrigation in evaluating culture outcomes [143]. A similar study by McGurkin-Smith et al. at the same institution used 5.25% NaOCl and the culture negative results following instrumentation and irrigation were 62% and 47%, respectively [144]. The increase in NaOCl concentration did not result in a higher negative culture rate. Interestingly, in another prospective clinical study study done by Wang et al. (2007), evaluating the use of a chlorhexidine gel as an irrigant at the same teaching institution, the negative culture rate after chemo-mechanical instrumentation and irrigation was 90%, similar to the results found in this study[160]. Similar to this study, the addition of calcium hydroxide did not result in

a statistically significant increase in negative culture rate at 92%. The authors speculated, among other factors, that the difference between this result and the two previous studies cited (Shuping et al., McGurkin et al.) could be related to the irrigation needle and depth of penetration as well as irrigant types. While a direct comparison between the current study and Huffaker et al. is not possible the increased irrigant volume and depth of irrigant needle penetration is similarly the most likely cause of such a divergent result. Importantly there is a common factor between the two clinical studies of Huffaker et al and this investigation. When culturing teeth for analysis of bacterial content it is understood that the sample collected represents those bacteria that are in a “planktonic” form in the solution[161]. While some bacteria may remain in planktonic solution after instrumentation and irrigation it is necessary to displace any bacteria that are in contact with the canal wall either through instrumentation or in biofilm communities. The simplest way to perform this is through the use of the pumping maximum recovery method (PMR) by first filling the canal with sampling solution and then instrumenting as much of the canal surface as possible with a sterile stainless steel hand file to working length. This fluid, containing the bacteria and hard tissue filings in suspension, is then sampled and allows for retrieval of planktonic and non-planktonic forms of microorganisms.

In this study the addition of calcium hydroxide and a second visit of instrumentation and irrigation increased the percentage of teeth without culturable bacteria from 82% to 91%. This difference was not significant. The second-visit negative culture rate of 87% after medicament and 91%

corresponds well to other bacteriologic sampling studies evaluating the use of calcium hydroxide in a multi-visit treatment [55,57,58,148]. This finding is evidence that the use of calcium hydroxide does result in bacterial reduction but that the exposure to a second visit of instrumentation and irrigation is also a factor in the overall bacterial reduction. The lack of statistical significance in this effect could be due to the already high level of disinfection seen after both irrigation protocols and could also be due to the small sample size. These findings are of clinical significance in that a second visit with calcium hydroxide can improve the negative culture rate by 9% and could affect long-term outcome.

Conclusions

1. In both a Passive Ultrasonic Irrigation protocol and a Non-Ultrasonic Irrigation protocol a high percentage of root canal systems, 84% and 80% respectively, had no cultivable bacteria.
2. There was no statistical difference between the protocols employed for bacterial removal.
3. While a second visit did increase the percentage of negative culture results for bacterial removal from 82% to 87% following CaOH medication and to 91% following second instrumentation, this difference was not statistically significant.
4. The high first visit culture negative rate is most likely a result of high volume of irrigant and depth of needle, consistent in both protocols. Further investigation is warranted.

Tables & Graphs

Figure 1

Study Design

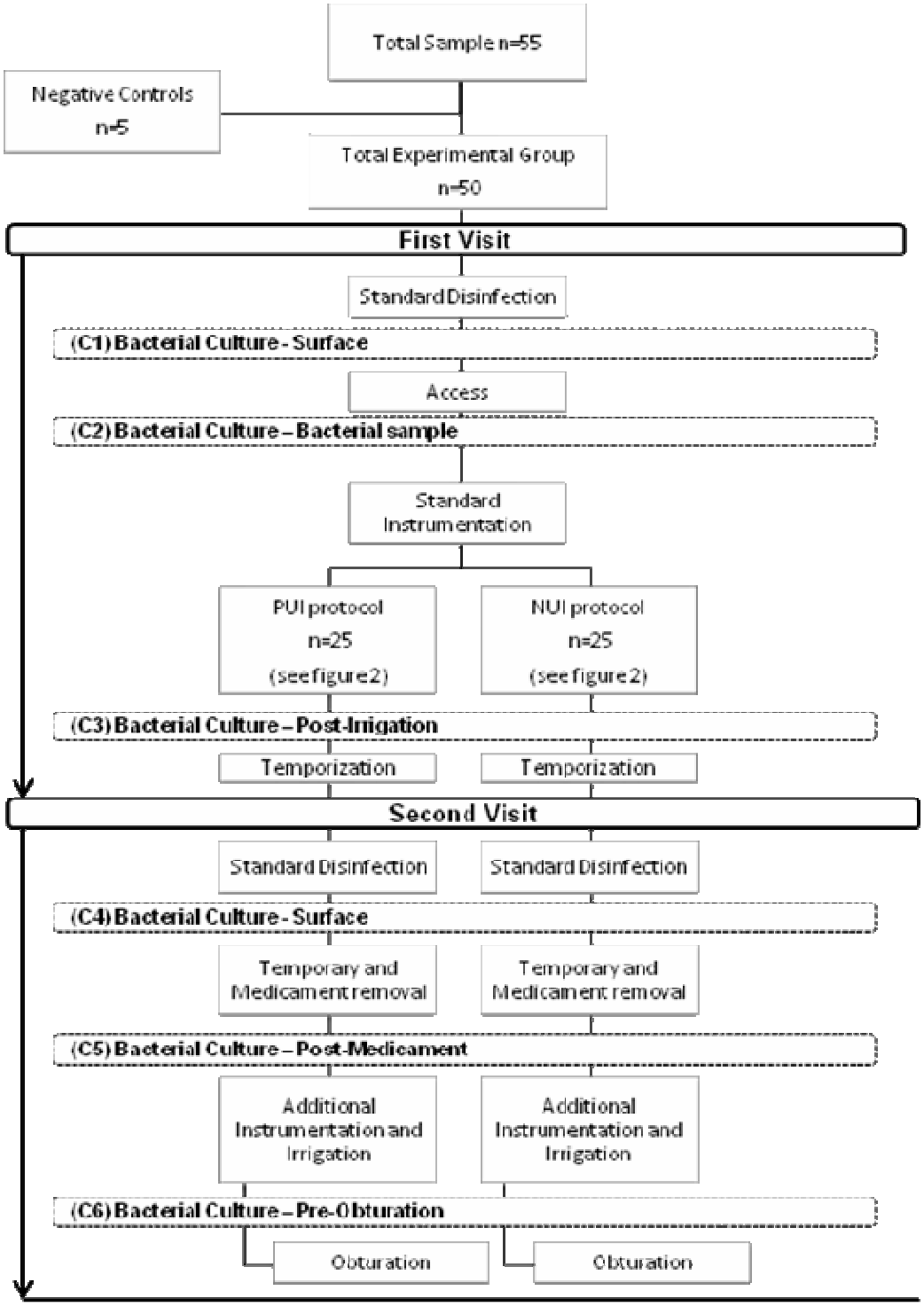


Figure 2

PUI and NUI irrigation protocol

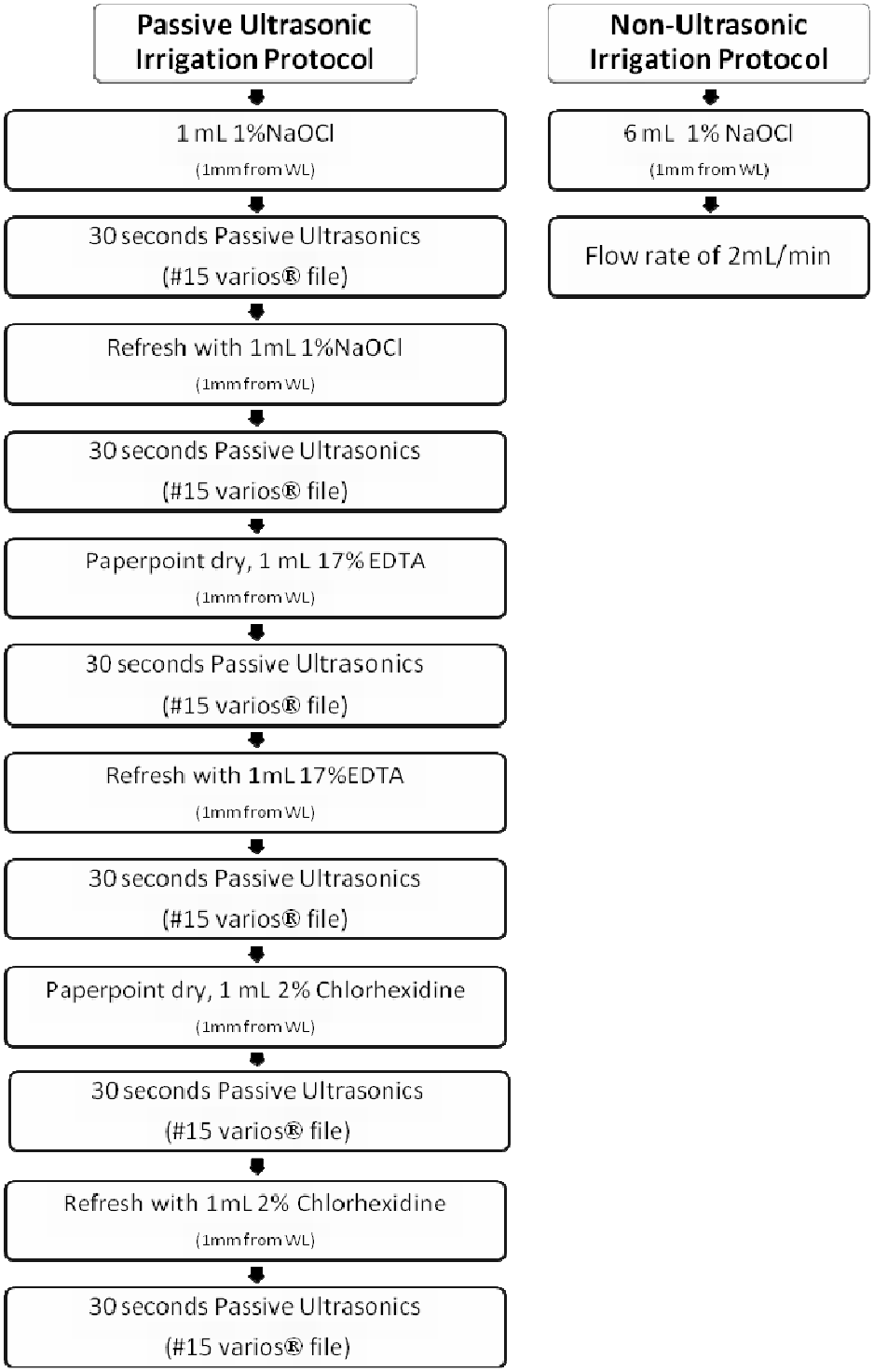


Figure 3

Histogram of age distribution

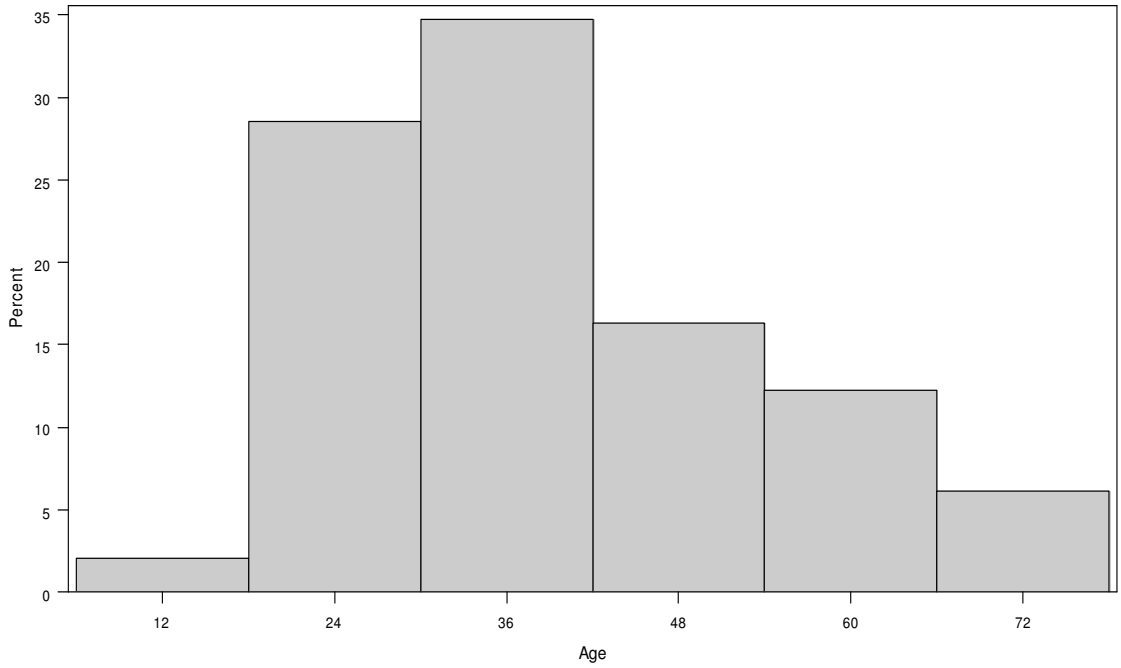


Table 1

Descriptive statistics

Descriptive Statistics		
	n=50	**%**
Male	23	46%
Female	27	54%
Presence of Sinus Tract	8	16%
Pre-Operative Pain	15	30%
Maxillary arch	25	50%
Mandibular arch	25	50%
Molar	30	60%
Premolar	20	40%

Table 2

PUI vs. NUI culture results: 1st visit

Bacterial Culture (C3) Result 1st visit					
			Culture Result 1st visit		
			Negative	Positive	Total
Protocol	PUI	Count	21	4	25
		% within Protocol	*84.0%*	*16.0%*	*100.0%*
	NUI	Count	20	5	25
		% within Protocol	*80.0%*	*20.0%*	*100.0%*
Total		Count	37	47	50
		% within Culture Result	*82.0%*	*18.0%*	*100.0%*
					(p>.05)

Figure 4

PUI vs. NUI culture comparison 1st visit

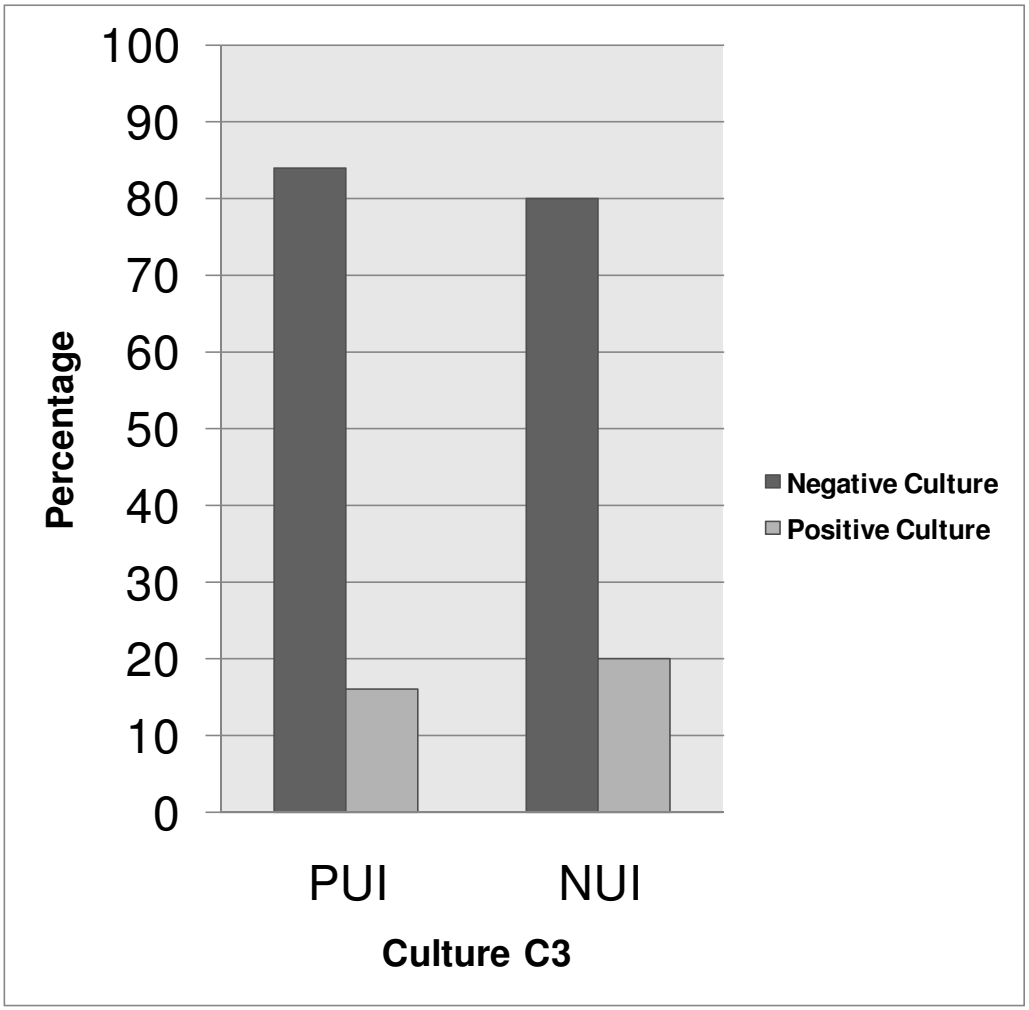


Table 3

PUI vs. NUI culture results: 2nd visit access

Bacterial Culture (C5) Result 2nd Visit Access					
			Culture Result 1st visit		
			Negative	Positive	Total
Protocol	PUI	Count	20	2	22
		% within Protocol	*91.0%*	*9.0%*	*100.0%*
	NUI	Count	20	4	24
		% within Protocol	*83.3%*	*16.7%*	*100.0%*
Total		Count	37	47	46
		% within Culture Result	*87.0%*	*13.0%*	*100.0%*
					(p>.05)

Figure 5

PUI vs. NUI comparison at 2nd visit access

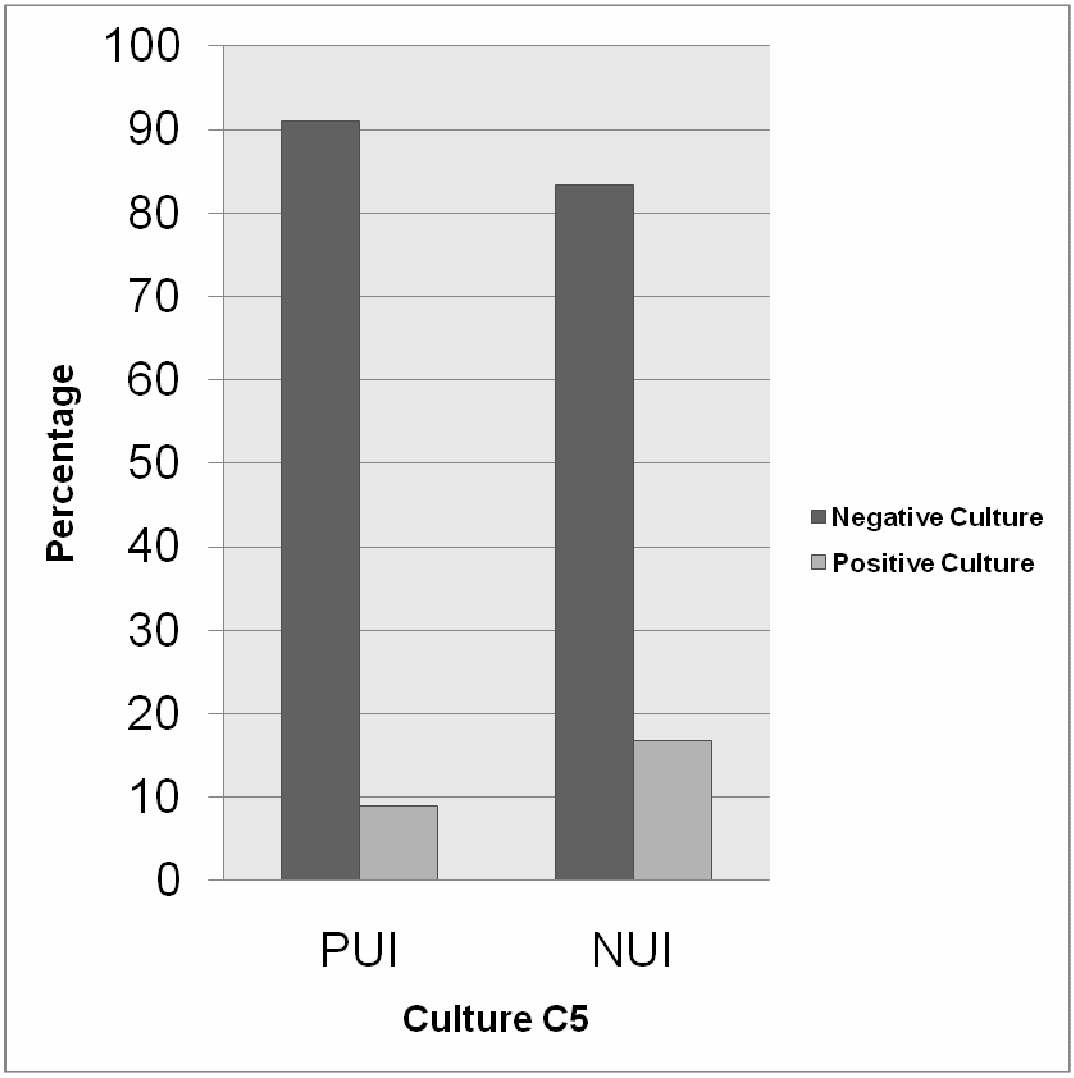


Table 4

PUI vs. NUI culture Results: 2nd visit pre-obturation

Bacterial Culture (C6) Result 2nd Visit Pre-Obturation					
			Culture Result 1st visit		
			Negative	Positive	Total
Protocol	PUI	Count	20	2	22
		% within Protocol	*91.0%*	*9.0%*	*100.0%*
	NUI	Count	22	2	24
		% within Protocol	*91.7%*	*8.3%*	*100.0%*
Total		Count	42	4	46
		% within Culture Result	*91.3%*	*8.7%*	*100.0%*
					(p>.05)

Figure 6

PUI vs. NUI comparison at 2nd visit pre-obturation

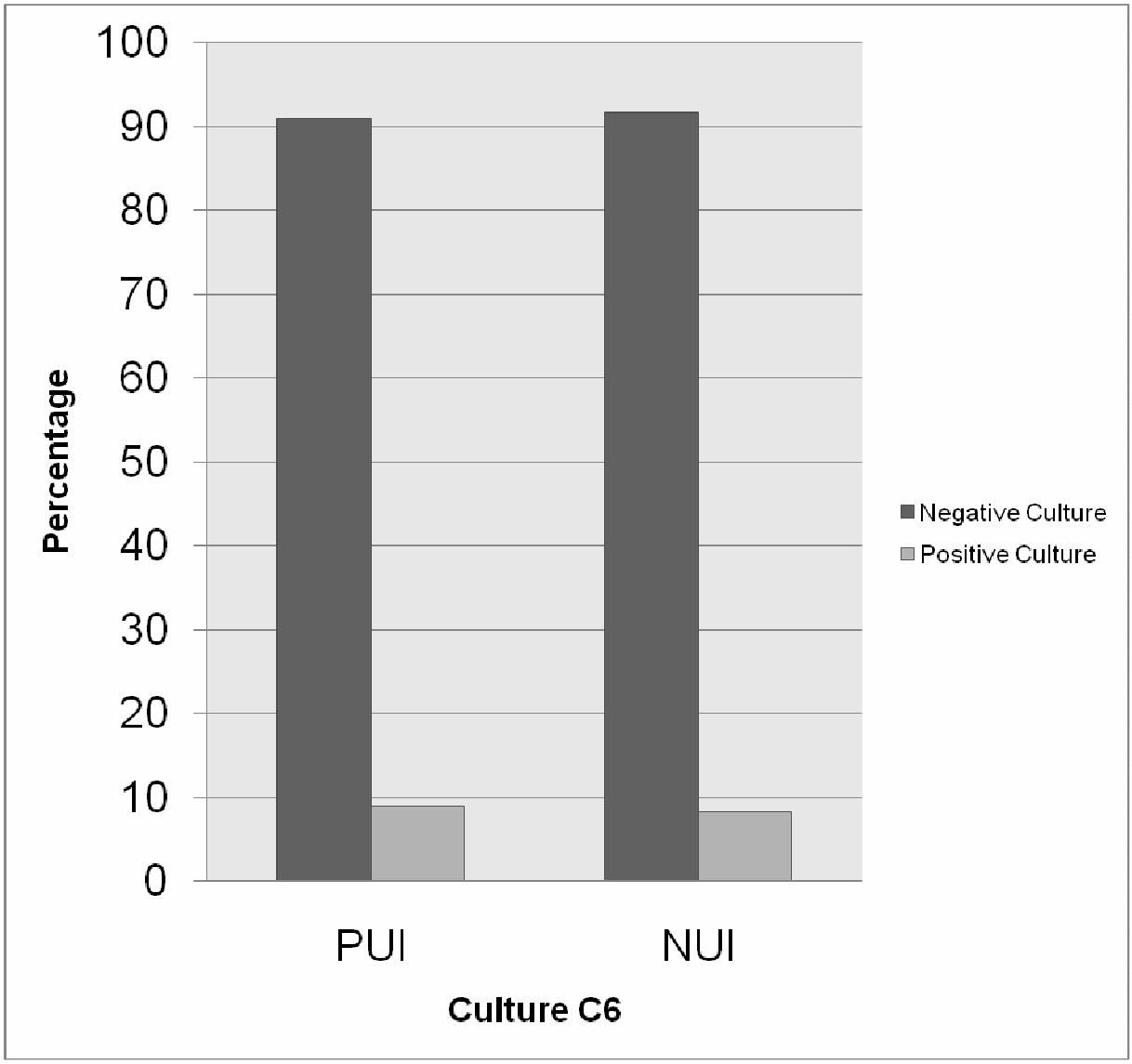
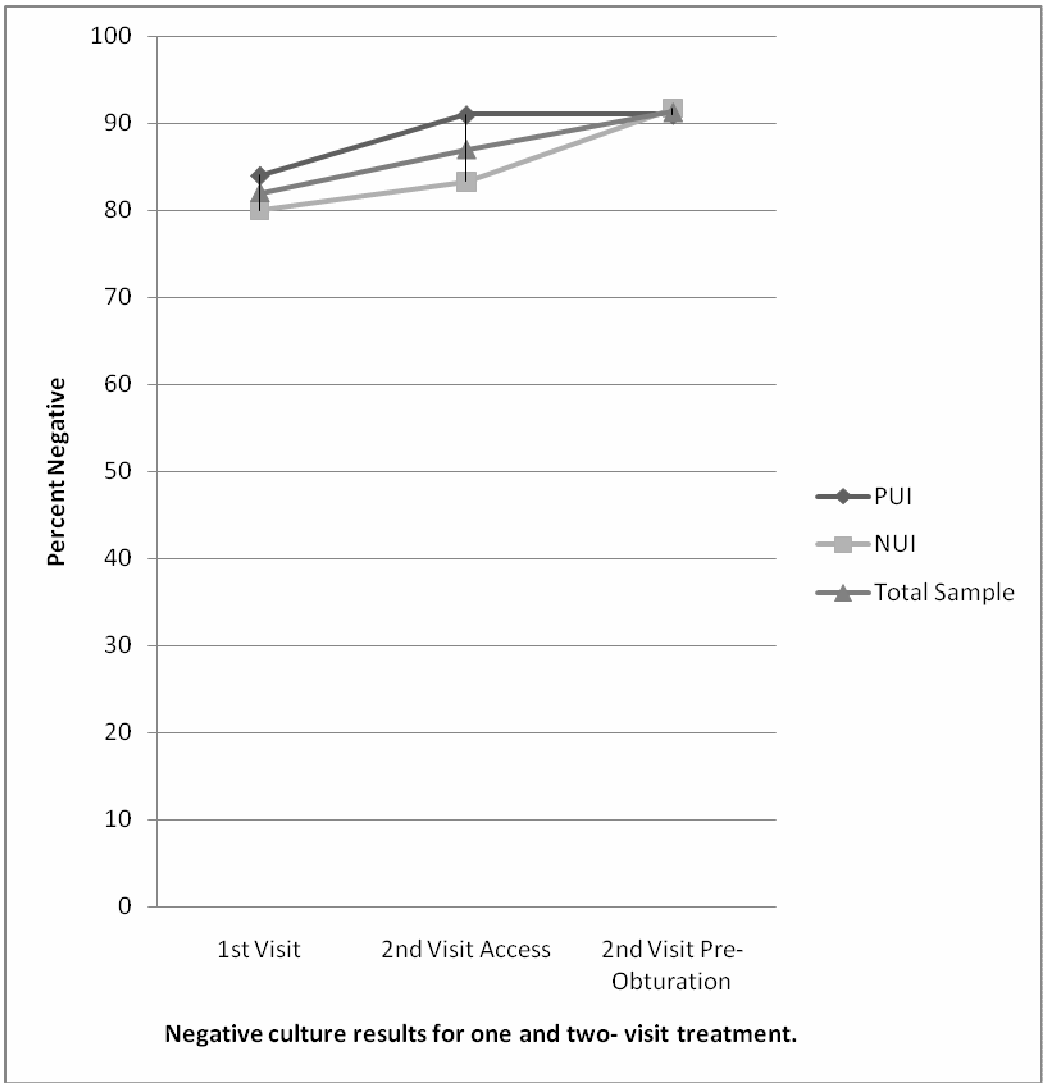


Figure 7

Comparison of first visit, post-medication, and pre-obturation



Appendix. Informed Consent Form

Principal Investigator (PI): Dr. Blythe Kaufman
Study Coordinators: Dr. Christopher Beus
Study Co-investigator: Dr. Daniel Fackrell

PI Phone Number: (860) 679-2454

Title of Research Study: *A comparison of ultrasonic and standard irrigation models on elimination of bacteria from root canal systems: a clinical study*

Expected Duration of Subject's Participation: 2 Visits and standard recall visits

Name of Research Participant: _____

What is the Purpose of This Research Study?

We are currently conducting a research study to help determine if different flushing or cleaning techniques used to destroy root canal bacteria could result in greater success. The purpose of the study is to clinically evaluate the use of an ultrasonic irrigation protocol when completing the final rinse of the root canal. Ultrasonic irrigation has become a widely used method to irrigate and clean the inside of a tooth in an effort to more completely remove bacteria from the root canal. Ultrasonic irrigation has been shown to create greater fluid movement and bubble formation in root canal cleaners as well as improving the action of antimicrobial and irrigant solutions commonly used in endodontic treatment. This study seeks to evaluate the clinical efficacy of a protocol which combines ultrasonic irrigation and commonly used root canal cleaners in comparison to conventional root canal irrigation protocols in use today.

Why Am I Invited to Participate?

You are invited to take part in this study because you are here today for routine treatment of an infected root canal. Your tooth has a necrotic (dead) pulp and your x-ray shows evidence of apical periodontitis, which means that you have an inflammation of the periodontal ligament, or connective tissue, surrounding the root apex of a tooth.

How Many Other People Do You Think Will Participate?

We are hoping to enroll a total of 50 patients who present to Dental Clinic #2 for routine endodontic treatment. At the time of the visit the patient would agree to participate in the

study, need root canal treatment of a restorable tooth and the tooth must contain dead pulp caused by disease or injury which will be confirmed by clinical test and a dental radiograph of the tooth.

Is Participation Voluntary?

Participation in this study is voluntary. Before making a decision about whether to participate in this research study, please read this consent form carefully and discuss any questions you have with the researcher.

How Long Will My Participation in This Study Last?

Your participation in this study will not decrease the amount of treatment normally received in Dental Clinic #2. Participating in this study will add approximately 5 minutes to the normal visit time for typical root canal treatment. Root canal treatments are typically performed during a total of two visits to Dental Clinic #2. Standard recall visits are scheduled every six months following completion of root canal treatment.

What Are the Costs to Me for Participating in This Study?

Participating in this study will not result in a decrease or increase to the cost of your root canal treatment.

What Procedures Will Be Done? Are They Safe?

Participants of this study will be randomly assigned to one of the two root canal treatment protocols. The first is a standardized cleaning and shaping technique using standard needle irrigation with 1.0%NaOCl (sodium hypochlorite) as an irrigant. The second is the same cleaning and shaping method with the addition of an ultrasonically activated antimicrobial rinse. Bacteriologic cultures (samples from the root canal) will be taken multiple times during each of the two visits. The procedures will follow Dental Clinic #2 protocol with the addition of the ultrasonic irrigation protocol. The risk of being involved in this study is possible anxiety of being exposed to few extra minutes of treatment.

What Are the Benefits of Participating in This Study?

The advantages of being involved in this study are the possibility of having the infected root canal cleaned to a greater degree with ultrasonic irrigation and knowing whether or not your canal is bacteria free prior to finishing the case. Root canal treatment of infected root canals has greater success when a negative bacterial culture can be achieved before the tooth is permanently closed up. You may or may not benefit from this study.

Your participation in this study would help researchers to determine if using an ultrasonic irrigation protocol during endodontic therapy will produce a greater number of negative microbiologic cultures than using standard needle irrigation alone. If the results of this study show that ultrasonic irrigation increases the removal of bacteria from root canal systems, it is possible that endodontic therapy will be more successful.

Will I Be Compensated for Participating in This Study?

There is no monetary compensation for participation in this study.

What Alternative Procedures or Treatments Are Available to Me?

If you choose not to participate in this study you will receive standard root canal treatment according to Dental Clinic #2 protocol.

How Will My Personal Information be Protected?

The following procedures will be used to protect the confidentiality of your data. Each tooth in the experiment will receive a number (1-55). This number will be printed on each bacterial sample taken and will be recorded on the treatment card. The patient's name or hospital ID number and any personal information will never be recorded on the card. A code will exist on a sheet kept locked in a secure location. It will have the experimental number and the patient's corresponding hospital ID number. Nowhere else will the two numbers exist together. Treatment cards which contain only the experiment number and do not contain hospital ID number will also be kept locked in a secure location. The study information will be kept as a research record, apart from your dental record. No names or personal information other than the experiment number will be found on this card. The study staff (principal investigator, research coordinator, co-investigators etc.) will keep all study records (including any codes to your data) locked in a secure location. All electronic files (e.g., database, spreadsheet, etc.) containing identifiable information will be password protected. Any computer hosting such files will also have password protection to prevent access by un-authorized users.

We will do our best to protect the confidentiality of the information we gather from you but we cannot guarantee 100% confidentiality. You should know that the Health Center's Institutional Review Board and the Human Subjects Protection Office may inspect records. They may inspect records to ensure that the study is being done correctly.

At the conclusion of this study the researchers may publish their findings. Information will be presented in summary format and you will not be identified in any publications or presentations.

Will I Find Out the Results of This Research Study?

You will be provided with the results of your culture.

What If I Decide to Stop Participating in The Study?

If you decide to participate in the study, you are free to withdraw from it at any time. If you decide not to participate or you withdraw from the study, your decision will not affect your present or future medical care at the University of Connecticut Health Center/John Dempsey Hospital and there will be no penalty or loss of benefits to which you are otherwise entitled.

If you decide to withdraw we ask that you let us know by calling the Principal Investigator, Dr. Blythe Kaufman, (860) 679-2454,or Study Coordinator, Dr. Christopher Beus, (860) 679-8310, or by sending a written notice to either Dr. Kaufman or Dr. Beus at the University of Connecticut Health Center, Division of Endodontology, 263 Farmington Avenue, MC-1715, Farmington, CT, 06032.

What if I Experience An Adverse (Bad) Event Related to My Participation?

The University of Connecticut Health Center (UCHC) does not provide insurance coverage to compensate for injuries incurred during this research. However, compensation may still be available. A claim may be filed against the State of Connecticut seeking compensation. For a description of this process contact a representative of the UCHC Institutional Review Board at 860-679-1019 or 860-679-8729.

The UCHC does not offer free care. However, treatment for a research related injury can be obtained at the UCHC for the usual fee.

What if I Have Questions?

Dr. Blythe Kaufman or Dr. Christoher Beus are willing to answer any questions you have about the research. You are encouraged to ask questions before deciding whether to take part. You are also encouraged to ask questions during your study participation. If you have questions, complaints or concerns about the research, you should call Dr. Blythe Kaufman, (860) 679-2454, or Dr. Christopher Beus, (860) 679-8310, If you have questions about your rights as a research subject you may contact the Institutional Review Board at 860-679-1019 or 860-679-8729 Call this number if you want to talk to someone who is not a member of the research team or if you need assistance contacting

someone on the research team.

If you have questions about your rights as a research subject you may contact a coordinator at the Institutional Review Board at 860-679-1019 or 860-679-4851. You may also call a coordinator at the Institutional Review Board if you want to talk to someone who is not a member of the research team in order to pass along any suggestions, complaints, concerns or compliments about your involvement in the research, or to ask general questions or obtain information about participation in clinical research studies.

Please do not call the IRB number for medical related issues or to schedule or cancel an appointment.

Consent To Participation:

By signing this form you (the participant, legally authorized representative, parent(s) or guardian) acknowledge that you have read, or have had read to you, this informed consent document, have talked with research personnel about this study, have been given the opportunity to ask questions and have them satisfactorily answered, and voluntarily consent to participate in this project as described in this form.

By signing this form the individual obtaining consent is confirming that the above information has been explained to the subject (and/or legally authorized representative, parents or guardians) and that a copy of this document, signed and dated by both the person giving consent and the person obtaining consent, along with a copy of the Research Participant Feedback Form, will be provided to the participant. The handout regarding the Genetic Information Non-Discrimination Act has also been provided to the subject.

Role	**Name**	**Signature**	**Date**
Subject Signature			
Person Obtaining Consent			
Witness:			

Assent Statement for Children:

Dr. Beus has talked to me about being part of a research study that will see if a rinsing method using a vibration technique rids the root canal of germs better than other rinsing methods. He also explained to me why he asked me to be in the study. If I agree to be in the study this means that I will be put into one of two groups. One group will receive root canal treatment on a tooth using regular rinsing methods. The other group will receive root canal treatment on a tooth along with the rinsing method that involves vibration of the rinse . For each tooth, many germ samples will be taken at each dental visit. The information that is received from these germ samples will help the dentists learn which rinsing method is better in making a germ-free root canal system. There is a chance that I will need to be in the dentist's chair approximately 5 minutes longer than the normal visit.

I can ask questions about this study whenever I want. Being in this study is voluntary. I can say no now, or change my mind later, and still get the same care. Whatever I decide, Dr. Beus will not be upset with me.

Signatures:

Role	**Name**	**Signature**	**Date**
Subjects Signature:			
Parent/Guardian:			
Person Obtaining Consent:			
Witness:			

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