EFFECTIVENESS OF GENTLEWAVE CLEANFLOW ON MULTISPECIES ENDODONTIC BIOFILM REMOVAL IN SINGLE ROOTED EXTRACTED TEETH

By

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INTRODUCTION

It has long been understood that bacteria are the primary etiological factor causing periapical and pulpal pathology[1]. Armed with this knowledge, the goal of standard RCT is to remove the bacteria to the greatest extent possible from the root canal system and allow the body to heal. However, achieving 100% sterility following RCT is not possible[2]. The complex anatomy of root canal systems allows for micro-organisms to reside in areas of the tooth that are difficult, if not impossible to fully clean[3]. Endodontists have relied on mechanical and chemical means to remove and disinfect canals; utilizing instrumentation and irrigation to remove the microbial load causing infection. Canal enlargement using Ni-Ti rotary instruments has become the most common means of mechanical preparation since their introduction in the early 90's, replacing stainless steel files with improved properties[4]. Peters showed that despite improvement in filing techniques, 35% of the canal's surface remains area remained unchanged following enlargement[5]. Similarly, Machado found an 81-84% reduction in bacterial load following canal preparation, but this means that 16-19% of the bacteria remains[6]. The untouched areas of the canal anatomy are often isthmuses, fins, and ramifications[3]. These areas which cannot be accessed by canal preparation still need to be disinfected and rely on irrigation protocols to do so. Sodium hypochlorite is the gold standard of irrigants due to its excellent antimicrobial/antibiofilm properties and its ability to dissolve organic tissue[7, 8]. NaOCl ionizes in water to form Na^+ and OCl^-; the hypochlorite ions reach equilibrium and hypochlorous acid (HOCl) forms which has a stronger antimicrobial effect than OCl^-. Hypochlorous acid directly affects the vital functions of cells leading to cell death. Because of sodium hypochlorite's toxicity, some practitioners choose to use a lower concentration, 1.5%, but the most commonly used is full strength 6%[9]. The organic tissue removal and antimicrobial effect by NaOCl is supplemented by the inorganic removal qualities of ethylenediaminetetraacetic acid (EDTA) which is generally used at a 17% concentration[10]. Classically, irrigation protocols have utilized these two irrigants which are alternately introduced via syringe and needle to each canal. Many attempts have been made to increase the efficacy of endodontic irrigation. Passive ultrasonic irrigation (PUI) is a technique which uses an activated tip to pass energy through irrigants, improving their effectiveness[9]. And most recently, the GentleWave (GW) system by Sonendo has come to market which uses multisonic energy to remove the endodontic biofilm and ultimately lead to better RCT outcomes.

GW irrigation has been brought to prominence by marketing and its users who vouch for its effectiveness; and it has been accepted by many endodontists. However, it is one of many techniques that aim to improve traditional endodontic irrigation protocols. Numerous techniques have been explored to find the best method to kill bacteria and remove the bacterial biofilm. Alternative irrigants such as chlorhexidine, maximizing irrigant disinfection with electromagnetic simulation, using diode laser, gaseous ozone and photodynamic therapy are all adjuncts aimed at improving disinfection[11-14]. GW improves irrigation by using a unique fluid delivery system that flushes large amounts of fluid through the canal system to disrupt the adhered bacterial biofilm and allow for removal[15]. The two innovations that they attribute this improved cleaning to are called SoundFlow and SoundBAR. SoundFlow is described as a fluid optimization process that degasses the liquid irrigants, thus improving the ability of the fluid to reach all areas of the canal system and increasing the energy proliferation through the liquid. SoundBAR is the process by which the energy is transferred into the irrigant solutions. When the solutions collide with the SoundBAR in the procedure instrument, microbubbles form. As these bubbles collapse in solution acoustic energy is generated leading to improved canal cleaning. This process also creates a vortical flow that is important as it creates a negative apical pressure, preventing solutions from being pushed out the apex of the tooth[15].

The GW system itself consists of a moveable console with touchscreen and utilizes single use procedure instrument (PI), i.e. handpiece. In the past GW has offered an anterior/premolar procedure instrument (APM PI) that was indicated for anterior and premolar teeth, a Posterior CleanFlow procedure instrument (PCF PI) indicated for premolars and molars, and a molar procedure instrument (M PI) that was indicated for molars only. However, as they look to improve the ease of use, they are now exclusively using a CleanFlow PI (CF PI) that is suitable for use on all teeth. The GW console has reservoirs for NaOCl, EDTA, distilled water, and for the evacuated fluids. During treatment the handpiece is positioned over the access and a complete seal must be obtained to prevent the high pressure irrigants from escaping. Caries, poor margins, or perforations are a few of the contraindications for GW use. The occlusal seal is created using “SoundSeal” (SS), GW’s light cure resin that covers the coronal portion of the tooth and on which the handpiece is placed for treatment[15]. The touchscreen is used to choose the appropriate treatment cycle and gives detailed instructions on how to attach the procedure instrument and prepare for use. Finally, treatment is initiated by pressing down on the GW foot pedal.

This study is using the Posterior CleanFlow Procedure Instrument (PCF PI), the newest release that can be used on molar and premolar teeth. Once correctly positioned with the appropriate seal the cycle can be initiated. The irrigants flow from the handpiece at a rate of 45mL/min[16]. This is important because regardless of the irrigant used, more fluid flushed through the canal system results in improved debris and bacterial removal[17]. All irrigating solutions are evacuated through the handpiece using a vented suction system[18].

In addition to improved intracanal cleaning, the GW boasts a few other benefits as well.

Conserving coronal and cervical tooth structure is a factor when considering an endodontic access. Contracted endodontic cavities (CEC), or "ninja" accesses are a current trend in endodontics. While there is currently a lack of clinical studies to show that a CEC have improved outcomes compared to traditional endodontic cavities (TEC), much research is currently focused in this area[19, 20]. One of the drawbacks of a smaller access is the challenge of complete debridement. GW is able to overcome this with its previously mentioned technology and provides adequate debridement even through CEC. Root dentin is also able to be conserved because the GW cleans the canal anatomy so that enlargement with rotary files is not considered necessary. GW manufacturer suggests that the canal can be enlarged to a final size of as small as 20.04. Users are pushing this to the limit by even foregoing instrumentation all together and utilizing GW as the sole method of canal disinfection[21]. While this technique is not endorsed by GW at this time, it is clear that the improved disinfection techniques are leading to innovation in obturation as well.

GW has caused the specialty of endodontics to re-think irrigation protocols; the previous gold standard of rotary files and 6% sodium hypochlorite irrigation are being questioned. For this reason, GW has been the focus of many studies since it was brough to market. One such paper by Zhang et al. 2018, compared GW and PUI[22]. They found both GW and PUI to be highly effective at reducing intracanal bacteria but noted that residual bacterial DNA was significantly lower when using GW. Another market leading technology in irrigation is the EDGEPRO (EP) BIOLASE built Er,Cr:YSGG laser. With similar goals to GW the EP laser looks to improve irrigation, increase biofilm removal and ultimately improve RCT outcomes. While both systems are being utilized by endodontists across the country and world, they are both also the focus of many research projects. Few studies however have utilized the same protocols to directly compare GW irrigation to EP laser assisted irrigation. One recent publication by Liu found conventional irrigation techniques were less effective at dissolving tissue than both the GW CleanFlow handpiece and lasers[23]. While my project is centered on the GW and its ability to remove a multispecies biofilm, my co-resident Dr. Samuel Patterson, is using the same protocols to assess the EP laser allowing for a direct comparison in bacterial load reduction.

Objective:

- The aim of this study is to evaluate the GentleWave Posterior CleanFlow instrument's ability to remove a multispecies biofilm from a single canaled extracted tooth compared to traditional irrigation techniques

Hypothesis:

- *Null*: GW PCF treated teeth will not demonstrate significant bacterial biofilm removal compared to those treated with traditional irrigation techniques.
- *Alternative*: GW PCF treated teeth will demonstrate significant bacterial biofilm removal compared to those treated with traditional irrigation techniques.

REVIEW OF LITERATURE

HISTORY OF ENDODONTICS

Despite being recognized relatively recently in 1963, the specialty of Endodontics has a long and unique history[24]. Currently, as defined by the AAE Glossary of terms, endodontics is "The branch of dentistry concerned with the morphology, physiology and pathology of the human dental pulp and periradicular tissues. Its study and practice encompass the basic and clinical sciences including the biology of the normal pulp and the etiology, diagnosis, prevention and treatment of diseases and injuries of the pulp and associated periradicular conditions." To better understand how this definition came to be, we can go back to the beginning of dentistry and endodontics.

The first theories about tooth pain were that it originated from "tooth-worms"[25]. Belief in a parasitic tooth-worm was the prevailing idea in cultures as far back as Ancient Egypt, the Roman Empire, and throughout the Middle Ages. It took until 1728 when Piere Fauchard, a French dentist, challenged the superstitious idea of a tooth-worm and set out to scientifically study the origins of tooth pain and decay[25]. In his book, *The Surgeon Dentist*, he accurately described pulp cavities, basic root canal anatomy, and the process of relieving an abscess by opening up the pulp to allow pus to release[26]. Though rudimentary in his understanding, Fauchard found ways to relieve tooth pain by removing the pulp, applying clove oil or cinnamon and treating tooth pain with opium mixtures[26]. Prior to Fauchard's research into dental pain and treatment, another vital discovery would prove to be one of the most useful in all of history and incredibly important to the development of modern medicine, dentistry, and specifically, endodontics.

It was in 1683 when Antonie van Leeuwenhoek, who would come to be known as the father of microscopy, found 'animalcules' in dental plaque and created the first drawings of bacteria[27]. It is van Leeuwenhoek who is considered the inventor of the modern day microscope. However, despite the use of his lenses he incorrectly backed the “tooth-worm” theory in a letter to the Royal Society of London[28]. His position held weight in the scientific community due to his reputation as a thorough observer and his powers of deduction[28]. It wasn’t until the aforementioned Piere Fauchard that these ideas were met with questioning.

Following Fauchard were a number of dentists who utilized different techniques in attempts to treat the dental problems that arose in their patients. Phillip Pfaff was a German dentist who documented the first pulp-cap procedure. He used gold or lead and fashioned it such that the inside surface was concave and didn’t come in contact with the pulp[26]. In 1757 Etienne Bourdet, another French dentist, described treatments where he would extract a carious tooth, fill the root canals with lead or gold, then replace the teeth[26]. The first recorded endodontic procedure described in the United States was performed by Robert Woofendale who in 1766 alleviated pain by cauterizing the pulp with a hot instrument and filling the open canals and chamber with cotton[26]. He also detailed a method for slowly killing an exposed nerve by applying a mixture of oil of cinnamon, cloves, turpentine, or other chemical oils and found that this gave patients relief from their pain[26]. Finally, Frederick Hirsch, a German dentist, was the first to correlate percussion sensitivity, a key diagnostic factor in modern endodontics, with diseased teeth[26]. Similar to Woofendale’s technique, he used red-hot probes inserted into the pulp via a perforation at the neck of the tooth as a means to relive pain[26]. While these techniques are all a bit barbaric compared to the treatment we are capable of today, they laid the foundation for the modern endodontics of today.

In the decades that followed the experimental treatments of the 1700’s there were more protocols, techniques and instruments used for the first time that we continue to use today.

Previously mentioned was Hirsch's percussion test; but other clinical tests to determine the status of the pulp and surrounding periodontium were also being discovered. Heat and ice tests were used to determine the vitality of the pulp, but in 1867 Magitot of France suggested using an electric current to test for pulp vitality[(29)]. The first endodontic file was fashioned by Edwin Maynard in 1838 who created a broach style file using the mainspring from a watch [(30)]. This lead to the first published account of a completed root canal consisting of: pulp removal, debridement, cleaning, and filling[(31)]. Other key advances in endodontics were the first use of a rubber dam rubber dam by S.C. Barnum in 1862, rubber dam clamp and forceps by Bowman in 1873, and the use of gutta percha as a filling material by G. A. Bowman in 1867[(26, 31, 32)].

What would prove to be one of the most important discoveries in medicine and dentistry was made by Konrad Roentgen in 1895 who discovered X-rays[(29)]. Prior to this, transillumination of teeth in a dark room was the only method to help determine if periapical disease was present[(29)]. Two weeks after the discovery of X-rays, a brave Professor by the name of Otto Walkhoff took the first radiographs of teeth, his own! X-rays at this time had an exposure time of 5-15 minutes, and required another 30-60 minutes to develop the images[(29)]. It would be another 20 years before X-ray machines were available that allowed for use in the dental office, even then fewer than a dozen dentists in the country utilized X-rays in their practices[(29)]. Dr. Edmund Kells was the first to utilize radiographs during endodontic treatment, using lead wires placed in canals to determine the fit[(29)]. In 1908 M.L. Rhein made the first working length radiograph to verify the length of the canal[(33)]. Roentegen's discovery of radiographs was a major advancement and one that would catapult the fields of medicine and dentistry forward as it allowed for greater information to be available to the clinician.

Advancements in endodontics continued as well. A move away from caustic agents for root canal sterilization occurred when sodium hypochlorite was introduced. It was in the 1940's that Grossman and Meiman showed the ability of sodium hypochlorite as a solvent for pulp tissue[33]. Newer obturation techniques were being explored as well. The old methods of using gold, or wood soaked in creosote with chloroform as a cement gave way to chloropercha, paraffin, and rosin filling material as practitioners sought after a hermetic seal of the root end[26, 34, 35]. A better understanding of the value of length control and root canal fill was suggested by G.V. Black who started using measurement controls to achieve better control over working length and apical size[34]. Silver points were introduced in 1933 by E. A. Jasper and were beneficial because they could be made to the same size and taper of the files and reamers that were used[34]. Silver points would remain a viable option for obturation for many decades.

As important as technique and material advances for RCT were the advances made in anesthesia. Early anesthesia was not safe and probably only partially effective. In 1836 Shearjashub Spooner used arsenic to devitalize the pulp prior to removal[33]. Although providing some level of anesthesia, the surrounding gingiva, periodontal ligament and bone could be damaged[33]. Carl Koller, an ophthalmologist, first discovered the anesthetic properties of cocaine in 1884[29]. In an attempt to use the drug for dental anesthesia, Dr. William Halsted used an injectable cocaine solution for the first ever mandibular nerve block[29]. Not only were local anesthetics being discovered, but general anesthesia as well. Horace Wells used nitrous oxide, "laughing gas" as he called it, which caused a loss of consciousness and some level of pain control[29]. To prove the effectiveness of the "laughing gas", Wells had his own tooth extracted by his colleague Dr. John Riggs and claimed the procedure was painless[29]. Wells would go on to have a failed demonstration of the anesthetic capabilities of "laughing gas", but nonetheless inhaled nitrous oxide became used for dental procedures, including pulp extirpation[29]. The big break through in local anesthetics came in 1905 when procaine (Novocain) was developed, and again in 1925 when it became available in an injectable form[29].

But for all the advancements in dentistry and endodontics, the early 1900's brought about a time of major questioning following the introduction of the focal infection theory in 1909[36]. The theory is built on the assumption that infections, i.e. tooth infections, have the ability to spread and cause chronic and acute disease elsewhere in the body[37]. Dr. William Hunter, an English physician, pioneered the introduction of the focal infection theory, and in a lecture at McGill University in Canada, he referred to the dental gold crown as, "a mausoleum of gold over a mass of sepsis."[24]. The medical community and subsequently the dental community subscribed to this theory and the unfortunate result was many patients being left to edentulism due to the misinformation. It would take 30 years before dentistry would recover from the setback caused by the focal infection theory[29].

By the 1940's studies were coming out that the extraction of infected teeth did not in fact lead to a decrease in systemic diseases. A major contributor to debunking the focal infection theory was Logan who believed that the presence of bacteria does not imply infection, and that healthy tissues can still have bacteria present[24]. Slowly the pendulum swung from extractions back towards root canal treatment. With greater care given to aseptic technique and the use of penicillin to treat tooth infections, root canals were once again an accepted treatment[24].

The first organized meeting of endodontics took place in Chicago in 1943 and resulted in the create of the American Association of Endodontists (AAE)[38]. As more dentists limited their practice to endodontics there became a push for it to be recognized as its own specialty, and in 1963 the American Dental Association officially recognized endodontics as a specialty within the field of dentistry. Indiana University's history is also intertwined with the AAE as five IU graduates have been president of the organization; Maynard Hine 1946-47, Harry Healey 55-56, Samuel Patterson 68-69, Donald Arens 83-84, and Carl Newton 98-99[39].

Writing this history of endodontics has given me a greater appreciation for how far the specialty has come, and the difficulties endured to get us to where we are today. I'd like to recognize the men and women who contributed to the foundation of endodontics and laid the groundwork for the modern endodontics of today. As a specialty we need to continue to grow, to push the boundaries of innovation, and make advances in treatment to better serve our patients and to increase oral health outcomes for all.

FOUNDATIONS OF ENDODONTICS

One of the most central research papers that lays the foundation for all that we do in endodontics was completed by Kakehashi, Stanley and Fitzgerald in 1965. In the study both germ-free (gnotobiotic) rats and conventional rats with normal oral microflora were given pulp exposures. Following the exposures it was observed that the gnotobiotc rats remained free of pulpal and periapical disease, while the conventional rats developed pulpal necrosis and periapical disease. These findings cemented that bacteria are responsible for the development of pulpal and periapical pathosis[1].

More studies were conducted which aimed to further understand exactly how bacteria initiate pulpal and periapical disease. Bacterial virulence factors such as lipopolysaccharide (LPS) and lipoteichoic acid (LTA) were found to be major mediators of pulp inflammation[40, 41]. Inflammation, when left untreated, persists causing micro-abscesses which ultimately leads to pulp necrosis and periapical pathology[42]. Understanding the sequence of events that leads to sustained infection in the root canal system leads us to identify the main goal of endodontic treatment: to remove bacteria and their byproducts from within the canal system to allow for the body's natural immune response to overcome any remaining bacteria and return to a state of health[43].

With that goal in mind Stewart identified what is known as the "endodontic triad". The three aspects of endodontic treatment: 1) chemo-mechanical preparation, 2) elimination of microbes, and 3) obturation of the root canal[44]. Of these three phases, Siqueira dubbed chemo-mechanical preparation the most important as it eliminates the cause of apical periodontitis, bacterial infection of the root canal[45]. However, before any of these goals of treatment can be accomplished, it is vital to understand the anatomy of the root canal system.

ROOT CANAL ANATOMY

While we have traditionally relied upon radiographs to give us information about the number of roots and the canals present in teeth, the information garnered from a radiograph is simplistic. Fortunately, CBCTs have made canal identification an easier task, but still the intricacies of root canal anatomy are not completely appreciated by a CBCT scan. Hess et al. in 1925 first explored the complexities of canal anatomy by injecting vulcanized rubber into the canals of teeth and identified anatomic variations such as fins, grooves, isthmuses, apical deltas and other irregularities[46].

Today there are two widely used classification systems used to describe canal anatomy. The first developed in 1969 by Weine is more simplified and describes four possible canal variations[47].

- Type I: A single canal from the pulp chamber to the apex
- Type II: Two separate canals that merge near the apex
- Type III: Two separate canals with their own apical exits

- Type IV: One canal that splits into two canals near the apex

The second was developed by Vertucci in 1984 and takes into account more complex possibilities [48].

- Type I: A single canal from the pulp chamber to the apex
- Type II: Two separate canals that merge near the apex
- Type III: A single canal that separates into two canals but join at the apex
- Type IV: Two separate canals with their own apical exits
- Type V: One canal that splits into two canals near the apex
- Type VI: Two separate canals that join mid root but separate again near the apex
- Type VII: A single canal that separates into two before joining mid root, then separating again near the apex
- Type VIII: Three separate canals leaving the chamber with their own apical exits

These classifications detail the major canal anatomy present in most roots. C-shaped canal configurations have their own separate classification, a system developed by Cooke and Cox[49]. All of these classifications only take into account the main canals. As mentioned above the fins, isthmuses, ramifications and other micro anatomy within root canal systems remain a challenge for clinicians as they often cannot be instrumented and rely on irrigants and interappointment medications for disinfection[45].

CLEANING AND SHAPING

Mechanical instrumentation serves to remove debris, allow irrigant penetration to the apex and prepares the canals for obturation[50-52]. Multiple techniques can be used to achieve these objectives, based on operator experience and preference. Popular techniques and the authors to whom they are attributed are: Step-down (Goerig), passive step-back (Torabinejad), anticurvature filing (Abou-Rass), balanced force (Roane), and crown-down (Morgan and Montgomery)[53-57]. The size to which the canals should be prepared is a topic constantly being discussed, but Saini et al found improved outcomes when the master apical file was three times larger than the first file to bind apically[58].

Regardless of what types of files or technique is used, or what size a canal is taken to, when mechanically preparing a canal we are not effectively removing dentin from all walls of the canal[59]. Although preparation alone can reduce microbial load by 100 – 1000 fold it does not eliminate all bacteria and their byproducts (i.e. virulence factors) from the canal system[60]. Peters found that over 35% of the canal anatomy remains untouched following instrumentation[5]. This percentage jumps to somewhere between 10% and 80% in severely curved canals and oval shaped canals[45]. Even when our instrumentation does plane the dentin walls of the canal, bacteria have been shown to penetrate up to 300 microns into dentin tubules, making them less likely to be fully removed by mechanical means[61]. And the complex anatomies mentioned previously (fins, lateral canals, apical deltas, anastomoses, etc.) also are a reservoir for bacteria and are generally unreached by our filing protocols[62]. Despite the shortcomings when it comes to bacterial removal by mechanical means, we must remember that the other main goals of canal preparation are to create space for irrigants and to prepare the canals for obturation.

IRRIGATION

Irrigation is part of the second objective in Stewart's endodontic triad; elimination of microbes [44]. It's the only way to clean areas of the canal that remain untouched by mechanical instrumentation. However, the goal of irrigation is not only to eradicate endodontic microbes but also to flush out debris, dissolve organic material, open dentin tubules and remove the smear layer, and to lubricate. Peter's created a list of all the ideal properties that an irrigating solution might have[(63)]:

- Be an effective germicide and fungicide
- Be nonirritating to the periapical tissues
- Remain stable in solution
- Have a prolonged antimicrobial effect
- Be active in the presence of blood, serum, and protein derivatives of tissue
- Have low surface tension
- Not interfere with repair of periapical tissues
- Not stain tooth structure
- Be capable of inactivation in a culture medium
- Not induce a cell-mediated immune response
- Be able to completely remove the smear layer, and be able to disinfect the underlying dentin and its tubules
- Be nonantigenic, nontoxic, and noncarcinogenic to tissue cells surrounding the tooth
- Have no adverse effects on the physical properties of exposed dentin
- Be convenient in its application
- Be relatively inexpensive

No single irrigant is currently capable of achieving all of these objectives, but by combining a few different solutions during irrigation protocols we can accomplish most of the goals listed.

Sodium hypochlorite (NaOCl) is the gold standard of irrigants and fulfills many of Peter's criteria. One of its most important objectives is to kill bacteria and NaOCl does this well. It has broad spectrum antimicrobial activity and is also able to kill spore-forming bacteria, fungi, protozoa and viruses(64). It kills planktonic bacteria, those in biofilms and bacteria that have penetrated up to 0.3mm into dentine tubules(65, 66). NaOCl works by dissociating into hypochlorous acid (HOCl-) and hypochlorite ions (OCl-) which lead to amino acid degradation and disruption of oxidative phosphorylation and DNA synthesis(67). Sodium hypochlorite can be used at different concentrations. According to a 2012 clinical survey, 57% of endodontists use "full strength" NaOCl(68). Lower concentrations have shown to still be effective but require more time to disinfect to the same level as 5.25% (full strength) NaOCl(64, 69). They do, however, have the added benefit of being less toxic to tissues. At any concentration sodium hypochlorite does need to be replenished often as it is inactivated by dentin(70).

Chlorhexidine gluconate (CHX) is an alternative broad-spectrum antimicrobial that can be used for disinfection of the root canal system. CHX has a cationic component that attaches to and penetrates the negatively charged cell wall leading to cell lysis(63). While 2% CHX has been show to be as effective as 5.25% NaOCl in its antibacterial activity, it is not as commonly used. Mainly because in addition to its antimicrobial profile, sodium hypochlorite dissolves organic tissue, something that CHX does not do(8). CHX does have the property of substantivity: that is, it binds to dentin and its antibacterial effects persist for up to 48 days, whereas NaOCl only works instantaneously upon contact(71).

Ethylenediaminetetraacetic acid (EDTA) is a non-antimicrobial irrigant that is used to remove the smear layer present after filing. EDTA is used at a 17% concentration and removes the inorganic debris and smear layer which opens up the dentinal tubules[72]. By opening up the tubules NaOCl can get better penetration, and when used as part of the final rinse allows for better sealer penetration into the tubules as well[73]. All solutions mentioned; NaOCl, CHX and EDTA act as lubricants when filing in the canal and assist in gross debris removal.

In order for any irrigation solution to be effective it must physically come in contact with the target areas of the canal; areas with microbial biofilms including fins, isthmuses, accessory canals etc. The current adopted protocol for delivering irrigation fluids into the root canal system is standard needle irrigation (SNI). To aid in the process of irrigation several adjuncts have been explored. One of the most popular is the use of ultrasonic activation. The term passive ultrasonic irrigation (PUI) was first used in 1980 by Weller and is a technique in which irrigation solutions within the canal system are activated by an ultrasonic tip for a period of time to add energy to the solutions, causing acoustic streaming[74, 75]. Acoustic streaming is the "rapid movement of fluid in a circular or vortex-like motion"[75]. This rapid fluid movement theoretically allows solutions to reach areas of the root canal system that would be otherwise untouched. And PUI has been shown to be more effective than standard needle irrigation in removal of bacteria[75].

In addition to PUI, other methods of disinfection that have been researched as well. Ozone (O_3) is a naturally occurring gas that when energized dissociates to oxygen (O_2) and a single oxygen atom (O_1), which is highly reactive and can deoxidize cells[13]. Photodynamic therapy is another method of disinfection that works by using a specific wavelength of light which activates a photosensitizer which can reaction with bacteria and lead to production of oxygen reactive species that disrupt cellular functions[11]. These canal sterilization techniques are not widely used but represent innovative ways that after further research may become more viable options for clinical treatment.

OBTURATION

Once the canals have been appropriately shaped and cleaned the final step of root canal treatment and the third aspect of Stewart's endodontic triad is obturation. Obturation is the process by which a clean canal is filled with a core filling material, often gutta percha, with a root canal sealer. The goal of obturation is to eliminate potential leakage by hermetically sealing the root canal system to prevent any ingress or egress of bacteria(76). The highest success rates of root canal treatment occur when the obturation ends in the appropriate location at the apex of the root, which is generally 0-1mm from the radiographic apex(77).

MICROORGANISMS

Not all microbes are pathogenic. Our bodies, both inside and out, are covered with complex microbiomes that function in symbiosis to keep us healthy. It is when opportunistic, pathogenic bacteria can proliferate that we develop infections. As mentioned previously, Kakehashi established this is true for endodontic infections(1).

Bacteria can enter the root canal system in a number of ways: direct exposure from caries or fracture, through microcracks present following coronal injury, and through dentin tubule invasion(78, 79). Once in the root canal system bacteria form biofilms which can be up to 1000 times more resistant than planktonic bacteria(80). Biofilms are an arrangement of bacteria that grow together attached to a surface and to each other that from a self-produced extracellular matrix that gives them their antimicrobial resistance(80). Quorum sensing, exchange of genetic material (particularly virulence factors), metabolic diversity, and overall density of bacteria also contribute to a biofilm's ability to defend itself(81).

The microorganisms present in endodontic infections differ based on the type of infection and how the infection occurred. Primary infections have a different microbial composition than secondary infections and infections resulting from a carious exposure have different compositions than those from trauma[82]. Classic endodontic literature attributed the genus of *Bacteroides* with being commonly found in endodontic infections and described them as "black pigmented Bacteroides"[83] However, the majority of microorganisms found in endodontic infections are gram-positive anaerobic rods and cocci[84]. Some common examples would include *Actinomyces naeslundii*, *Peptostreptococcus* sp., and *Streptococcus* sp. *Fusobacterium nucleatum*, and *Porphyromonas gingivalis* are also commonly found in endodontic infections and are gram-negative anaerobic rods. [85]. One bacteria of particular note due to its antimicrobial resistance and presence in many secondary infections due to said resistance is *Enterococcus faecalis*[86]. *E. faecalis* is a gram-positive facultative cocci that possesses a proton pump that makes it particularly resistant to calcium hydroxide medicament. It also thrives in biofilms, as evidence by Retamozo et al who found that a mature mono species biofilm of *E. faecalis* required at least 40 minutes of 5.25% NaOCl in order to achieve sterilization[87].

GENTLEWAVE IRRIGATION

Endodontics is constantly evolving and finding ways to improve RCT and provide better outcomes to patients. The GentleWave (GW) system by Sonendo aims to enhance RCT by improving irrigation protocols to better clean infected and debris filled canal systems. Currently most practitioners use standard needle irrigation (SNI) to deliver irrigants into teeth. However, SNI has limitations, mainly that in order to get fluid dynamics to occur at the apex of the tooth the canal must be opened up to a size 40.04 for a 27-guage irrigation needle to reach within 3 mm of the apex[88]. As previously mentioned in the anatomy section above, intricate anatomy also harbors bacteria that SNI cannot reliably remove. The GW irrigation system uses degassed irrigation solutions and patented technology which delivers acoustic energy and creates vortex fluid dynamics to clean apically and within complex canal anatomy.

The GW is not the only product available that aims to improve irrigation apically. EndoVac and Quantec-E are other irrigation devices that work to both deliver irrigation solutions to the apical third of teeth and also vacuum them out. The novel GW system delivers degassed NaOCl, EDTA and sterile water into the canal system and uses a suction system to remove the fluids as well. The GW procedure instrument has a SoundBAR which transfers energy into the solutions which forms microbubbles. The microbubble implosion within the canal system creates acoustic energy that cleans canal walls of debris and biofilm. The fluid dynamics within the canal create a negative apical pressure which prevents the high-pressure fluids from being extruded apically[(89)].

The literature supporting the GW is growing, with early in vitro and ex vivo studies showing positive results. Molina et al show GW to be as effective as SNI in debris removal in the main canal of mesial buccal and mesial lingual canals of lower molars and mesial buccal roots of upper molars, and with increased removal in isthmuses[(90)]. The high rate of irrigant flow, particularly of sodium hypochlorite, also leads to quick organic tissue dissolution. GW performed better than both SNI and PUI in this regard[(91)]. Ma et al demonstrated the use of GW to remove calcium hydroxide medicament which was accomplished predictably within a 90 second cycle using just water, highlighting the multiple ways in which the GW can be utilized during RCT[(92)].

A 2022 review article by Coaguila-Llerena et al assessed published GW research and described the outcomes [(16)]. GW excels at removing debris in isthmuses, organic tissue and calcium hydroxide as mentioned above[(90-92)]. However, it was found to be only marginally better

(2% better) than PUI when used in retreatments to remove gutta percha and sealer residue[93]. Other uses of the GW that have been explored are separated instrument and calcification removal. Calcifications present in uninstrumented lower molar distal canals were able to be opened up partially or completely, and overall 86.4% were reduced in size[94]. Instrument removal is also possible with the GW; 2.5mm fragments of size 10, 15 and 20 K files in the middle and apical thirds of extracted molars were removed with 83% and 61% success respectively[95]. GW was less successful in curved canals (42%) than it was in straighter canals (91%). Instrument removal had an average treatment time of less than 11 minutes[95]. These studies highlight the multiple ways in which the GW irrigation system can be utilized. However, it should be noted that these are in vitro and ex vivo studies.

The possibility of better irrigation with less instrumentation when using the GW is an exciting possibility to endodontists who could achieve the same, or improved, levels of disinfection without compromising tooth structure and time used for instrumentation. Better penetration of irrigants into dentin tubules can be achieved with GW, which we know is beneficial because bacteria reside within dentin tubules in necrotic teeth[16, 66]. Better irrigation penetration allows for better sealer penetration and can lead to better obturation as well. Zhong et al minimally prepared teeth to size 15.04 using Vortex Blue, used the GW, and evaluated quality of single cone technique obturation with 3 different sealers. Two of three sealers provided high-quality obturation with 94.7– 98.9% filled canal[96]. Notably, the MTA based sealer was lower at 89.4-89.5%[96].

After reading all the positive evidence in support of GW it may seem like it would be adopted across the board. But that isn't the case. A number of limitations exist, and one of the first often mentioned by clinicians is the cost. Endodontists are unwilling to invest in a new product unless they are certain it will be beneficial for their patients and their practice. Clinical limitations exist as well. The GW uses 3% NaOCl and 8% EDTA, significantly lower than the concentrations many endodontists are accustomed to (6% NaOCl and 17% EDTA). Another drawback is the platform that needs to be built in order to achieve a sealed connection with the handpiece. Building the platform takes time and materials that otherwise wouldn't be needed. These limitations combined with the overall lack of outcome studies proving GW root canals are more successful than the current standard of treatment gives many endodontists pause.

The outcome studies that are available provide a compelling but albeit short sighted case for GW treatment. A one year follow-up by Asgeir Sigurdsson et al showed a 92.9% and 98.4% success rate in necrotic (n=14) and irreversible pulpitis (n=61) cases respectively[97]. In this study teeth were accessed, patency achieved, and instrumented to 20.07 (ProTaper F1 file) before GW cycle ran and obturation with warm vertical technique. In a separate study, also by Sigurdsson, a similarly high success rate of 97.4% was found at a six month follow-up. I think it is important to note that the researchers Asgeir Sigurdsson and Randy Garland, also a cited author on both of the aforementioned outcome studies, are consultants for Sonendo Inc. It is also acknowledged, in the case of the 12-month follow up study, that Sonendo Inc. provided the funding for the study. This doesn't delegitimize the research but is of note. One other clinical trial assessed postoperative pain following GW RCT compared to standard endodontic treatment and found no significant difference between the two[98].

Biofilm removal by GW is an area that has been studied by Choi et al and Kim et al in separate studies. Both have similarities and differences to my research[15, 99]. Choi assessed removal of a mono-species *E. faecalis* in molars using the GW molar PI. Canals were minimally instrumented following biofilm growth to size 15.04 using EndoSequence rotary files followed by GW treatment. The GW treatment group was compared to conventional instrumentation (size 35.04 EndoSequence rotary file) with PUI. Biofilm removal was assessed by fixing remaining biofilm using formalin, teeth were sectioned and stained and independent evaluators examined remaining biofilm and scored teeth accordingly. SEM images were also taken. GW out performed conventional instrumentation with PUI in this study(15).

The Kim study was the first GW study to utilize the CleanFlow PI(99). Similar to Choi it used a mono-species *E. faecalis* biofilm but in the mesial roots of mandibular molars. Kim compared biofilm removal of un-instrumented canals to those minimally instrumented with SSwhite 20.06v. Colony forming units (CFU) were collected as well as samples for SEM viewing. All canals were found to have remaining *E. faecalis* and debris was also found in the instrumented teeth isthmuses. Based on their SEM photos they also called into question the ability of paper points to adequately sample canal systems for remaining bacteria after noting the remaining bacteria was primarily not in the main canal. Their findings were that there was a significant difference between both groups and a positive control but not a significant difference between the un-instrumented and minimally instrumented GW groups(99).

My study continues research to assess the GW's ability to remove an adhered bacterial biofilm in an extracted tooth model. Key differences are that a multi-species biofilm was used, taken from an adult tooth with pulpal necrosis. No instrumentation occurred following inoculation as occurs in the Choi study, and for one treatment group in the Kim study. Lastly the GW CleanFlow PI was used, as is done in the Kim study, which is an important consideration as GW moves away from different PIs for molar vs anterior teeth and will only produce the CF PI.

MATERIALS AND METHODS

SAMPLE SELECTION

Thirty six deidentified single rooted premolar teeth with single canals were collected from the Oral Health Institute and stored in 0.1% thymol solution at room temperature. Selected teeth had fully formed roots with intact cementum, closed apices and no signs of fractures or other developmental anomalies. They were caries free and extracted for non-endodontic reasons. Teeth were straight or had a slight curvature (< 30°). Expedited IRB approval was obtained for this study due to use of human tissue.

TOOTH PREPARATION

Teeth were prepared to a standardized length of 16mm by removing the coronal aspect with a diamond bur and water irrigation. The single canal was negotiated with an ISO 10.02 K file and patency achieved with visual confirmation. An ISO15.02 K file was taken to length, approximately 0.5mm short of the minor foramen as determined by visualization. The canal was then sequentially enlarged with Edge-X7 Nickel – Titanium files to size 25.06 with a ProMark endodontic motor (300 rpm and 300 g/cm). Patency was maintained by using a #10 K file between rotary files. Saline (0.9% NaCl) irrigation via standard needle irrigation (SNI) with a 27-guage side vented needle was used throughout to prevent packing of debris. An artificial chamber, approximately 4mm deep was prepped into each tooth to act as an artificial chamber and irrigant reservoir, as well as to create space for the GW Clean Flow handpiece tip. Once fully prepared, teeth were irrigated using SNI with 6% NaOCl and 17% EDTA to remove organic and inorganic debris respectively. A final rinse with sterile saline was completed before storing back in 0.1% thymol at room temperature until inoculation. All work was performed by experienced endodontic residents.

INOCULATION AND BIOFILM FORMATION

The bacteria used for inoculation was obtained from an adult tooth with pulpal necrosis which was isolated as a part of a previous endodontic residents' research "Antibacterial Effects of Antimicrobials Used in Regenerative Endodontics against Biofilm Bacteria Obtained from Mature and Immature Teeth with Necrotic Pulps" by Jacobs and Troxel[(100)]. Per their materials and methods, the bacteria were isolated from an adult tooth with pulpal necrosis under sterile conditions using paper points prior to root canal treatment. The paper points were placed in 5mL of brain-heart infusion broth with 5g/L yeast extract and 5% vitamin K and hemin and vortexed before a 48 hour incubation period at 37°. Samples were then frozen for future use at -80°.

The same tooth sample was utilized in a second study assessing the antimicrobial effect of double antibiotic paste on mature compared to immature biofilms[26]. As a part of that project the necrotic culture was profiled to determine what bacterial species were most prevalent. *Streptococci* and *Staphylococci* were predominant in the mature necrotic biofilm (Table 2).

In preparation for inoculation of the prepared teeth for this study, the bacterial sample was removed from the freezer, thawed and incubated anaerobically via use of gas-generating sachets (Gas-Pak EZ; Becton, Dickinson and Company) for 24 hours at 37°C. 2 mL of growth media consisting of brain-heart infusion broth with yeast extract (BHI-Y; Becton, Dickinson and Company, Franklin Lakes, NJ), 5% vitamin K and 5% hemin was prepared and pipetted into 12 well plates where the prepared teeth were placed following a sterile saline rinse. Each tooth in its individual well in the 12 well plate was submerged in the growth media (Figure 5). 50 µl of the necrotic tooth biofilm was then pipetted into each well. The well plates were anaerobically incubated for 9 days at 37°C with transference into new well plates with fresh growth media occurring twice within the 9-day growth period (Figure 6).

EXPERIMENTAL GROUPS

Following the 9-day incubation period the well plates were transported to the IUSD endodontic clinic where the irrigation protocols were carried out. Teeth were transported in the same airtight containers they were incubated in and only removed once they were in the endodontic clinic. The 36 teeth were randomly assigned to one of 3 possible groups: negative control, positive control and experimental.

1. Negative Control Group – Sterile water irrigation
2. Positive Control Group – Traditional irrigation with 3%NaOCl and 17%EDTA
3. Experimental Group – GentleWave Posterior CleanFlow irrigation (3% NaOCl, 8%EDTA, and sterile water)

All teeth were mounted in white paper cups using alginate impression material prior to treatment (Figures 9 and 10).

NEGATIVE CONTROL TREATMENT

The negative control treatment group consisted of standard irrigation with a 27-gauge side-vented needle used approximately 1mm from the apex to deliver 5mL of sterile saline (0.9%) over the course of 2 minutes while moving the needle in and up-and-down motion.

POSITIVE CONTROL TREATMENT

Positive controls underwent irrigation with a 27-guage side-vented needle; first with 5mL of 3% NaOCl delivered over 3 minutes followed by 5mL of 17% EDTA delivered over 1 minute. A final rinse with 5mL of sterile saline was completed to remove any remaining NaOCl or EDTA.

GENTLEWAVE POSTERIOR CLEANFLOW

The experimental group was treated with the GW PCF handpiece under the necrotic tooth setting. The standard settings for the necrotic cycle remained unchanged and include a 30 second initial H2O rinse which is termed the “leakage test” and allows for the operator to adjust the handpiece until a proper seal is achieved. This is followed by 5 minutes of 3% NaOCl, a 30 second distilled water rinse, 2 minutes of 8% EDTA, and a 15 second distilled water final rinse. The GW unit automatically dilutes the 6-10% NaOCl that is in its reservoir to 3%, as well as the 17% EDTA to the appropriate 8%. The irrigant reservoirs were refilled and the waste reservoir emptied as needed between treatments to prevent unnecessary stoppages mid cycle.

Prior to running the GW cycle the teeth were prepared for the GW handpiece by building a platform. A ring of GW’s “SoundSeal” (SS) was dispensed directly around the tooth, in contact with the tooth and the alginate impression material and light cured. A plastic platform builder which comes packaged with each individually wrapped handpiece was then used build the platform itself. To do that, SS was dispensed onto the plastic platform builder so that it covered the entire platform except for the tip. The platform builder with the SS was then placed onto the tooth with the tip going into the access, and light cured before gently removing the platform builder using an explorer. A final light cure was completed to ensure the SS was fully set.

Platforms were built on all 12 teeth prior to any of the teeth having the GW cycle ran, this was for efficiencies sake as the instrumentation of all teeth took place on the same day and time was a concern. Two platforms had to be removed and built again directly prior to treatment as the GW handpiece dislodged the SS from the tooth upon placement.

To begin treatment, the CleanFlow Posterior handpiece was placed onto the platform and the cycle initiated (Figure 11). A complete seal was obtained during the initial 30 second leakage test and maintained throughout the cycle and adjusted when necessary.

BACTERIAL COLLECTION AND PLATING

Following completion of treatment protocols, a sterile VersaBrush was used in a slow speed rotary handpiece, placed as far apically as possible and ran for 10 seconds on medium speed to remove any remaining bacteria and/or biofilm and then placed in a sterile vial. To remove any remaining bacteria sterile paperpoints, one size 25 and one size 30 were placed into the canal, twisted and moved in an up and down motion for 5 seconds each before removal and placed in the same vial as the VersaBrush for that tooth. Debris collection occurred at the end of treatment for each treatment group.

Following completion of all treatments the sealed vials were transported to a microbiology lab in the Oral Health Institute. Quantification of bacteria for each sample was done by assessing colony forming units (CFU). The 15mL vials with the VersaBrushes and paperpoints were sonicated for 10 secs at 30 amp power, and vortexed for 10 seconds to suspend bacteria in solution. 100 µl of the sonicated and vortexed solution was mixed with 900 µl of sterile saline to make a 1:10 base concentrate for each tooth. For the GW and positive control treatment groups the 10% concentration was plated on blood-agar plates (CDC, BioMerieux, Durham, NC). For the negative control group, for which a far greater concentration of bacteria was expected, the 10% solution was further diluted to a 1:100 concentration for each tooth before plating.

Plating was completed by placing 100 µl of solution in the middle of a blood-agar plate. A glass L-shaped cell spreader was dipped in alcohol and flamed before being used to spread the sample evenly on the blood agar plate with the use of a spiral spreader for even coating.

Blood-agar plates were stacked and incubated anaerobically for 48 hours at 5% CO_2 and 37°C after which CFU's were counted twice by two separate individuals.

STATISTICAL ANALYSIS

Comparisons among the GW, positive control, and negative control groups for differences in CFU/mL were made using Wilcoxon Rank Sum tests. A two-sided 5% significance level was used for each test. Analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA).

SAMPLE SIZE

Utilizing 12 teeth per treatment group allowed for 80% power to detect a 2.25x difference between groups. This is consistent with other studies of similar style that look to assess differences in treatment protocols for endodontic techniques.

RESULTS

Table 1 shows the CFU/mL for each blood agar plate for each treatment. CFUs were compared between the GW, positive control, and negative control groups for differences in CFU/mL. Comparisons were made using Wilcoxon Rank Sum tests. A two-sided 5% significance level was used. Both the GW and positive control groups demonstrated significantly lower CFU/mL than the negative control ($p<0.001$). However, the GW and positive control groups were not significantly different from one another ($p=0.132$). Figure 1 demonstrates the exponentially greater CFUs detected in the negative control group. In Figure 2 the negative control group has been removed so that a clearer comparison can be made between the positive control and the GW groups.

FIGURES AND TABLES

Plate #	GW (CFU/mL)	Negative Control (CFU/mL)	Positive Control (CFU/mL)
1	200	228250	0
2	1875	1015750	4200
3	2700	730000	450
4	5675	634000	2250
5	2750	2276000	2600
6	100	1047750	9375
7	100	12000	4675
8	0	95500	300
9	2075	1500000	325
10	1500	1980000	400
11	225	1240000	5525
12	200	458000	26275

Table 1. Mean CFU/ml of biofilm remaining on teeth treated with GW compared to positive and negative controls.

Organism Name	IM1	IM3	M1	M3
*Streptococcus*_Genus_probe_4	2.53%	1.54%	90.03%	4.17%
Enterococcus_faecalis	88.11%	0	0	0.02%
*Staphylococcus*_Genus_probe_3	0.00%	0	0	76.73%
Streptococcus_anginosus	2.38%	32.67%	0.00%	0
Unmatched Reads	6.61%	9.79%	9.14%	8.29%
Veillonella_dispar	0.04%	14.79%	0.00%	0
Dialister_invisus	0	4.02%	0	10.45%
*Veillonella*_Genus_probe_2	0.11%	13.16%	0.00%	0.00%
Neisseria_elongata	0	11.40%	0	0
Atopobium_parvulum	0	6.14%	0	0
Veillonella_parvula	0.00%	3.57%	0.00%	0
Veillonella_denticariosi	0.00%	1.95%	0	0

Table 2. Necrotic tooth biofilm polymicrobial makeup.

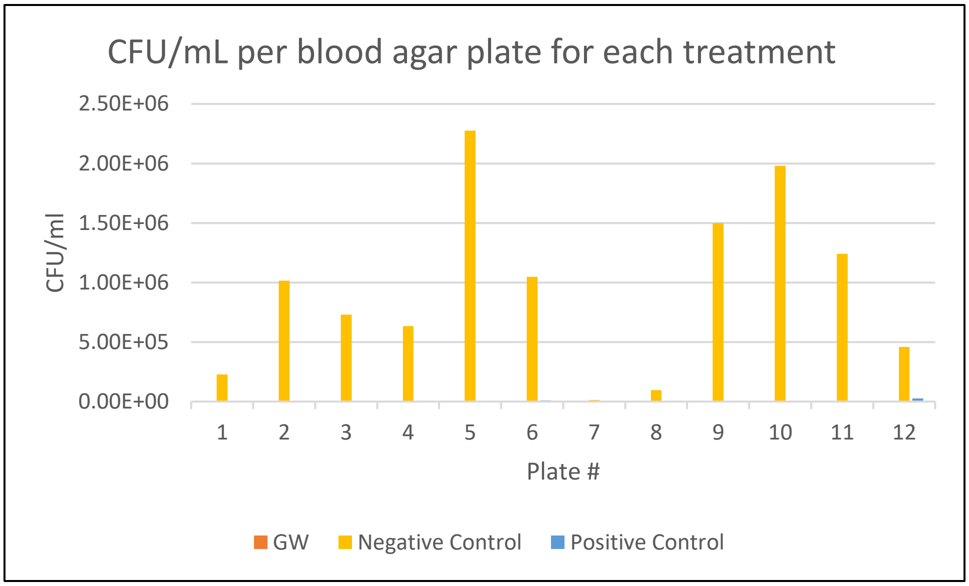

Figure 1. CFU/mL per blood agar plate for each treatment.

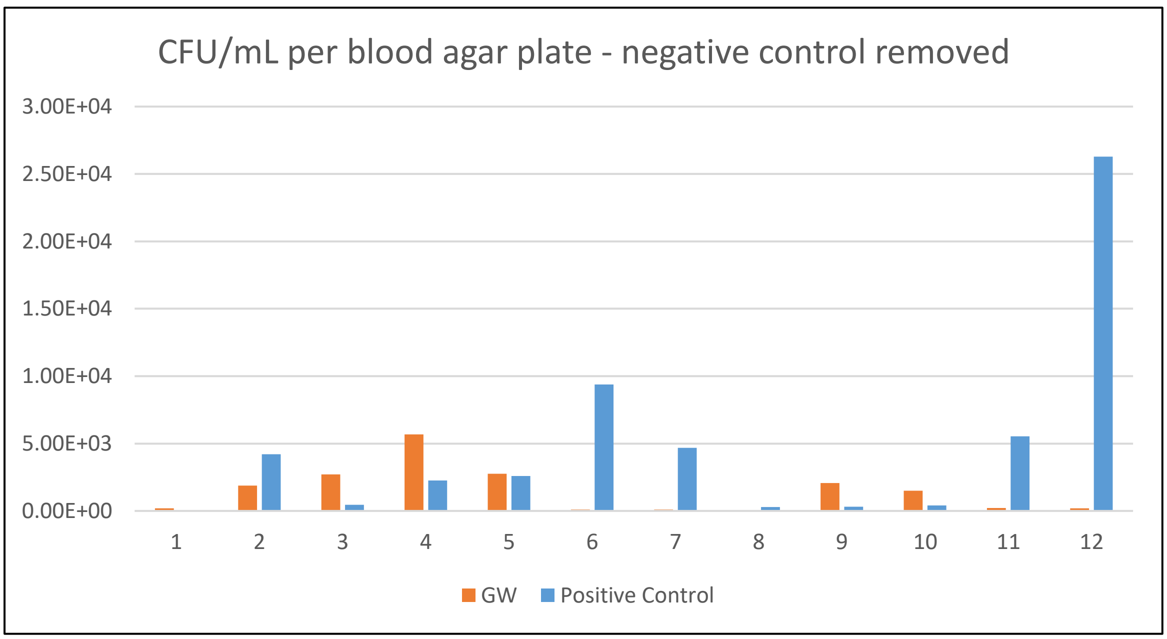

Figure 2. CFU/mL per blood agar plate - negative control removed

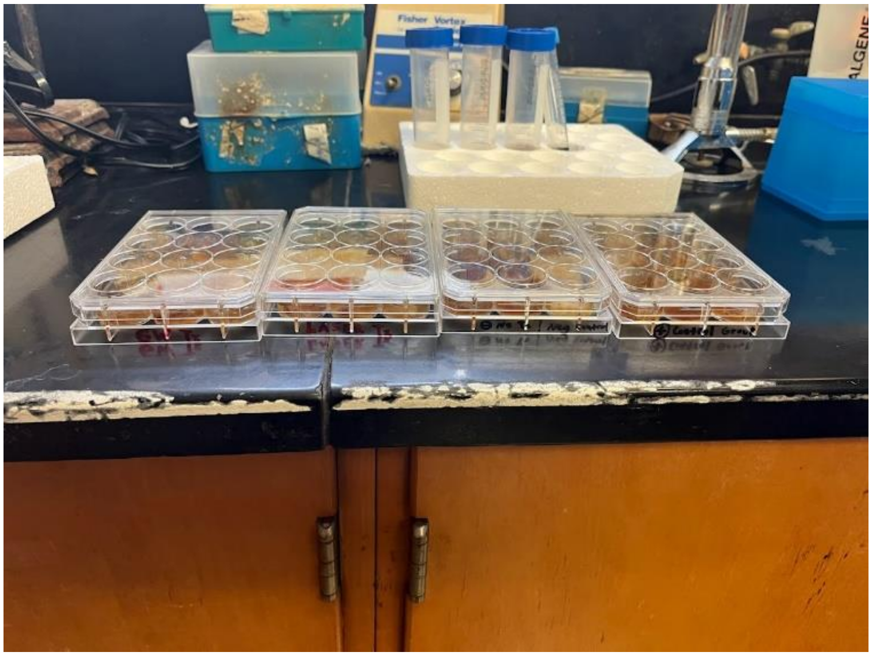

Figure 3. Teeth in 12 well plates.

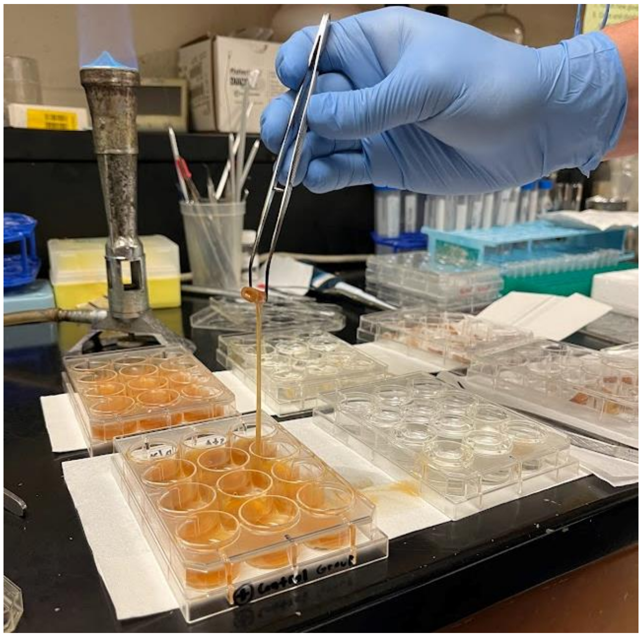

Figure 4. Teeth exhibiting biofilm growth.

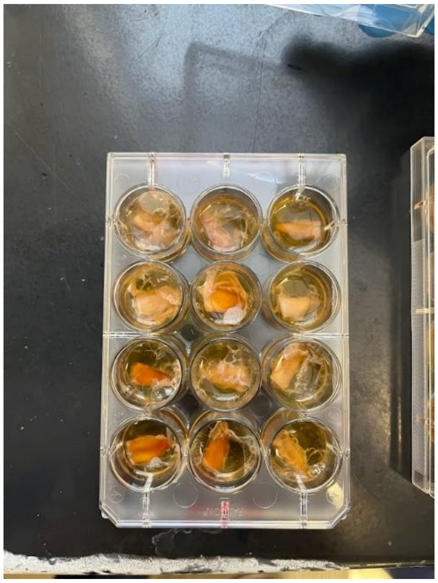

Figure 5. Teeth in 12 well plate with biofilm growth.

Figure 6. Anaerobic growth chamber.

Figure 7. Example of 12 well plate used in pilot study.

Figure 8. GentleWave console touch screen

Figure 9. Teeth mounted in alginate

Figure 10. Teeth mounted in alginate.

Figure 11. Treatment of tooth with GW PCF PI.

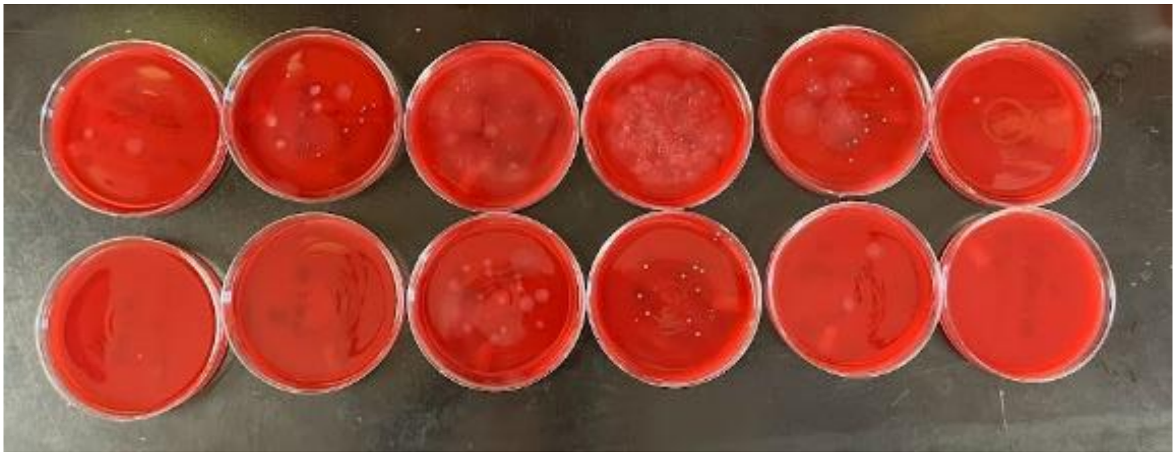

Figure 12. GW blood agar plates 48 hours after plating.

Figure 13. GW blood agar plate #2.

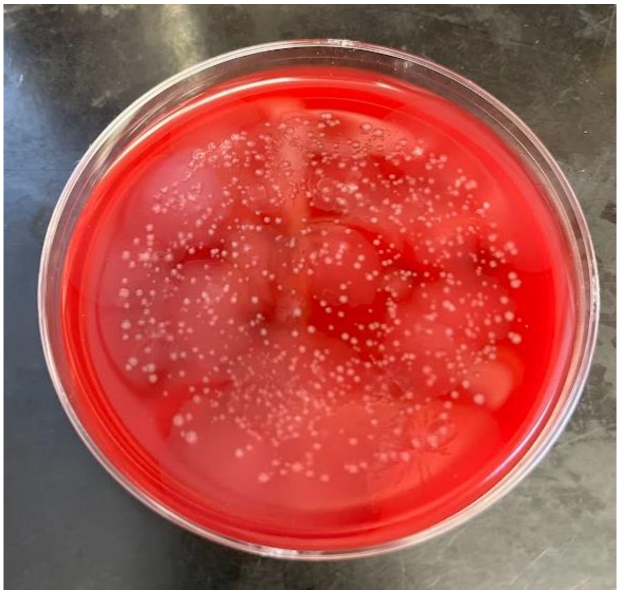

Figure 14. Negative control plate #2.

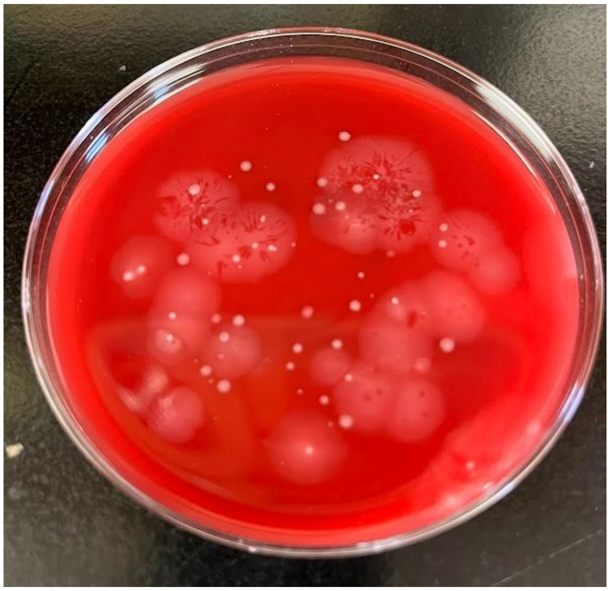

Figure 15. Positive control plate #5.

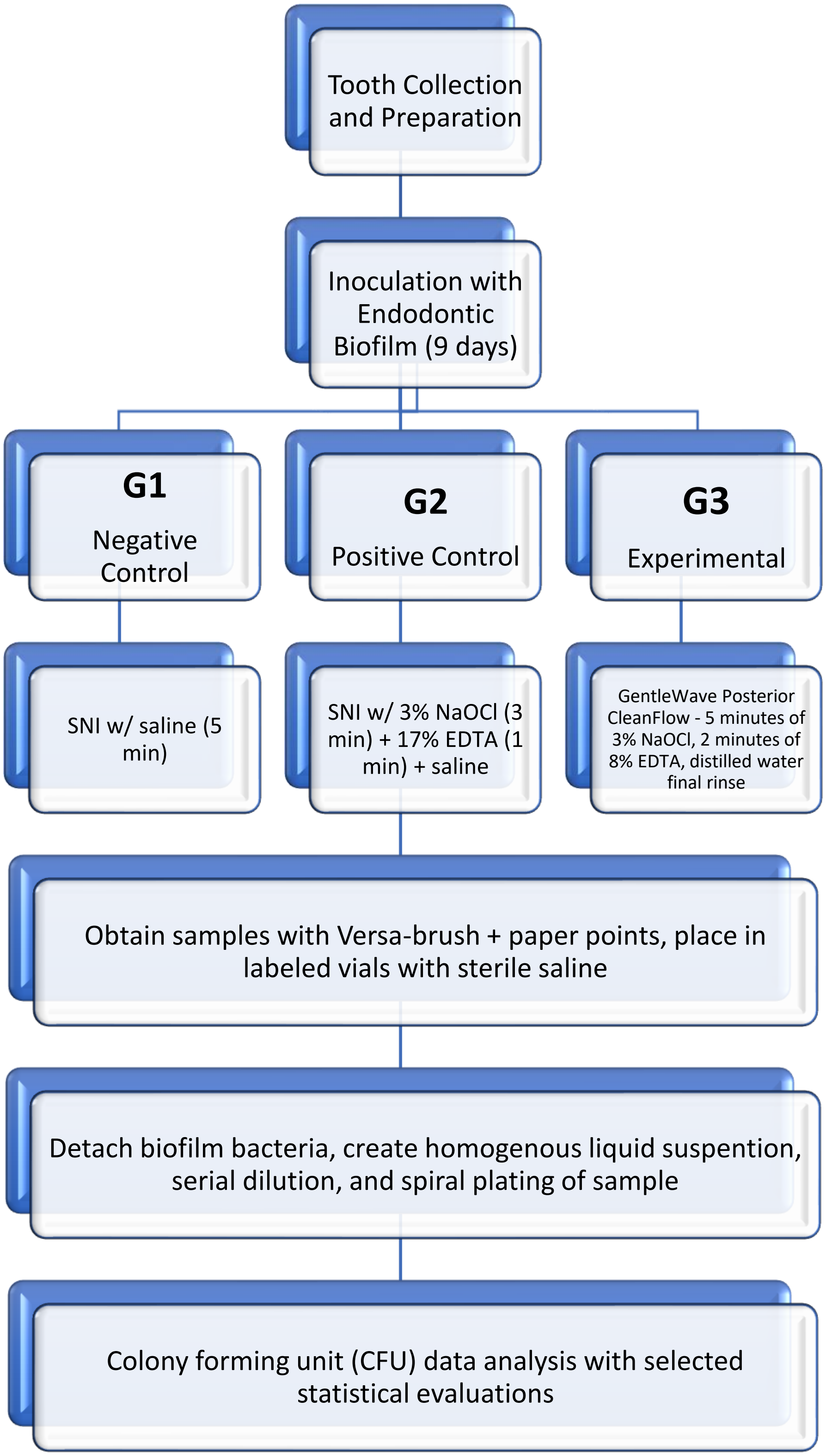

Figure 16. Flow Chart of study design.

DISCUSSION

The success or failure of RCT hinges on the ability to disinfect the root canal system. The gold standard has been SNI with sodium hypochlorite to remove organic tissue and kill bacteria with the addition of EDTA for smear layer removal. This in vitro study assessed the GW (Posterior CleanFlow) PCF's ability to remove a multispecies biofilm compared to SNI. Previous studies have addressed the GW system and its capacity for biofilm removal. However, this study is unique because it utilizes a multispecies biofilm taken from a tooth with pulpal necrosis. It also uses the newest GW procedure instrument, the GW Posterior CleanFlow (GW PCF), which has not been used in the majority of literature on GW biofilm removal.

Based on the results, the GW PCF is not a superior alternative compared to SNI at removing a multi-species biofilm in a single canal extracted tooth. Although the overall number of CFU's across all 12 teeth for the positive control was more than three times that of the GW group (4,698 CFU vs. 1,450 respectively) it was not statistically significantly different. This result goes against previous in vitro research that would suggest GW is a superior method for cleaning canal systems[15, 90-92]. For that reason, it is appropriate to review previous studies with a critical eye as well as to assess possible deficiencies in this study design and implementation.

Choi et al used a single species *E. faecalis* biofilm and compared PUI to GW[15]. Although not stated in their paper, it can be assumed that the PI that was used was the GW Molar PI which, at the time of publication, was the only GW PI available for use in molar teeth. Because the Molar PI has a sound bar that extends into the chamber of the tooth it is hypothesized that it can deliver more energy into the apical third of a root canal system. They did find that the GW had better cleaning in the apical third and in isthmuses compared to PUI. They made this assessment by splitting the teeth following treatment and viewing them using an optical stereomicroscope.

Choi pointed out the need for more studies, specifically ones carried out with a multispecies biofilm, stating “Oral heterogeneous biofilms can be even more resistant and it is important to utilize multi-species biofilm to understand the disinfection ability of the GentleWave System”(15). One GW study that did use a multispecies biofilm was carried out by Zhang et al. Although the biofilm was curated from supra and subgingival plaque, not from a necrotic tooth, and the Molar PI was used as in the Choi study. Paper points were used as the sampling technique, and samples submitted for PCR analysis in addition to being plated and CFU’s counted. This similar technique to the current study yielded different results. No growth was present on plates for PUI or GW groups. However, PCR analysis showed that PUI reduced bacteria by 98.01% compared to pretreatment levels, and GW by 99.91%. Although both are impressively high levels of disinfection, the results were statistically significantly different(22).

Zhang showed that both GW and PUI can greatly reduce intracanal biofilm from pre-treatment levels, and PCR analysis gave an accurate measure of how much reduction occurs. But by using paper points they may only be sampling the main canal and missing remaining bacteria in intricate anatomy, thus not fully being able to assess the GW’s capacity to clean apically.

Because results have differed across multiple biofilm studies, more research is needed. The future of GW is with its newest CleanFlow PI. For that reason, future studies should utilize the CF PI as opposed to the older models. A multispecies biofilm is also important for an accurate study. Multi-species interactions within a biofilm form a synergistic effect through cooperation between different species, which enhances the survival of the biofilm. We know that infections in patients are multi-species, so we should reflect that in in vitro studies. Ideally this biofilm should be procured from a tooth with pulpal necrosis.

Lastly, a challenging aspect of a biofilm study, but one that is important to get right is how to appropriately assess amount of biofilm removal. A combination of the protocols used in the aforementioned studies would be ideal. Using paper points as a means of collecting a sample has limitations as it cannot accurately sample intricate anatomy that may be present. However, it is simple to do and should still be utilized. In addition to paper pointing and culture teeth should be split and viewed microscopically, as done by Choi et al, to visualize any biofilm remaining in apical anatomy. Finally, quantifying bacterial load by using PCR analysis, as done by Zhang et al, would be a third method to determine biofilm removal. Combining all of these approaches would yield the most informative study on biofilm removal using GW.

SUMMARY AND CONCLUSIONS

The findings of this study suggest that the GentleWave Posterior CleanFlow procedure instrument does not exhibit improved biofilm removal compared to standard needle irrigation. However, based on mixed results when comparing this study to previous GentleWave biofilm removal studies, it is clear that more research is necessary. Future studies should considering using a multispecies biofilm, the GentleWave CleanFlow procedure instrument and multiple techniques to assess biofilm removal.

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ABSTRACT

EFFECTIVENESS OF GENTLEWAVE CLEANFLOW ON MULTISPECIES ENDODONTIC BIOFILM REMOVAL IN SINGLE ROOTED EXTRACTED TEETH

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Introduction: One of the challenges of non-surgical root canal treatment is disinfection. Bacterial biofilms adhere to canal walls and invade the intricate anatomy present within root canal systems. Traditional irrigation methods are unable to deliver irrigation solutions to all parts of the canal system. The GentleWave system is an advanced irrigation method designed to improve irrigation and disinfection, ultimately leading to more successful root canal outcomes.

Objective: The aim of this study is to evaluate the GentleWave CleanFlow posterior instrument's ability to remove a multispecies biofilm from a single canaled extracted tooth compared to traditional irrigation techniques.

Materials and Methods: Thirty-six single rooted premolar teeth with single canals were prepared to a uniform size, instrumented to size 25.06 and inoculated with a multispecies bacterial biofilm taken from an adult tooth with pulpal necrosis. Teeth were incubated and biofilm established before teeth were disinfected. Three disinfection groups included: GentleWave irrigation using the Posterior CleanFlow Procedure Instrument on the necrotic tooth cycle, standard needle irrigation with 2.5% NaOCl and 8% EDTA, and needle irrigation with sterile water. Following treatment, canals were swabbed and plated on blood agar plates and incubated for 48 hours when colony forming units were counted.

Results: Both GW and standard needle irrigation demonstrated significantly lower CFU/mL than the negative control ($p<0.001$). However, the GW and positive control groups were not significantly different from one another ($p=0.132$).

Conclusion: The findings of this study suggest that the GentleWave Posterior CleanFlow procedure instrument does not exhibit improved biofilm removal compared to standard needle irrigation. However, based on mixed results when comparing this study to previous GentleWave biofilm removal studies, it is clear that more research is necessary. Future studies should

considering using a multispecies biofilm, the GentleWave CleanFlow procedure instrument and multiple techniques to assess biofilm removal.

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