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Influence of a Passive Sonic Irrigation System on the Elimination of Bacteria from Root Canal Systems

S. Kirk Huffaker
University of Connecticut Health Center

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S. Kirk Huffaker

B.S., Brigham Young University, 2001

D.M.D., Case Western Reserve School of Dental Medicine, 2006

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Presented by

S. Kirk Huffaker, D.M.D.

Major Advisor: Blythe Kaufman

Associate Advisor: Patricia Diaz

Associate Advisor: Kamran Safavi

University of Connecticut

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Introduction

Apical periodontitis is the defense mechanism the human body has developed to keep destruction of the dental pulp and microbial infection of the root canal system from spreading beyond the apical foramen. Even though Miller[1] in 1890 described the presence of several different types of bacteria in the necrotic pulp, the role of microorganisms as the cause of apical periodontitis was uncertain for many years. During that time necrotic pulp,[2,3] stagnant tissue fluid,[4] or overextended root fillings were considered causative factors for apical periodontitis. More than 60 years later, Kakehashi et al. performed a study involving germ-free rats in which pulps exposed to the oral cavity did not develop apical periodontitis, while control rats with normal oral microflora developed the disease.[5] Sundqvist added to the work of Kakehashi et al. by sampling caries-free, human teeth needing root canal treatment due to trauma. These teeth had necrotic pulps, but only the teeth harboring bacteria in their root canal systems developed periapical radiolucencies, which are the hallmark of apical periodontitis.[6] Möller et al. further established the important role of obligate anaerobes in endodontic infections.[7] Since that time researchers have accepted the role that bacteria and their byproducts play as the principal cause of apical periodontitis.[8,9,10,11,12]

Endodontic Microflora

There are an estimated 10^{10} of bacteria with over 500 different species living in the human oral cavity.[13,14] All of these bacteria have the opportunity to invade

the root canal space, but there are only a few species that have been routinely isolated. The infected root canal space is largely dominated by obligate anaerobes usually belonging to the genera *Fusobacterium, Porphyromonas, and Prevotella.*[15,16,17] This is especially true in the case of traumatized teeth with intact crowns. When carious exposure of the pulp space is the cause of apical periodontitis, the microflora is dominated by strict anaerobes to a lesser extent.[18]

The unique local environment of the root canal provides the necessary factors that select for the strict anaerobic genera found therein. Oxygen and its products play an important role in the determination of which species will thrive at the different levels of the root canal system.[19,20,21] Traveling apically in the root canal system, oxygen is consumed with an increase in production of carbon dioxide and hydrogen. The developing low reduction-oxidation potential, together with the lack of oxygen selects for the growth and development of anaerobic species.

The nutrients available to the invading bacteria also play a role in deciding which bacterial species will thrive in the root canal. When there is carious exposure of the pulp space, the coronal third of the root canal contains exogenous nutrients such as fermentable carbohydrates which promote the growth and development of those species which derive their energy by carbohydrate fermentation. Facultative anaerobes such as streptococci derive their nutrients in this manner.[22] However, further down into the middle and apical third of the root canal system, the availability of these carbohydrates decreases and the bacteria found there must derive their nutrients from different endogenous sources. As will be discussed later, certain bacteria cause

inflammation of the periapical tissues and this inflammation leads to an influx of protein-containing exudate. Anaerobic bacteria have the ability to ferment proteins and glycoproteins into amino acids which they can use as nutrients for growth and development.[23] Many bacteria also receive their nutrients from the by products of other species found in the infected root canal system. It is known that mixed bacterial populations contain a food chain in which one species receives its sustenance from another bacterial species.[24] For example, black-pigmented anaerobic rods such as *Porphyromonas* have a very specific requirement for Vitamin K and hemin in order to grow. Vitamin K is produced by the *Veillonella* genus and this in turn is used by the black-pigmented rods for growth and development.[25] Species belonging to the Porphyromonas and Veillonella genera are commonly found together in the infected root canal system.

The complex interactions between microbial species found in the infected, necrotic root canal system leads to a mixed microbial population of different types, shapes, and sizes. In the undisturbed, infected root canal system these bacteria usually do not live as a single microbial species but as a collection of "matrix embedded...multispecies organisms in microecosystems that may be immobilized on the dentinal wall".[26] Today these groups of bacteria living together protected by a hydrated exopolysaccharide complex are known as biofilms.[27,28] These biofilms are known to be resistant to antibiotics and antimicrobial solutions even when directly exposed *in vitro*.[29,30] The inability to

eradicate biofilms from the root canal system may be the underlying factor in persistent, post-treatment apical periodontitis.

Pathogenicity of Endodontic Microflora

As mentioned earlier, the unique environment of the infected root canal system selects for Gram negative anaerobes such as *Porphyromonas* and *Prevotella* species. An integral part of the cell wall of Gram negative bacteria is a large molecule consisting of a lipid and a polysaccharide joined by covalent bonds. This large molecule is known as endotoxin or lipopolysaccharide (LPS). LPS contributes greatly to the structural integrity of Gram negative bacteria by increasing the negative charge of the cell membrane and thus helping to stabilize the overall membrane structure.[31]

LPS is capable of eliciting an intense immune response. LPS is released when a Gram negative bacterium dies and its cell wall disintegrates. LPS is also shed from the cell wall as Gram negative bacteria grow and multiply. It has been shown that when LPS comes in contact with mammalian cells, it has the ability to activate the complement cascade,[32] lead to the formation of antibodies,[33] and signal endothelial cells to release the cytokine TNF-α.[34] TNF-α is the primary mediator of the damaging effects of LPS as it attracts polymorphonuclear leukocytes (PMNs), macrophages, and stimulates osteoclastogenesis.[35] LPS signals the presence of Gram negative bacteria, and the body's defense cells react violently. This reaction, with all of its destructive sequelae, is seen in apical periodontitis. It has been shown that the amount of LPS removed from infected

root canals is directly correlated with the presence and number of Gram negative bacteria.[36] Removing these bacteria, along with the severely reactive LPS, is paramount in endodontic therapy.

Treatment of apical periodontitis

The presence of bacteria and their destructive byproducts inside of the normally sterile root canal sets in motion the body's defenses, and apical periodontitis is the result. It becomes clear then, that the primary goal in treatment of this disease is the removal of the foreign contents from the infected canal system.[37] Once the necrotic contents of the canal have been removed and the canal system completely disinfected and filled, healing of the damaged periradicular tissue can occur and normal biologic structure be restored.[38,39]

Root canal disinfection usually involves two distinct processes that can be explained by the term chemomechanical instrumentation. There are two parts to this term, the mechanical part and the chemical part. The mechanical aspect of root canal treatment involves the physical scraping of material (dentin, bacteria, LPS, etc.) from the root canal walls. This mechanical aspect also involves creating a particular canal shape that both follows the original canal morphology and aids in the effective delivery of antimicrobial solutions and medicaments. That being said, the chemical aspect of treatment involves the flushing of infected contents out of the root canal with an antimicrobial solution.

Before delving into the different materials and methods involved in canal disinfection it is important to note something that Walter Hess recognized in

1921.[40] The root anatomy of human teeth is much more complicated than the simple tapering canal with a single foramen. Weine[41] categorized root canal systems into four basic types. Vertucci[42] took an even closer look by using cleared teeth that had been injected with hematoxylin dye. He found the anatomy to be much more complex and identified eight possible root canal configurations. Some of these configurations can be extremely difficult to mechanically clean and chemically disinfect. In addition to the different canal patterns, there are other areas inside of the root that are challenging, if not impossible to cleanse of irritants and microorganisms. There are multiple foramina, additional canals, fins, deltas, intercanal connections, loops, C-shaped canals, and furcation and lateral canals that researchers have shown to be present in most teeth.[43,44] Removing bacteria from root canals with an orthograde technique, through the crown of a tooth that is still present in a patient's mouth can prove to be a very formidable task considering the multitude of unseen variations in canal morphology. It is surprising that the success of root canal treatment has such a predictable outcome.[45]

There have been many different instruments designed to remove infected material from root canals. Both hand-held and engine-driven instruments have been devised with the intent of physically scraping microorganisms and carious root dentin from the canal wall. For much of the past century, endodontic instruments were made of stainless steel. These files are strong, stiff, and durable, but they were also prone to cause many clinical endodontic problems such as blocks, ledges, transportation, and perforation. Nickel-titanium

instruments were introduced to endodontics in the 1990's.[46,47] There are two properties of the NiTi alloy that are beneficial to the dental operator: superelasticity and resistance to cyclic fatigue. Superelasticity is the ability to undergo a certain amount of elastic deformation when a load is applied and return to original shape when the load is removed. Clearly this is a property that would aid the clinician in cleaning and shaping root canals with all of their different configurations. Cyclic fatigue in endodontics refers to the structural damage occurring when a material is rotated around a curve. Nickel-titanium is able to resist this cyclic fatigue due to its martensitic transformation.[48] This entails changing its crystalline structure back and forth during loading and rotation, thus resisting the possibility of fracture around sharp curves.

Stainless steel hand instruments have been and are currently used for removal of necrotic material and microorganisms from root canals as well as establishing working length and glide path for subsequent rotary instrumentation. These hand instruments come in several different configurations, each bestowing a different characteristic to the file. K-files are manufactured by twisting square or triangular metal blanks along their long axis, producing horizontal cutting blades. Moving the file in a careful clockwise and counterclockwise rotational and translational working stroke is the recommended manner in which to use the file.[49] Hedström files are milled from round, stainless steel blanks. They are designed for a linear, filing motion and thus, are most efficient for translational strokes.[50] They cut in one direction only, in a withdrawal stroke. Compared with K-files, Hedströrn files show a high risk of torsional fracture due to their low core

diameter and the fact that they are milled from blanks. Because of this they are not suitable for use in a rotary reaming motion.[51] They have sharp edges and are more efficient than K-files at cutting dentin, relocating canal orifices, and removing overhangs.

Many of the classic prognosis studies found in the literature were performed using only hand instruments.[52,53,54] These studies put the success rate of teeth with infected pulps at around 80-85%. These hand instruments can be quite effective at removing bacteria from root canal systems. However, there have been studies that show instrumentation with hand instruments in curved canals is not as effective as most clinicians would like them to be. Stainless steel hand instruments have been shown to be unsatisfactory in removing debris from curved canals.[55] Hand instruments have also been shown to create pronounced zips and elbows and they tend to remove an excessive amount of tooth structure from the inner aspect of the canal curvature.[56]

Curved canals can now be treated in a consistent way with engine-driven nickel-titanium instruments. These instruments were introduced to dentistry in the 1990's and have been embraced rapidly by clinicians performing root canal treatment.[57] There are many different systems and configurations of files. Generally most files fit into two categories: Landed and non-landed. Landed nickel-titanium rotary instruments were some of the first types to be released to the market. The ProFile system, introduced in 1994, was the first of this type. It has an increased taper compared to conventional hand instruments, exhibits radial lands, and has a parallel central core. The action of the radial lands is one

of grinding or scraping rather than cutting. The lands also act to keep the instrument centered in the root canal which has been shown to prevent many of the procedural errors discussed above.[58,59,60] Non-landed nickel-titanium rotary instruments, as their name describes, have no radial lands. The cross section of these instruments is much like a K-file with sharp cutting edges. This gives the file the ability to cut dentin quicker and more aggressively. That being said, non-landed files have been associated with canal transportation.[61,62] Being made of nickel-titanium makes these instruments more resistant to deformation and loss of sharpness when compared with stainless steel handfiles.[63] It has been shown, that when placed in an engine-driven, rotary handpiece, nickel-titanium instruments remove debris quicker and stay more centered in the root canal than handfiling with stainless steel instruments.[64]

As far as the effectiveness of stainless steel handfiling vs. nickel-titanium rotary instrumentation in debriding infected root canals, various studies have shown it to be similar.[65,66,67] As effective as mechanical instrumentation is, recent studies have shown that endodontic files cannot touch a large proportion of the canal wall.[68,69] Add this to the apical deltas, webs, and lateral canals that can never be physically instrumented, and it appears that mechanical instrumentation alone would be somewhat ineffective.

A very important part of mechanical instrumentation is not only to cut dentin from the canal walls, but also to create a shape that will allow antimicrobial chemicals to effectively reach the apical areas of the root canal system. The correct shape also allows necrotic debris to be flushed from the canal by these

chemicals. Endodontic chemicals can be described as irrigants which are chemical disinfectants used during treatment; or as intracanal antimicrobial dressings placed in the canals for a certain period of time for disinfection purposes.[20]

Historically, sterile saline was used to flush the debris loosened by mechanical instrumentation from the root canal and was somewhat effective.[12] Today however, the antimicrobial irrigant of choice is sodium hypochlorite (NaOCl).[70] In 1915 NaOCl was recognized as an antimicrobial solution suitable for medical use because it would flush away necrotic tissue and at the same time not cause damage to vital tissue.[71] In 1919, Coolidge[72] suggested that NaOCl be used as an antimicrobial irrigant for endodontic use. Since that time it has been shown that NaOCl improves the ability of nickel-titanium rotary instruments to remove bacteria from root canals.[73] Sodium hypochlorite has also been shown to increase the cutting efficiency of rotary instruments and the rate of root canal volume removal.[74] This is most likely due to its exceptional ability to dissolve organic matter.[75,76] NaOCl has been shown to kill bacteria and remove debris from the root canal significantly better than saline.[77]

Despite widespread use of NaOCl in endodontics, surprisingly little is known about its mechanism of action. We do know that the active ingredient in NaOCl is hypochlorous acid (HOCl). This acid is produced by the mammalian host defense to kill invading microorganisms.[78] HOCl has the ability to stop DNA synthesis, disrupt oxidative phosphorylation, and disrupt bacterial membrane activites,[79] However, until just recently the mechanism behind these

antimicrobial activities was unknown. In 2008, Winter et al. were able to show that in low concentrations, HOCl causes oxidative protein unfolding which targets thermolabile proteins for irreversible aggregation.[80] By causing essential bacterial proteins to unfold, the unfolded proteins begin to aggregate. The aggregation of essential proteins then kills the microorganism.

Many different concentrations of NaOCl have been used in root canal systems for irrigation purposes. It is a source of controversy in the endodontic literature as to whether one should use commercially available, full strength (5.25%) NaOCl or a diluted concentration. Hand et al found that 5.25% NaOCl was significantly more effective that all other diluted concentrations in dissolving necrotic tissue.[81] Harrison et al showed that dilution of NaOCl significantly reduced its antimicrobial activity.[82] Another study showed that infected root canals treated with differing concentrations of NaOCl could only be rendered bacteria free in the undiluted form.[83] On the other hand, it has been shown that a lowered concentration does not reduce the ability to remove and kill bacteria from root canals.[84,85,86] Spångberg showed that full-strength NaOCl is very cytotoxic to living cells. He also showed that by diluting NaOCl down to 0.5% there is an acceptable balance between antimicrobial affect and cytotoxicity.[87] Basically, a 0.5% NaOCl solution dissolves necrotic tissue, but not living tissue, and still maintains its antimicrobial effect. Another study using electron microscopy showed that the amount of debris removed from canals was not significantly different when using dissimilar concentrations of NaOCl.[88] The amount of HOCl present in 5.25% NaOCl is greater, but with the increased amount comes more

cytotoxicity and more opportunity to cause series biological damage to living structures.[89] If a decreased concentration has sufficient antimicrobial effect and can effectively remove necrotic tissue from canals, it would appear wise to use a reduced concentration of NaOCl in the treatment of apical periodontitis.

Chemomechanical instrumentation with hand/rotary instruments and NaOCl is effective at reducing but not eliminating intracanal bacteria.[39,73] In attempt to reduce the bacterial load of infected root canal systems above that attained with conventional instrumentation and irrigation, the addition of an interappointment, intracanal medication has been recommended.[90,91,92] Many different intracanal medicaments have been suggested, but Hermann[93] in 1920 introduced the use of calcium hydroxide in endodontic treatment, and this compound has become the interappointment, intracanal dressing of choice.[94] Calcium hydroxide is a strongly alkaline compound, which has a pH of approximately 12.5. Many species of bacteria commonly found in infected root canal systems are very susceptible to changes in pH and are eliminated after a short exposure to this substance.[85,90] Calcium hydroxide is normally used as a slurry of calcium hydroxide in a water base. In an aqueous environment, calcium hydroxide dissociates into calcium and hydroxide ions. The antimicrobial activity of this compound is directly related to the release of hydroxide ions which maintains the alkalinity of the aqueous environment. Hydroxide ions are also highly reactive free radicals that react with biomolecules such as cytoplasmic membranes[95], DNA[96], and enzymatic proteins[97] essential for the survival of a cell. In addition to killing bacteria, calcium hydroxide has the ability to hydrolyze the lipid moiety of

LPS, thus reducing its effect.[98,99] This is a beneficial property of calcium hydroxide because even though bacteria may not be viable, the material from their cell wall may be present and continue to stimulate inflammation in the periradicular area. Calcium hydroxide has also been found to improve the ability of NaOCl to dissolve necrotic tissue, thus facilitating its removal from the root canal system.[100] It appears that from a microbiological standpoint, a multi-visit approach to treatment of teeth with necrotic pulps, with the use of calcium hydroxide, would be beneficial to the prognosis of the treatment.

Despite all of the efforts to kill and remove bacteria from root canal systems by chemomechanical instrumentation and interappointment medication, the success rate of treatment is not 100% and there are failures in which the apical periodontitis does not heal.[101] There are several reasons for the failures that occur, but usually it is due to residual bacteria still alive in the root canal system even after thorough root canal therapy.[102] Nair et al, analyzed, histologically, the effectiveness of thorough instrumentation, irrigation, and even obturation of infected root canal systems.[103] It was found that a majority of teeth treated endodontically, still harbored bacteria in inaccessible areas of the root canal system and that these bacteria existed as biofilms. It was concluded that nonantibiotic chemomechanical measures are very important in the disruption of biofilms so as to reduce the intraradicular microbial load to as low as possible.

Passive Ultrasonic Irrigation

In recent years the addition of ultrasonic devices to the irrigation regimen has been proposed as a means of disrupting the microbial flora of the infected root canal and removing more necrotic debris.[104,105,106] Irregularities exist in the root canal that endodontic instruments cannot mechanically debride such as oval extensions, isthmuses, and apical deltas.[101,107,108] The effectiveness of irrigation depends on the antimicrobial and tissue dissolving mechanism of the solution and the ability to bring the solution in contact with microbes and tissue.[109,110] Ultrasonic energy produces two phenomena that may be able to debride and bring irrigant to those areas that cannot be cleaned with traditional endodontic instruments in orthograde treatment. These two phenomena are cavitation and acoustic streaming.

Ultrasonics were introduced to endodontics by Richman in 1957[111]. At first they were used in the instrumentation of root canals in place of stainless steel handfiles. Unfortunately, it was too difficult to produce consistent root canal shapes during canal preparation and perforations were common.[112] However, it was found that ultrasonically activated hand files could increase the effectiveness of irrigation during root canal treatment. Weller et al.[113] was the first to describe the term passive ultrasonic irrigation (PUI). 'Passive' does not accurately describe the action of the process because an endodontic file is being activated. 'Passive' refers to the 'noncutting' action of the ultrasonically activated file. PUI depends on the transmission of acoustic energy from the activated file to a solution in the root canal. The energy is transmitted through ultrasonic waves,

which induce the phenomena of cavitation and acoustic streaming in the irrigating solution.[114]

Acoustic streaming and cavitation is used in the manufacturing industry to clean electronics of micro-debris at a very high level.[115] Acoustic cavitation is the creation and collapse of tiny air bubbles created by acoustic sound waves. Sound waves are produced in closed cavities by sonic or ultrasonic energy. When this energy is transferred into a liquid, a pressure change occurs in the liquid which causes it to momentarily drop below vapor pressure, producing air bubbles. In the presence of these sound waves, the air bubbles contract and expand to a point where the surface tension of the bubble can no longer be sustained and the bubble will collapse. The energy released when the bubble collapses is transferred to the wall of the cavity and any debris found thereon is liberated from the wall.[116] Acoustic streaming involves the creation and movement of sound waves inside of a closed channel. The sound waves are created at small sources called nodes.[114] Ultrasonic energy radiated into a small hand file can produce several nodes along the length of the file.[112] The momentum created by these waves enhances the detachment and removal of particles away from their substrates much like the current in a river carrying dirt and sediment downstream. In the case of root canal irrigation, the current carries the debris coronally[117] and into a high-vacuum suction.

PUI is used following hand and rotary instrumentation of the root canal system. After the shape of the canal has been prepared, the canal is filled with irrigant and a small ultrasonically activated file is introduced in the center of the

canal. Because the canal has already been shaped to a size and taper larger than the activated file, the oscillating file can move freely and the irrigant can infiltrate deeper into the apical recesses of the canal,[118] and the cleansing effect will be more vigorous.[112] If the canal is not prepared sufficiently the file may touch the canal wall excessively dampening the sideways movement of the file. This dampening of movement will result in decreased node production, decreased acoustic streaming and cavitation and thus decreased cleaning power.[119] van der Sluis et al.[120] showed that by increasing the taper of the canal, more debris was removed during PUI. Also, if the activated file is too large (>ISO 20) it may be too stiff to oscillate sufficiently to produce enough acoustic streaming to clean effectively.[121] As with any technique, the proper application is necessary to achieve the desired effect.

Martin et al[122] found that ultrasonically energized hand files removed a significantly greater amount of dentin than hand filing alone. PUI was found to be more effective than syringe irrigation in removing pulp tissue and dentin debris when using NaOCl as the irrigant.[123] PUI was also more effective at removing planktonic bacteria when compared to syringe irrigation.[124] Cavitation has been shown to remove and destroy biofilm.[125] In curved canals, when activated files are pre-bent, PUI is found to clean canals better than syringe irrigation.[126] The portion of the root below the curve was examined histologically in one study and PUI was more effective at removing debris than syringe irrigation.[127] The efficacy of PUI to debride the isthmus which runs between canals was evaluated compared to syringe irrigation. PUI performed significantly better.[128]

The EndoActivator® is a new device designed to produce sonic sound waves (10,000 cycles per minute) inside of a root canal system[129] Energy transfer occurs between the activator's polymer tip and the irrigating solution used in the root canal system. The manufacturer claims that "the EndoActivator System is designed to provide a safer, better, and faster way to achieve success in ... the debridement and disruption of ... biofilm."[129] The EndoActivator® is advocated for use following standard instrumentation and irrigation techniques just prior to obturation of the root canal. A review of the literature revealed that, to date, there has been no *in vivo* study published involving the EndoActivator® and increased removal of bacteria from infected root canal systems over standard irrigation and instrumentation protocols. There have, however, been studies comparing passive ultrasonic irrigation with passive sonic irrigation (PSI), in which PUI removed more debris than PSI.[130,131] Sonic irrigation is different from ultrasonic irrigation because it functions at a lower frequency. This will produce a lower streaming velocity. In addition, the oscillating patterns of sonically activated instruments are different. There is one node near the attachment of the file and one antinode at the tip of a sonically activated file. This means that if the sonically activated file in anyway is constrained during PSI, the sideways movement will disappear, eliminating the one node along with cavitation and acoustic streaming.[132] An *in vivo* study testing the ability of the EndoActivator® to improve irrigation in root canal treatment is warranted.

One-visit versus multi-visit endodontics

As mentioned ealier, Nair et al.[103] concluded that biofilms found in the canal system cannot be removed by instrumentation and irrWigation alone in one-visit treatment. This conclusion is not a new one. It has been recommended by many researchers and clinicians over the years that treatment of necrotic pulps be finished in at least two visits with the use of an intracanal medicament such as calcium hydroxide.[9,23,38,39,90,91] This recommendation was made on the basis of animal, human, and culture studies.

In animals, teeth exhibiting necrotic pulps that were medicated with calcium hydroxide and then obturated were compared to teeth of the same type obturated in one-visit. These teeth were then studied histologically. It was found that teeth medicated with calcium hydroxide exhibited more healing and less inflammation than the teeth treated in one visit.[133] Another study evaluated teeth with radiographic signs of apical periodontitis. Teeth having received interappointment calcium hydroxide medication were compared to teeth where root canal treatment was completed in one-visit. Teeth treated with calcium hydroxide prior to obturation showed a higher healing rate.[38] There have been other follow-up studies that have shown that a multi-visit approach for the treatment of teeth with apical periodontitis results in improved healing rates.[91,134]

On the other hand, there are other studies that have shown no difference in the radiographic healing between one- and two-visit treatment. Weiger et al.[135] could not show a statistical difference between one- and two-visit treatment with a 5-year follow-up. Peters et al.[136] found radiographic healing of periapical

lesions 81% of the time with single-visit endodontics and 71% of the time with two-visits and calcium hydroxide. Other studies have also failed to show a significant difference in radiographic healing between one- and two-visit treatments of apical periodontitis.[137,138] It has even been suggested that cases with soft-tissue swelling and symptoms can be completed in one visit with meticulous endodontic treatment, incision and drainage, and an appropriate antibiotic regimen.[139]

In 1965, Engström and Lundberg[140] reported a lower success rate for root canal treatment when teeth were obturated in the presence of cultivable bacteria. Sjögren et al.[39] also reported the same findings, with successful periapical healing being seen 94% of the time when bacteria were not able to be cultured from the infected root canal. When obturated in the presence of cultivable bacteria the healed rate dropped to 68%. Other studies have also shown that cultivable bacteria present in the canal at the time of obturation have a negative impact on radiographic healing.[66,134] From a literature standpoint, it appears that successful healing of apical periodontitis does not depend on whether an infected tooth is treated in one- or multiple visits, but it depends upon whether bacteria can be eliminated from the canal to a level that is not cultivable. If necrotic pulps could be consistently rendered "culture-negative" in one visit that would be beneficial to both clinician and patient. On the other hand, if a more consistent result is produced with two-visits of instrumentation/irrigation and interappointment calcium hydroxide disinfection, then the doctor must provide the more effective care to his/her patient.

Microbiological sampling of root canals

All of the above mentioned treatment protocols are directed toward the elimination of bacteria from the root canal. As mentioned before, bacteria present in the normally sterile root canal system, are the principal cause of apical periodontitis.[5,6] Because of the intimate relationship between bacteria and clinical endodontics, many of the past and current philosophies and treatment protocols are based upon bacteriological root canal sampling. The rationale behind it is quite simple and logical. If bacteria are the main cause of apical periodontitis, then elimination of the bacteria from the root canal system should usually result in healing of the apical periodontitis. If a negative bacteriologic sample can be obtained from an infected root canal system prior to obturation, then our prognosis for healing and our confidence in a particular treatment protocol should improve. Surely, a healed periapical lesion as seen on a radiograph is the ultimate indicator of success, but follow-up studies of this type require large sample sizes, are time consuming, and are costly. Even though it may not be sensitive enough to predict treatment outcome effectively,[141] sampling is an easily measured variable that may predict a successful treatment result.[15,65,142,143]

Onderdonk was the first to advocate sampling of the infected root canal system.[144] His opinion at the time was that there were two tests available to a clinician to predict a successful outcome. One was the physical test, which he said was the "absence of pain". Then he proposed that clinicians use a "scientific

test" so that they could know when they have obtained an "aseptic root" and be more assured of a successful outcome. He suggested using sterile cotton to obtain a bacteriologic sample from the root canal. When no bacteria could be sampled from the canal, i.e. a negative culture, the cleaning portion of the treatment was finished and the clinician should proceed to obturation. Following Oderdonk's proposal, clinicians began to sample infected root canal systems following cleaning and shaping procedures. These samples were taken aerobically, with no thought of the anaerobic bacteria that, unknown at that time, were the principal cause of the disease. There is no doubt that many teeth in that time were obturated in the presence of bacteria that could be cultured today.

Out of the era of routine bacteriologic sampling of root canals came many of the recommendations and treatment protocols that we have today,[145,146,147] and endodontic therapy turned into a very reliable treatment of apical periodontitis.[53,148] With that reliability, clinicians and even some educators stopped taking cultures and even began advocating treatment of teeth with necrotic pulps in one visit.[149,150]

Today, bacteriologic samples are not taken routinely in clinical practice or in educational institutions, and in fact, sampling has been called into question as a means of predicting successful treatment outcome.[141] Researchers have brought up the argument that sampling of root canal systems for positive or negative cultures may not be a good predictor of success as it has been shown that more than half of culture positive teeth show radiographic healing[39] and teeth with normal periapical status harbor living bacteria.[151] That being said, we

should remember that the purpose of bacteriologic sampling is not to predict healing. Sampling root canals is simply an attempt to recover viable bacteria from root canals as they are the cause of apical periodontitis.[5,6] The goal of endodontic therapy is to eliminate viable bacteria from the root canal system. If a certain treatment protocol can render the majority of root canals clean of cultivable bacteria, then the protocol can be recommended to clinicians as a means to eliminate cultivable bacteria from the infected root canal system. Follow-up studies involving radiographic healing can be performed later to strengthen the recommendation.

The oral cavity is a challenging environment from which to obtain an accurate bacteriologic sample as bacteria are ubiquitous, increasing the possibility of false positives due to contamination. Möller established the gold standard for effective sampling of the root canal system.[152] He showed that if certain steps are strictly adhered to, contamination can be avoided, and the process can be simple and effective. These steps are the following: 1) The tooth receiving the treatment needs to be scaled and polished with a rubber cup and pumice and effectively isolated with rubber dam. 2) The rubber dam, retainer, and tooth must be disinfected with 30% hydrogen peroxide followed by 5% tincture of iodine. 3) Sterility of the operating field needs to be confirmed by sampling tooth surfaces with sterile cotton. If the sample taken from the surface is positive for bacteria, the data obtained from that tooth must be discarded. Researchers have followed these steps in their sampling procedures and have avoided contamination.[39,134]

Rationale for the present study

The goal of endodontic treatment is to eliminate the causes of apical periodontitis from the infected root canal system. Apical periodontitis is caused almost exclusively by bacteria and their byproducts. Conventional chemomechanical instrumentation with hand/rotary instruments and NaOCl is effective at killing bacteria and removing necrotic debris from the root canal system. This has been seen in studies utilizing microbiological sampling in which the bacterial load is significantly reduced. However, bacteria found in inaccessible areas of the root canal and biofilms cannot be killed and removed effectively by conventional means and may cause failures. Interappointment medication with calcium hydroxide has been found to increase the probability of obtaining a negative bacterial sample from infected root canal systems. Passive ultrasonic irrigation has also been found to be effective in the removal of debris from root canals due to the unique phenomena of cavitation and acoustic streaming. The EndoActivator®, used as a passive sonic irrigation system, boasts "a better, safer, and faster" way to eliminate biofilms from root canal systems. The ability of this device to kill bacteria and remove necrotic debris from canals *in vivo* has not been tested.

It would be beneficial for the doctor and the patient if teeth exhibiting apical periodontitis could be treated in a single visit. This would mean only one injection, one hour in the chair, one day off from work, and only one day of possible anxiety. To the doctor this would mean that he/she could streamline the treatment to be more efficient, and thus increase productivity and be able to see

more patients. If the addition of the EndoActivator® to standard chemomechanical instrumentation can increase the clinician's ability to achieve negative bacterial cultures more than standard irrigation alone then it is a valuable tool and should be added to the endodontic armamentarium. If its ability to produce negative cultures is comparable or better than that of interappointment calcium hydroxide disinfection, then two-visit treatment becomes obsolete and unwarranted.

The aims of this study were the following: (1) to evaluate if the addition of the EndoActivator® to standard chemomechanical instrumentation could improve the ability of endodontic residents to achieve a negative bacteriologic sample from teeth exhibiting radiograph signs of apical periodontitis. (2) To compare the ability of the EndoActivator® or standard irrigation (SI) to eliminate bacteria with that of a second-visit with calcium hydroxide disinfection. (3) To evaluate possible factors that might contribute to or influence the ability to achieve a negative bacteriologic sample such as: operator, amount of irrigation, master apical file size (MAF), and tooth type.

Materials and Methods

This study, as it involves living human beings, was approved by the Institutional Review Board of the University of Connecticut Health Center. The DHHS Federal Wide Assurance Number is 00006064.

Our goal was to detect a "clinically" significant difference in the ability to culture viable bacteria from canals. In 1988 Cohen[153] suggested effect sizes for

detecting differences in independent samples. According to his recommendation, we chose an effect size of 0.8 which would detect a large difference between means of independent samples. With an effect size of 0.8 decided upon, a power analysis using G*Power statistical software[154] gave us a sample size of 42 for each group. Therefore, 84 was the number chosen for our sample size.

Participant selection

Any patient presenting to the endodontic clinic for root canal treatment 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

Treatment protocol

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 was disinfected following the endodontic clinic standard disinfection protocol.[152] This includes swabbing the tooth with 30% hydrogen peroxide followed by 5% iodine tincture. After removal of any restoration and caries, the tooth was disinfected again following the same disinfection protocol as described above. The iodine was then inactivated using 5% sodium thiosulphate and a bacteriologic sample was taken to confirm that we

had elirninated cultivable bacteria from the surface of the tooth. All bacterial cultures were taken by the same operator throughout the duration of the study so as to standardize the culturing technique.

Cultures were taken with sterile paper cones and 8mm tubes containing liquid thioglycollate broth enriched with vitamin K-1 and hemin which enhances the growth of anaerobic species.[155] Following sampling, the cultures were placed in an incubator for seven days at 37° C and 100% humidity and observed for turbidity.

The pulp chamber was entered and observed for pulp vitality. All canals were then located and slightly reamed with hand files to create space for paper points. No irrigation was used up until this point. Following this, each canal was filled with sterile saline and a second bacterial culture taken. This culture confirms the presence of bacteria in the canals.

Standard clinical instrumentation protocol followed the second bacterial culture. This involves the preflaring of canals, obtaining working length approximately 1 mm short of the radiographic apex, and full instrumentation with rotary and hand instruments used in a crown down fashion under copious irrigation with 0.5% sodium hypochlorite (NaOCl). EndoSequence rotary instruments were used on all teeth with the MAF being determined by the clinician. 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.

When the endodontic resident performing the treatment determined that chemo-mechanical instrumentation was complete, a card was brought to the

resident indicating whether the tooth would be sonically irrigated or not. These cards had been randomized by a computer program before the beginning of the experiment, so that the resident performing treatment and the operator taking the bacterial samples were blinded to the treatment protocol following instrumentation. The resident then recorded on the card: the tooth being treated, MAF, and the total amount of irrigation used in milliliters. If the card indicated passive sonic irrigation (PSI), the following protocol was followed:

- Each canal is filled with NaOCl
- EA inserted into the canal and activated for 30 seconds in each canal. After 30 seconds of activation in each canal, a fresh solution of NaOCl is delivered into each canal and is activated for 30 more seconds.
- Following activation, canals were flushed with sterile saline and dried with paper points.

If the card indicates standard irrigation (SI), the following protocol was followed:

- Each canal is filled with NaOCl.
- The NaOCl is left in each canal for 30 seconds. After 30 seconds a fresh solution of NaOCl is delivered into each canal and left there for 30 more seconds.
- Canals are then flushed with sterile saline and dried with paper points.

Subsequent to this post-instrumentation irrigation protocol, each canal was 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. Each canal was then sampled for viable bacteria.

Following this third bacterial sample, each canal was filled with calcium hydroxide, and the tooth temporarily restored according to standard protocol: a 3-4 rnm layer of cavit covered with Fuji IX. The patient was then scheduled for a second appointrnent at least two weeks later.

At the second visit, the tooth was isolated, disinfected, and sampled as before to rule out false positive contamination. The teeth were reaccessed and the canals irrigated completely with NaOCl along with any additional instrumentation. Five percent Sodium Thiosulphate was again delivered into each canal to inactivate any remaining NaOCl, canals flushed with sterile saline and a final bacterial sample taken. At this point all clinical information had been gathered for this tooth and the resident was free to obturate the canals in the manner of his/her choosing.

All samples were observed by the study coordinator for signs of turbid broth signaling a culture positive for bacteria. The day that the broth turned turbid was noted for statistical analysis.

Six teeth scheduled for root canal treatment with no signs of periapical destruction as seen on a radiograph, and vital pulps confirmed by the presence of heme upon entry into the pulp chamber were used for negative controls. Three teeth were assigned to the PSI group and three to the SI group. The entire experiment as recorded above, including use of EA and taking of bacterial

samples was performed on these six teeth. Control teeth were also observed for turbidity for seven days.

Each tooth in the experiment was assigned a number (1-90). This number was printed on each bacterial sample taken and was recorded on the treatment card. At the end of the first visit, the card was stapled to the patient's record found in the patient's chart. At the second visit, this card was removed from the patient's chart and submitted to the principal investigator so that the information found thereon could be analyzed.

All statistical analysis was performed using SPSS Statistical package 17.0. Cross-tabular calculation was used to compare the different data sets for each sample. Independent and paired-sample T-tests were performed for analysis of turbidity results for PSI vs. SI groups and first-visit vs. second-visit groups respectively. Chi-square analysis was performed to analyze if the differences seen in treatment parameters were significant.

Results

A negative culture was obtained for surface disinfection in 96.5% of the samples. All controls showed negative cultures at the end of the first and second visits.

In comparing PSI and standard irrigation, tables 1 & 2 and graphs 1 & 2 show the results. A total of 84 first-visit bacteriologic samples were obtained, 42 for each group. PSI produced 17 negative cultures (41%) and standard irrigation produced 20 negative cultures (48%) and the difference was not significant (p

>.05). Ten patients would not present for the second visit appointment and were excluded from that data set for a total of 74 second-visit samples. Of the 74 bacteriologic samples obtained 36 were from the PSI group and 38 from the SI group. There were 27 negative cultures obtained at the second-visit from the PSI group, and there were 27 negative cultures obtained from the SI group. Comparing the first visit with the second visit, there were a total of 37 negative cultures obtained at the first visit and 54 negative cultures obtained after calcium hydroxide disinfection at the second visit. The difference between first and second visit was significant ($p<.05$).

Due to the inconsistent number of cases contributed to the study by each operator in the resident endodontic clinic, the influence of operator on the ability to produce a negative culture could not be evaluated.

Table 3 outlines the influence of MAF on culture results after one visit. The negative culture result for an MAF of 25 was 2/17 or 12% negative. The negative culture result for an MAF of 30 was 12/22 or 55% negative. The negative culture result for MAF sizes of 35, 40, 45, 50 were 4/11 (37%), 4/10 (30%), 2/5 (40%), and 4/5 (80%) respectively. Of the samples taken from canals finished with hand files above MAF size 50 there were 5/7 (71%) negative cultures obtained. These differences were found to be significant ($p< .05$).

Table 4 outlines the influence of MAF on culture results after the second visit. The negative culture result for an MAF of 25 was 12/15 or 80% negative. The negative culture result for an MAF of 30 was 17/32 or 53% negative. The negative culture result for MAF sizes of 35, 40, 45, 50 were 7/10 (70%), 5/9

(56%), 3/4 (75%), and 5/5 (100%) respectively. Of the samples taken from canals finished with hand files above MAF size 50 there were 5/7 (71%) negative cultures obtained. With the addition of a second visit of instrumentation and irrigation and calcium hydroxide disinfection, the significance in the differences vanished ($p > .05$).

Table 5 outlines the influence of amount of irrigation on culture results. If an operator used 0-12 ml of irrigation a negative culture was obtained 6/11 (55%) times. For 13-24 ml, 25-36 ml, and >37 ml the results were 13/36 (36%), 10/25 (40%), and 2/5 (40%) respectively. These differences were not significant ($p > .05$).

The culture results for amount of irrigation at the second visit are found in Table 6. If an operator used 0-12 ml of irrigation a negative culture was obtained 17/20 (85%) times. For 13-24 ml, 25-36 ml, and >37 ml the results were 25/36 (69%), 10/14 (71%), and 1/2 (50%) respectively. As with the first visit, the results for amount of irrigation used during the second visit are not significant ($p > .05$).

Culture results for the influence of tooth type on cultivable bacteria present after chemomechanical instrumentation at the first visit are found in Table 7. Of the 36 incisor/canine teeth sampled, 22 (61%) had a negative culture. Negative culture results for Premolar and Molar teeth were 6/26 (23%) and 4/16 (25%) respectively. These differences were found to be significant ($p < .05$).

Table 8 shows the influence of tooth type on culture results for the second visit. Of the 34 incisor/canine teeth sampled, 28 (82%) had a negative culture. Negative culture results for Premolar and Molar teeth were 9/14 (64%) and 16/24

(67%) respectively. The differences between tooth types were not found to be significant after calcium hydroxide disinfection at the second visit ($p > .05$).

Discussion

When dealing with non-surgical root canal treatment clinicians are usually faced with two situations. The first is a tooth present with a vital pulp in which the tissue found in the root canal is inflamed but not completely infected by microorganisms. When these types of cases present themselves, the treatment is relatively straightforward. In vital cases, all of the clinician's effort is spent removing the sterile tissue aseptically and not introducing microorganisms into the root canal. This type of treatment can usually be completed on one visit.[156] The other situation is much more complex. When a patient presents with a tooth that has been infected by bacteria and has begun to cause periapical bone breakdown, the clinician must use all the means he/she has to kill and remove the invading bacteria and their inflammatory byproducts from the canal system.

In recent years, it has been suggested that files attached to ultrasonic handpieces be used to aid in the irrigation and debridement of infected root canals.[113,157] Recently the EndoActivator® has been recommended[129] for use in the irrigation of root canals systems due to its proposed ability to create sonic waves inside of the root canal which may aid in the killing of bacteria and debridement of necrotic tissue. In the current study it was not shown that EA improved the ability to kill cultivable bacteria from infected root canals. Our study

found no significant difference between the negative cultures obtained by standard irrigation and those obtained with use of EA.

One reason for this may be that EA produces only sonic waves. Stamos et al.[130] compared the use of sonically and ultrasonically activated instruments and found that the ultrasonically activated instruments removed significantly more debris that those that were activated sonically. These results were duplicated in another study as well.[131] Node production along activated files is an important part of acoustic streaming, resulting in a strong current produced along the activated instrument. If the instrument touches the canal wall, the node in the immediate vicinity will be diminished. Because it is inevitable that the file will touch the canal wall, it is important to create several nodes along the instrument being activated. Sonic energy only has the power to produce one node along the length of the instrument, so any constraint of the instrument will significantly decrease if not eliminate the acoustic streaming necessary to dislodge and carry away necrotic debris.[132] EA is powered by one double-A battery. As the battery life is used up, only the audible "whir" of the handpiece will tell the clinician if the battery is low and the handpiece is losing power. Clearly, some power will be lost before the clinician can audibly detect it, which will lessen the ability of EA to produce the sonic waves needed for acoustic streaming and cavitation inside the canal.

EA may not be powerful enough to disrupt bacterial biofilms. Ahmad et al. showed that ultrasonically activated instruments could not disrupt bacteria but simply dispersed it to other areas of the canal.[158] Even with ultrasonics, Mayer et

al[119] found that only the coronal third of the canal was being cleaned. In that study there was no significant difference between syringe irrigation and PUI in the apical third of the canal. This may be due to the activated file touching the canal wall in the apical third and not being able to produce the necessary nodes for acoustic streaming and cavitation.[117] If ultrasonic instruments with their constant power supply and more powerful node-producing waves cannot effectively clean in the apical third, it is likely EA with its battery power and sonic engine will have the same problem.

The ability of a second visit of instrumentation and irrigation together with an interappointment medication of calcium hydroxide was compared to a single-visit of instrumentation and irrigation. Studies have shown that when treatment of necrotic pulps is performed in two-visits with calcium hydroxide disinfection that there is a reduction in intracanal bacteria and a greater likelihood of obtaining a negative culture.[73,90,142] Calcium hydroxide raises the pH of the root canal system to a level at which many microorganisms cannot survive.[142,159] It has also been found that when necrotic tissue has been in direct contact with calcium hydroxide, it becomes more soluble and susceptible to dissolution by NaOCl.[100,160] In the present study it was shown that the addition of calcium hydroxide together with another round of instrumentation and irrigation was effective at eliminating cultivable bacteria from significantly more teeth.

In the current study, two-visit treatment with calcium hydroxide disinfection was able to eliminate cultivable bacteria from about 75% of teeth exhibiting apical periodontitis. This is in line with some studies[66,161,162,163], and is a lower number

than other studies.[73,90] It is known that some bacteria are more resistant to the high pH of calcium hydroxide.[164] Bacteria living in biofilms are also more resistant to NaOCl and the alkaline stress of calcium hydroxide even when directly exposed *in vitro*.[165166] A very important factor in the effectiveness of calcium hydroxide is the ability of the operator to place it effectively. It is essential that the calcium hydroxide be placed in the instrumented canal as a thick, moist paste that completely obturates the canal space.[159] The manner in which the calcium hydroxide was mixed and placed was not observed or standardized, and this could be a confounder.

If a resistant species of bacteria is present, if bacteria has established itself as a biofilm inside of the canal, or if the operator is not careful about his/her placement of calcium hydroxide the effectiveness of the dressing will be compromised. That being said, one of the greatest benefits of multi-visit treatment may be the benefit of a second or third opportunity to disrupt biofilms and irrigate them out of the root canal.[30,103] Calcium hydroxide is not the only benefit in a multi-visit approach to apical periodontitis.

The size to which an operator should enlarge a canal apex is important in root canal treatment. It has been suggested that MAF sizes should be kept to a minimum to avoid apical perforation, ledge creation, apical zipping, over-weakening of the root, and/or apical splitting.[23] In addition, the ability to successfully obturate a canal without overfilling is made more difficult with a very large apical prep size. It is also more difficult to successfully retreat a tooth that has already been prepared to very large MAF without further weakening the root

structure by taking away precious root dentin. That being said, it has been shown that increasing the apical size during root canal preparation is effective at reducing intracanal bacteria.[143,167] In the current study, this was shown to be the case. 80% of canals that were only prepared to an MAF of 25 still harbored cultivable bacteria. At a size 30 this number dropped to 45% and of the teeth instrumented to an MAF of 50, only 20% harbored cultivable bacteria. These differences were found to be significant. When a second visit with calcium hydroxide disinfection was added, the significant difference disappeared, as only 20% of the teeth prepared to a size 25 now harbored cultivable bacteria. If the clinician wishes to keep his/her apical preparation small, a second appointment with inter-visit medication of calcium hydroxide may be an appropriate addition to the treatment.

Tooth type was shown to be associated with a greater likelihood of obtaining a particular culture result at the first visit. Molar and premolar teeth proved to be the most difficult from which to obtain a negative culture as 77% and 75%, respectively, still harbored cultivable bacteria at the first visit. This is not surprising when one thinks of the isthmuses, extra canals, and eccentric root anatomy found in molar and premolar teeth.[168,169,170] In 61% of single-canal incisors and canines a negative culture was obtained at the first visit. Isthmuses, extra canals, and eccentric root anatomy are found to a much lesser extent in these tooth types.[171,172] The differences in culture result between the different tooth types was significant at the first visit, but the significance disappeared with a second visit and calcium hydroxide disinfection. With all of the different places

for bacteria to live and hide in molar and premolar teeth, adding a second-visit of further instrumentation and irrigation seems to give the clinician an extra opportunity to remove them.

Amount of irrigation was not a significant predictor of culture status. Whether the operator used less or more did not seem to affect the culture results. One possible explanation for this goes back to the biofilm present in the canal. Unless biofilm is physically manipulated by a file or (possibly) ultrasonically created cavitation bubbles it will most likely survive NaOCl and calcium hydroxide exposure.[11,30,165] In light of biofilm's remarkable resistance to antimicrobial stresses, a multi-visit approach with added instrumentation seems to be advocated for teeth with apical periodontitis.

Conclusions

1. Passive sonic irrigation with the EndoActivator® did not perform better than standard syringe irrigation in the removal of cultivable bacteria from root canal systems.
2. A second visit of instrumentation and irrigation with inter-visit medication with calcium hydroxide eliminated cultivable bacteria from teeth to a significantly greater extent than a single visit of chemomechanical instrumentation.
3. A larger master apical file size removed significantly more bacteria at the first visit, but with the addition of a second visit and calcium hydroxide disinfection smaller preparations produce similar results.

4. It is more difficult to remove cultivable bacteria from molar and premolar teeth in one visit when compared to single rooted incisors and canines. Addition of a second visit and calcium hydroxide disinfection significantly improves the ability to remove cultivable bacteria from molars and premolars.

Tables & Graphs

Table 1 – PSI vs. SI Culture Results

Protocol * Culture Result 1st visit

			Culture Result 1st visit		
			Negative	Positive	Total
Protocol	PSI	Count	17	25	42
		% within Protocol	40.5%	59.5%	100.0%
	SI	Count	20	22	42
		% within Protocol	47.6%	52.4%	100.0%
Total		Count	37	47	84
		% within Protocol	44.0%	56.0%	100.0%

Independent Samples Test

Assumptions=Equal variances assumed

	Levene's Test for Equality of Variances		t-test for Equality of Means						
								95% Confidence Interval of the Difference	
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Day turbid 1st visit	**2.036**	**.157**	**1.612**	**82**	**.111**	**.66667**	**.41363**	**-.15618**	**1.48951**

Graph 1

Influence of Passive Sonic Irrigation (PSI) on culture results compared with standard irrigation (SI).

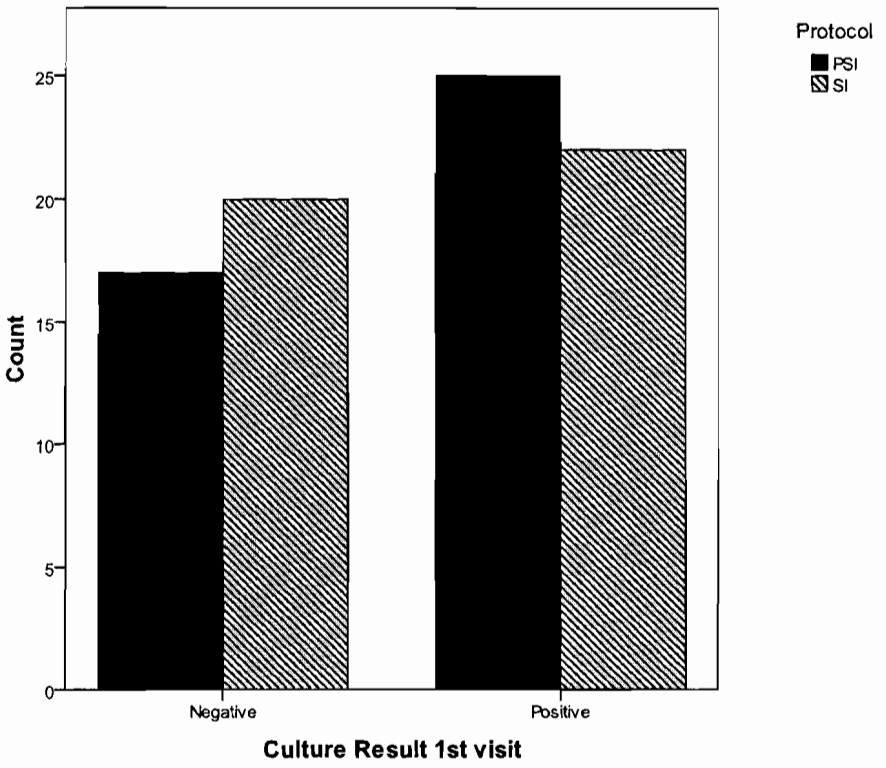

Table 2

1st vs. 2nd visit – Culture Results

Protocol * Culture Result 2nd visit

			Culture Result 2nd visit		
			Negative	Positive	Total
Protocol	PSI	Count	27	9	36
		% within Protocol	75.0%	25.0%	100.0%
	SI	Count	27	11	38
		% within Protocol	71.1%	28.9%	100.0%
Total		Count	54	20	74
		% within Protocol	73.0%	27.0%	100.0%

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Day turbid 1st visit - Day turbid 2nd visit	**1.13514**	**2.20413**	**.25623**	**.62448**	**1.64579**	**4.430**	**73**	**.000**

Graph 2

Influence of the number of visits on culture results.

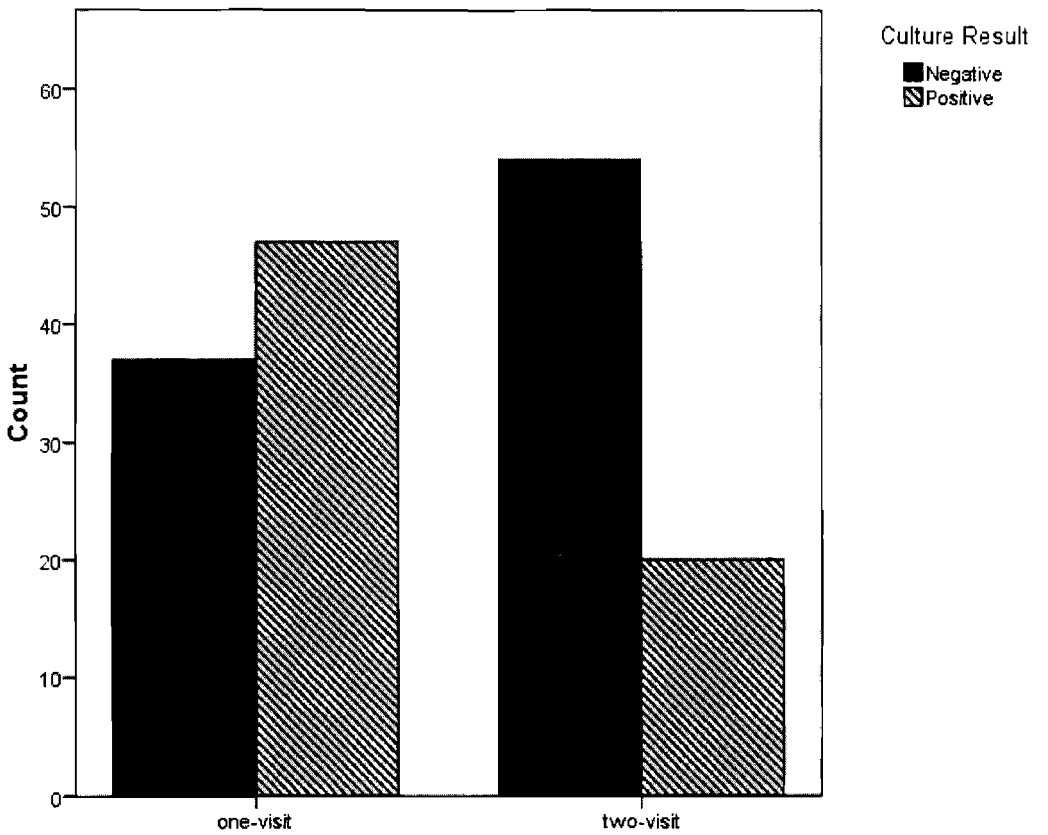

Table 3

Influence of Master Apical File Size on Culture Results – 1st Visit

			Culture Result 1st visit		
			Negative	Positive	Total
Master Apical File	25.00	Count	2	15	17
		% within Master Apical File	11.8%	88.2%	100.0%
	30.00	Count	12	10	22
		% within Master Apical File	54.5%	45.5%	100.0%
	35.00	Count	4	7	11
		% within Master Apical File	36.4%	63.6%	100.0%
	40.00	Count	3	7	10
		% within Master Apical File	30.0%	70.0%	100.0%
	45.00	Count	2	3	5
		% within Master Apical File	40.0%	60.0%	100.0%
	50.00	Count	4	1	5
		% within Master Apical File	80.0%	20.0%	100.0%
	60.00	Count	3	0	3
		% within Master Apical File	100.0%	.0%	100.0%
	70.00	Count	1	0	1
		% within Master Apical File	100.0%	.0%	100.0%
	80.00	Count	0	1	1
		% within Master Apical File	.0%	100.0%	100.0%
	90.00	Count	1	1	2
		% within Master Apical File	50.0%	50.0%	100.0%
Total		Count	32	45	77
		% within Master Apical File	41.6%	58.4%	100.0%

Statistical Analysis		**Value**	**df**	**Sig.**
	Pearson Chi-Square	17.855	9	.037

Table 4

Influence of Master Apical File Size on Culture Result – 2nd Visit

			Culture Result 2nd visit		
			Negative	Positive	Total
Master Apical File	25.00	Count	12	3	15
		% within Master Apical File	80.0%	20.0%	100.0%
	30.00	Count	17	5	22
		% within Master Apical File	77.3%	22.7%	100.0%
	35.00	Count	7	3	10
		% within Master Apical File	70.0%	30.0%	100.0%
	40.00	Count	5	4	9
		% within Master Apical File	55.6%	44.4%	100.0%
	45.00	Count	3	1	4
		% within Master Apical File	75.0%	25.0%	100.0%
	50.00	Count	5	0	5
		% within Master Apical File	100.0%	.0%	100.0%
	60.00	Count	2	1	3
		% within Master Apical File	66.7%	33.3%	100.0%
	70.00	Count	1	0	1
		% within Master Apical File	100.0%	.0%	100.0%
	80.00	Count	0	1	1
		% within Master Apical File	.0%	100.0%	100.0%
	90.00	Count	1	1	2
		% within Master Apical File	50.0%	50.0%	100.0%
	Total	Count	53	19	72
		% within Master Apical File	73.6%	26.4%	100.0%

Statistical Analysis		**Value**	**Df**	**Sig.**
	Pearson Chi-Square	7.637	9	.571

Table 5

Influence of the Amount of irrigation on Culture Results – 1st Visit

Amount of Irrigation-1st visit * Culture Result 1st visit

			Culture Result 1st visit		Total
			Negative	Positive	
Amount of Irrigation-1st visit	0-12 ml	Count	6	5	11
		% within Amount of Irrigation-1st visit	54.5%	45.5%	100.0%
	13-24 ml	Count	13	23	36
		% within Amount of Irrigation-1st visit	36.1%	63.9%	100.0%
	25-36 ml	Count	10	15	25
		% within Amount of Irrigation-1st visit	40.0%	60.0%	100.0%
	37-48 ml	Count	2	3	5
		% within Amount of Irrigation-1st visit	40.0%	60.0%	100.0%
Total		Count	31	46	77
		% within Amount of Irrigation-1st visit	40.3%	59.7%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.192[a]	3	.755
Likelihood Ratio	1.173	3	.759
Linear-by-Linear Association	.234	1	.629

Table 6

Influence of the Amount of Irrigation on Culture Results – 2nd Visit

Amount of Irrigation-2nd visit * Culture Result 2nd visit

			Culture Result 2nd visit		Total
			Negative	Positive	
Amount of Irrigation-2nd visit	0-12 ml	Count	17	3	20
		% within Amount of Irrigation-2nd visit	85.0%	15.0%	100.0%
	13-24 ml	Count	25	11	36
		% within Amount of Irrigation-2nd visit	69.4%	30.6%	100.0%
	25-36 ml	Count	10	4	14
		% within Amount of Irrigation-2nd visit	71.4%	28.6%	100.0%
	37-48 ml	Count	1	1	2
		% within Amount of Irrigation-2nd visit	50.0%	50.0%	100.0%
Total		Count	53	19	72
		% within Amount of Irrigation-2nd visit	73.6%	26.4%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.266[a]	3	.519
Likelihood Ratio	2.352	3	.503
Linear-by-Linear Association	1.506	1	.220

Table 7

Influence of Tooth Type on Culture Results – 1st Visit

Tooth type * Culture Result 1st visit

			Culture Result 1st visit		Total
			Negative	Positive	
Tooth type	Molar	Count	6	20	26
		% within Tooth type	23.1%	76.9%	100.0%
	Premolar	Count	4	12	16
		% within Tooth type	25.0%	75.0%	100.0%
	Incisor/Canine	Count	22	14	36
		% within Tooth type	61.1%	38.9%	100.0%
Total		Count	32	46	78
		% within Tooth type	41.0%	59.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	11.163[a]	2	.004
Likelihood Ratio	11.405	2	.003
Linear-by-Linear Association	9.512	1	.002

Table 8

Influence of Tooth Type on Culture Results – 2nd Visit

Tooth type * Culture Result 2nd visit

			Culture Result 2nd visit		Total
			Negative	Positive	
Tooth type	Molar	Count	16	8	24
		% within Tooth type	66.7%	33.3%	100.0%
	Premolar	Count	9	5	14
		% within Tooth type	64.3%	35.7%	100.0%
	Incisor/Canine	Count	28	6	34
		% within Tooth type	82.4%	17.6%	100.0%
Total		Count	53	19	72
		% within Tooth type	73.6%	26.4%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.560[a]	2	.278
Likelihood Ratio	2.610	2	.271
Linear-by-Linear Association	1.930	1	.165

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