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Comparison of STERIPLEX[™] HC and Sodium Hypochlorite Cytotoxicity on Primary Human Gingival Fibroblasts

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Dentistry at Virginia Commonwealth University.

By

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Abstract

COMPARISON OF STERIPLEX™ HC AND SODIUM HYPOCHLORITE CYTOTOXICITY ON PRIMARY HUMAN GINGIVAL FIBROBLASTS

By Jesse Bluford Harris, DDS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Dentistry at Virginia Commonwealth University.

Virginia Commonwealth University, 2012

Director: Karan J. Replogle, DDS, MS Department Chair, Department of Endodontics

This study examined the cytotoxic effects of STERIPLEX[™] HC (sBioMed, Orem, UT) and sodium hypochlorite (NaOCl) on human fibroblast cells in vitro. Fibroblasts exposed to various concentrations of NaOCl or STERIPLEX[™] HC were visualized via light microscopy. Dilutions of either NaOCl or STERIPLEX[™] HC that did not appear to disrupt the integrity of the cells were recorded for further analysis. Cells were then cultured and grown to confluence in five separate plates. A void was created down the middle of each plate. If the cells were viable, cellular confluence was seen. If nonviable, confluence of the cells did not occur. Both disinfectants showed absolute kill at all concentrations above 1%. The cells treated with 0.1% NaOCl were found to be nonviable. However, at 0.1% STERIPLEX[™] HC, the cells were viable and able to replicate, filling the void and returning to confluence.



Introduction

The ultimate goal in endodontic therapy is the healing and regeneration of periapical tissues.¹ In order to reach this level of healing, the root canal space must be chemomechanically cleansed of bacteria and debris without causing irreversible damage to the surrounding periapical tissues.

Irrigation is an important step in the eradication of bacteria, debris, and necrotic tissues from the canal space during endodontic treatment. Mechanical instrumentation alone has been shown to be insufficient in the reduction of bacterial counts and removal of necrotic tissue and debris.² Irrigation with a broad spectrum solution to provide asepsis in vital cases and antisepsis in non-vital cases is of utmost importance.² It is also a consideration that the number of irrigants used be as few as possible to decrease treatment time but also to cut down on unwanted interactions between irrigants, such as the interaction between sodium hypochlorite (NaOCI) and chlorhexidine, which can leave behind toxic and potentially carcinogenic compounds, coat the root canal walls, and occlude dentinal tubules.³⁻⁵

Walker was the first to suggest the use of NaOCl in root canal irrigation in 1936.⁶ Since then, dental clinicians have used NaOCl to aid in the debridement of the root canal system because of its ability to dissolve both vital⁷ and necrotic tissues⁸ and its antimicrobial properties.⁹

NaOCl has been used for over 100 years as a universal disinfectant.¹⁰ At a cellular level, hypochlorite (HOCl) is a biomolecule synthesized from hydrogen peroxide and chlorine ions in a chemical reaction taking place in zones of inflammation.³² The efficacy of HOCl as a microbicidal agent results from the inability of bacteria or



mammalian cells to counteract its toxic effects since they lack the enzymes required for its catalytic detoxification.¹¹ Chlorine itself, although one of the most widely distributed elements on earth, is not found in a free state in nature but in combination with either sodium, potassium, calcium, or magnesium.

Chlorine releasing agents, such as NaOCl, were first discovered in the late 18th century and used as bleaching agents. By the end of the 19th century NaOCl was being used as a disinfectant. Created by Henry Dakin, a French chemist, and Alexis Carrel, a French surgeon, Dakin's solution was an antiseptic containing NaOCl. During WWI, this buffered 0.5% NaOCl solution was used to irrigate the wounds of injured soldiers.¹²

The NaOCl used today as a household disinfectant, such as Clorox, is made by bubbling chlorine gas into NaOH to form equal amount of NaOCl and NaCl. This form of NaOCl usually comes in 5.25% NaOCl. Since equal amounts of NaOCl and NaCl are formed during the production process, about 5% NaCl is found in the very alkaline solution (pH 11-12) of NaOH. The presence of NaCl makes the solution hypertonic which contributes to its clinical efficacy. This gives the solution the ability to hydrolyze, oxidize, and even osmotically draw fluids out of tissues.¹³

One of the most popular debates in endodontics involving NaOCl is what concentration is most effective. Heggers et al.¹⁴ discovered that 0.025% NaOCl is the efficacious therapeutic concentration that is both microbicidal and non-toxic. He and his team were, in turn, awarded the Lindberg Award in 1991, changing the NaOCl concentration of Dakin's solution from 0.5% to 0.025%. While this discovery has proven to be very useful in medical field for cleaning the wounds of burn victims and the like, the necessity for complete dissolution of necrotic tissue demands a higher concentration.



The chemical removal of organic debris by NaOCl occurs by the release of hypochlorous acid which reacts with insoluble proteins to form soluble peptides, amino acids, and other by-products.¹⁵ 5.25% NaOCl has been shown to be most effective as both a broad spectrum antibacterial agent and in the dissolution of both vital and necrotic tissues.^{7,8} Others prefer lower concentrations^{16,17} or to use higher volumes of lower concentrated NaOCl.¹⁸ Using a scanning electron microscope, Baumgartner found that a 1% solution of NaOCl was enough to chemically remove organic debris from the root canal walls.¹⁵ In the same study, he also suggested the use of a higher volume of NaOCl to replenish its capabilities and to increase contact time. Whether the solution is used at 5.25% or 1%, it is still damaging to vital surrounding tissues and one still runs the risk of an accident.

It has been stated that the characteristics of an ideal root canal irrigant should have a broad antibacterial spectrum and high efficacy against anaerobic and facultative microorganisms organized in biofilms, dissolve necrotic tissues, inactivate endotoxin, and prevent the formation of a smear layer or dissolve it once it has formed.² It is this broad spectrum of properties of NaOCl that make it so attractive as an endodontic irrigant. In addition, an ideal root canal irrigant should also be systemically nontoxic and noncaustic to periodontal tissues.²

A major concern with using NaOCl during endodontic treatment is the possibility that the irrigating solution will come into contact with the surrounding periapical tissues. It is also possible for the irrigant to be pushed beyond the apical foramen during treatment. There are no formal statistics to be found on the incidence of irrigation accidents. There are only case reports and papers written outlining the possible incidents



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that may occur during root canal irrigation, along with the sequelae, prevention, and treatment options.

The main pathways for extrusion of irrigants are perforations and the apical foramen.¹⁹ Movement of an irrigant beyond the apical foramen can result from anatomically large foramina or in cases where the foramen has been destroyed due to overinstrumentation or resorption. In other situations, excessive pressure during irrigation or binding of the irrigation needle tip without lateral venting can result in increased hydraulic pressures, resulting in expulsion of irrigant into the surrounding tissues. When NaOCl is involved, the result is tissue necrosis.

It has been shown that the toxic effects of NaOCl exposure to vital tissues results in hemolysis, skin ulcerations, and necrosis.²⁰ The corrosive effects of NaOCl on metals have also proven to be problematic with endodontic instruments and rubber dam clamps.²¹ In addition to complications within the oral cavity, there is always the concern of splashing NaOCl onto the patients clothing, into the patient's eyes, or even mistaken use of NaOCl as an anesthetic solution.

In 2005, Witton et al.²² reported two cases where accidents occurred involving NaOCI. In the first case, a 43 year old female was referred to an oral and maxillofacial surgeon with right sided facial swelling and severe pain following endodontic treatment by her general dentist. Root canal treatment had been started two days prior on tooth number 12. The tooth canal had been irrigated with 10ml NaOCl of unknown concentration. During irrigation, the patient experienced immediate pain and cheek swelling. Treated was halted immediately, antibiotics were prescribed and a follow-up appointment was scheduled. After 24 hours the pain and swelling increased significantly,



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forcing the patient to seek treatment at a local hospital. Approximately 50 hours after the incident, there was a firm right-sided facial swelling, which extended from below the border of the mandible up to the right eye. Right infra-orbital nerve paraesthesia was noted together with weakness of the buccal branch of the facial nerve, resulting in some loss of upper lip and cheek function. Mouth opening was limited to 20 mm and intraorally marked necrosis of the labial mucosa and ulceration of the mucosa of the maxillary alveolus around tooth 12 was seen. The patient was admitted to hospital and given intravenous dexamethasone, 8 mg 3x/day 2 days, and intravenous amoxicillin 1.0 g 3x/day together with regular oral diclofenac, 50 mg 3x/day for 2 days. The swelling and pain gradually decreased over the next 2 days, although there was extensive facial skin bruising. No necrosis developed. At a 1 month follow-up, the swelling had almost completely resolved, mouth opening was gradually improving and the patient was pain free. The area of infra-orbital nerve paraesthesia had decreased since her original admission. However, there was no improvement of her buccal-branch facial nerve weakness, with persisting loss of the nasolabial grove and the down-turning of the angle of the mouth still evident. No surgical intervention was necessary. Complete resolution of her facial weakness occurred approximately 6 months after the incident.

In 1991, Gatot et al.²³ reported a case involving accidental injection of NaOCl beyond the apical foramen of a maxillary right central incisor during routine endodontic treatment. He reported that the patient immediately experienced severe pain and edema developed extending from the lip to the right eye. The patient received hydrocortisone intravenously and penicillin. Thirty-six hours later there was a large ecchymosis under the right orbit and diffuse ecchymosis over the upper lip, as well as epithelial necrosis.



Surgical debridement with excision of a large amount of necrotic tissue had to be performed under general anesthesia. Healing took more than 2 weeks, leaving a scar on the right cheek and right infraorbital nerve anesthesia.

As was stated earlier, the ideal endodontic irrigant should have a broad antibacterial spectrum and high efficacy against anaerobic and facultative microorganisms and be systemically nontoxic and noncaustic to periodontal tissues. STERIPLEXTM HC is a new sterilant/sporicide developed by sBioMed, LLC, located in Orem, UT. Geared towards the health care profession, thus HC (Health Care), it is the first broad spectrum sporicide to receive EPA registration officially November 2, 2009. According to the manufacturer, it is non-fuming, non-corrosive and destroys a large variety of endospores, bacteria, and viruses within seconds. In addition, it has been shown to penetrate biofilms, is biodegradable, and is dermally non-toxic. Since this is a new product, there is very little research outside of the manufacturer's labs to confirm these claims. The only literature available is a white paper published by the department of microbiology and molecular biology at Brigham Young University.

The main focus of the white paper was its sporicidal capabilities, specifically those against *Bacillus Subtilis, Bacillus Anthracis, and Clostridium Sporogenes*. It proved to be extremely effective against all three endospores. Complete kill was seen in 15 seconds for *C. Sporogenes* and about 3.5 minutes for *B. Anthracis*.

Peracetic acid-based disinfectants have more cellular targets due to their oxidative capabilites thus making them an effective broad spectrum disinfectant. STERIPLEXTM HC is a peracetic acid-based chemical disinfectant containing additional active components that work synergistically.²⁴ According to the MSDS provided by the



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manufacturer, it is composed of 0.25% peroxyacetic acid, 0.03% hydrogen peroxide, 0.19% acetic acid, 19% glycerol, 10% ethanol, 0.0004% sorbitol, 0.03% processed silver, and 70% water. It has a mild, vinegar like smell and appears as a clear to light yellow liquid with a pH of 2.8.

Peracetic acid, also known as peroxyacetic acid, or PAA is an organic compound. It is a colorless liquid with an acrid odor similar to acetic acid. PAA is formed by continuously feeding acetic acid and hydrogen peroxide into an aqueous reaction medium containing a sulfuric acid catalyst. PAA is also formed naturally in the environment through a series of photochemical reactions involving formaldehyde and photo-oxidant radicals, as found in acid rain.²⁵ Peracetic acid is always sold in solution with acetic acid and hydrogen peroxide to maintain the stability of the chemical. PAA kills microorganisms by oxidation and subsequent disruption of their cell membrane. Its broad spectrum capability is due to its ability to react with any oxidizable compound in its vicinity, damaging virtually all types of macromolecules associated with an organism.²⁶ Oxidation of PAA occurs through the generation of free radicals. Damaging carbohydrates, nucleic acids, lipids, and amino acids, it causes cell lysis and true microbial death. PAA also decomposes to safe by-products (acetic acid and oxygen) but has the added advantages of being free from decomposition by peroxidases.

Endodontists are always looking for an ideal irrigant that is not only bactericidal, destroys biofilms, dissolves necrotic tissues, but is also systemically nontoxic and noncaustic to periodontal tissues. The endodontist would like to have the peace of mind to know that when he or she is irrigating a canal, if the irrigant was accidentally expelled



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out the apex, the situation could be easily managed and the fear of tissue necrosis and severe post operative pain would not be an issue.

There are no known studies testing the cytotoxicity of STERIPLEX[™] HC but the hope is that the product is found to be as effective as NaOCl without the side effects. The purpose of this study was to examine the cytotoxic effects of STERIPLEX[™] HC and sodium hypochlorite (NaOCl) on human fibroblast cells *in vitro*.



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Materials and Methods

Human Gingival Fibroblast Cell Culture

Primary gingival fibroblasts were obtained from gingival tissue explant cultures from tuberosity reduction surgery. Primary gingival fibroblasts were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 0.1 mM non-essential amino acids, 10% fetal bovine serum, 100 units/ml penicillin, 0.1 mg/ml streptomycin at 37°C and 5% CO₂ in a fully humidified incubator in 100-mm cell culture dishes. Fibroblasts used in this study were never passaged more than seven times. Cells were grown to confluence and then placed in serum-free medium containing 0.1% bovine serum albumin for 12 hours prior to treatment. Cultures were then washed two times with Hank's balanced salt solution (HBSS) prior to challenge with various dilutions of NaOCl or STERIPLEXTM HC. All cell culture media, supplements and consumables were purchased from Thermo Fisher (Pittsburg, PA). STERIPLEXTM HC was obtained from sBioMed[®] (Orem, UT). Sodium hypochlorite (NaOCl, 5.25%) was purchased from the James Austin Company (Mars, PA).

Cytotoxicity of NaOCl and STERIPLEXTM HC on Gingival Fibroblast Cultures

NaOCl or STERIPLEX[™] HC was added to HBSS to achieve 50%, 25%, 10%, 1%, and 0.1% dilutions of both disinfectants. Each dilution was made fresh minutes before addition to fibroblast cell cultures. Fibroblasts were visualized via light microscopy using a Zeiss Axiovert 200M microscope at 10X magnification. Cells were photographed at times 0-seconds, 30-seconds, 1-minute, 3-minutes and 5-minutes following the introduction of diluted disinfectant.



At first glance it appeared as though the cells treated with STERIPLEX[™] HC were unaffected. In order to test this, cells previously exposed to STERIPLEX[™] HC were trypsinized for 60 seconds. Some of the cells detached in deformed clumps but a majority remained attached, or fixed. This confirmed that the cells were in fact nonviable. Dilutions of either NaOCl or STERIPLEX[™] HC that did not appear to disrupt the integrity of the gingival fibroblasts were recorded for further analysis.

Cells were then cultured and grown to confluence in five separate plates. A line was drawn on the bottom of each plate, splitting the plates in half. A cell scraper was then used to scrape the cells off of the plate following the line drawn, creating a void down the middle of each plate. One of each plate was treated with HBSS, 1% NaOCl, 0.1% NaOCl, 1% STERIPLEXTM HC, and 0.1% STERIPLEXTM HC for 5 minutes. The cells were then viewed every day for eight days to determine whether the cells were viable and capable of replication after treatment with each dilution. If the cells were viable, proliferation would be seen into the void created with the scraper and the cells would once again become confluent. If the cells were nonviable, confluence of the cells did not occur.



Results

The cytotoxic effects of NaOCl and STERIPLEX[™] HC were initially assessed *in vitro*, at concentrations ranging from 0.1% to 100%, under light microscopy. Cells treated with HBSS were used as controls and remained viable and healthy throughout the experiment.

NaOCl concentrations of 100%, 50%, 25% and 10% resulted in complete cell destruction at exposure times of 30 seconds, 1 minute, 3 minutes and 5 minutes. Cell lysis is illustrated in Figure 1 and Figure 2. Cell lysis is evidenced by loss of visible cell membranes across all four time periods of 5 minutes or less.

At concentrations of 1% and 0.1% NaOCl, it was difficulty under light microscopy to determine whether cell viability had been compromised (Figure 3). Cell membranes appeared visibly intact.

STERIPLEX[™] HC concentrations of 100%, 50%, 25% and 10% showed no apparent visible cell destruction at exposure times of 30 seconds, 1 minute, 3 minutes and 5 minutes. The lack of apparent change in cell structure is illustrated in Figure 1 and Figure 2. At concentrations of 1% and 0.1%, the cells appeared unharmed displaying no visible physical changes with cell membranes remaining intact under the microscope as illustrated in Figure 3.





Figure 1 The Cellular Effects of NaOCl and STERIPLEX[™] HC



Figure 2 The Cellular Effects of NaOCl and STERIPLEX™ HC





Figure 3 The Cellular Effects of NaOCl and STERIPLEX™ HC

To determine whether the apparent "normal" physical appearance of the cells treated with concentrations of 1% and 0.1% STERIPLEX[™] HC and NaOCl was evidence of cell viability or a picture of cell fixation, a cell viability test was performed. Cells were trypsinized and an attempt was made to pass the cells.

A comparison between healthy trypsinized cells and fixed cells can be seen in Figure 4. Healthy cells, when not fixed, appear spherical and are free floating in solution. Fixed cells maintain the shape held while attached to a substrate and either remain attached or appear as free floating, deformed clumps.

Cells previously treated with STERIPLEX[™] HC at a dilution of 50% were trypsinized. The cells treated with 50% STERIPLEX[™] HC either did not detach or detached in deformed clumps, proving that the cells were indeed fixed, and therefore, nonviable. The same results were seen with STERIPLEX[™] HC concentrations of 25% and 10%.



Trypsinized gingival fibroblasts following 5 minute incubation with HBSS.

Trypsinized gingival fibroblasts following 5 minute incubation with 50% STERIPLEX[™] HC



Figure 4 The Effects of Trypsin on Cells Treated with NaOCl and STERIPLEX™ HC

Results of trypsinization at a 1% concentration STERIPLEX[™] HC can be seen in

Figure 5. The image on the left shows a healthy group of cells and adjacent void created



Figure 5 The Cellular Effects of NaOCl and STERIPLEX[™] HC

to allow room for growth. The images on the right show the destruction of the cells and as a result, no growth. Figure 5 also shows the results of trypsinization of a 1%



concentration of NaOCl. The images on the right show the destruction of the existing cells and as a result, no cell growth. The cells were, therefore, nonviable after exposure at 1% concentration.

In Figure 6, a different comparable conclusion was found after trypsinization. Cells treated with 0.1% NaOCl did not grow into the void created and were thus deemed nonviable. However, at 0.1% STERIPLEXTM HC, the cells were viable and able to replicate, filling the void and returning to confluence.



Figure 6 The Cellular Effects of NaOCl and STERIPLEX[™] HC

In summary, complete lack of cell viability at concentrations of 10% and higher occurred for NaOCl and STERIPLEXTM HC over an exposure period of 5 minutes. Complete cell destruction resulted for cells treated with NaOCl and complete cell fixation occurred for cells treated with STERIPLEXTM HC. Cells treated with 0.1% NaOCl were found to be nonviable. Cells treated with 0.1% STERIPLEXTM HC were viable and able to replicate.



Discussion

As was stated earlier, an ideal root canal irrigant should have a broad antibacterial spectrum and high efficacy against anaerobic and facultative microorganisms organized in biofilms, dissolve necrotic tissues, inactivate endotoxin, and prevent the formation of a smear layer or dissolve it once it has formed. Debridement of a canal space, combining mechanical and chemical techniques, reduces the number of microorganisms and increases the success rate of endodontic therapy. Adequate tissue dissolution and removal is an important step in this process. Unfortunately, the use of toxic chemicals is the only way that endodontists have been able to eliminate vital and necrotic tissue from the canal space. The presence of these toxic chemicals increases the possibility of complications during the course of root canal treatment and can interfere with the process of repair. Thus, biocompatibility is a major concern and a desired trait of an endodontic irrigant. An ideal root-canal disinfectant would be a material with the least cytotoxicity and the strongest antimicrobial activity.²⁷

Potential irrigants with these characteristics may be tested by studying the antibacterial and cytotoxic properties of available medications and chemicals at various dilutions, thus identifying a medication or chemical that has both desirable properties²⁸. This study used a light microscope to examine the cytotoxic effects of STERIPLEXTM HC and NaOC) on human fibroblast cells in vitro. This study determined whether the cells were viable and capable of replication after exposure to varying concentrations of either NaOCl or STERIPLEXTM HC.

There have been numerous endodontically focused studies over the years, measuring the cytotoxic effects of NaOCl dating back to Walker in 1936. There have



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also been many studies comparing NaOCl as an endodontic irrigant to a multitude of proposed alternative irrigants. No irrigant has been found to replace NaOCl in the role of chemical root canal debridement.

Prior to this study, there had been no studies performed to date examining the cytotoxic effects of STERIPLEXTM HC on human fibroblast cells. No studies to date compared its cytotoxic effects with that of NaOCI. The hope was that STERIPLEXTM HC would prove to possess all of the positive characteristics of NaOCI, without the detrimental side effects. According to the manufacturer, STERIPLEXTM HC has been shown to be non-corrosive to the skin and eyes, which qualifies the STERIPLEX HC post-activated formula for a health and safety rating of '0' (Hazardous Materials Identification System = 0). It exhibits no oral or inhalation toxicities, and only mild irritation when sprayed directly into the eyes. It is also shown to be non-oxidizing to materials and is safe for direct application on stainless steels, plastics and polymers.

With all of these properties being the complete opposite of NaOCl, it was presumed that it would prove to be less cytotoxic as well. Unfortunately this was not the case. At first glance, the cells treated with STERIPLEXTM HC appeared unharmed. Upon further investigation, it was found that STERIPLEXTM HC fixed cells *in vitro*. It was determined that STERIPLEXTM HC was cytotoxic at concentrations ranging from 100% to 1%. The most likely reason for this would be the combination of ethanol and acetic acid found in STERIPLEXTM HC.

It is well known that alcohols, such as ethanol, are used in cell fixation. Alcohols, by themselves, are known to cause considerable shrinkage and hardening of tissue during



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fixation while acetic acid alone is associated with tissue swelling; combining the two may result in better preservation of tissue morphology.

Cell fixatives are still being used in dentistry today. Formocresol, although controversial, is still recommended for treatment in carious pulp exposures of primary teeth. Formocresol, which contains formaldehyde, works by denaturing the cell proteins rendering the cells nonviable and permanently fixed in place. Intracanal placement of formocresol is believed to reduce postendodontic treatment pain, decrease the intracanal bacterial count, mummify or fix residual pulp tissue, and decrease possible inflammatory reactions.³³

Fixing cells in the root canal during endodontic treatment violates one of Zehnder's characteristics of an ideal root canal irrigant, i.e. the ability to dissolve necrotic tissue.² By fixing the tissue in place, proper cleansing of the root canal would become more difficult. More controversy lies in the possible systemic carcinogenic effects of the formaldehyde found in formocresol than in the fixation of pulpal cells. For this study, the fixation of cells is an undesirable characteristic of STERIPLEX[™] HC.

At 0.1% concentration of STERIPLEXTM HC the cells were still capable of replication, though the long term effects are unknown. Dr. Jonathan Coudron, a second year endodontic resident at VCU, examined the antimicrobial effects of STERIPLEXTM HC at various dilutions. It was hoped that the results would show that STERIPLEXTM HC was a safer but equally effective root canal irrigant as NaOCI. Results have since proven to be conflicting. Dr. Coudron found that STERIPLEXTM HC was ineffective as an antimicrobial against *Enterococcus faecalis* at less than 1% concentration of



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solution.³¹ This being said, at 0.1%, STERIPLEXTM HC is nontoxic, but also ineffective as an antimicrobial.

The capacity to kill bacterial spores determines how a commercial product will be marketed. Disinfectants are not expected to kill all bacterial spores and are used to decontaminate devices that ordinarily do not penetrate tissues or that touch only intact skin.²⁹ Sterilants are expected to kill all microorganisms, including bacterial spores, and are used to treat devices that penetrate tissue or present a high risk if unsterile.²⁹ Viable spores still attached to various materials could remain undetected by current sporicidal tests, resulting in overestimation of the sporicidal activity of sterilizing agents.²⁹ Since surface disinfectants are most commonly used in the dental operatory, and as more research reveals nosocomial infections are related to spores³⁰, an alternative may be the use of STERIPLEX[™] HC.

Though STERIPLEXTM HC may not have a place as an endodontic irrigant; there are other potential applications for it in dentistry. STERIPLEXTM HC is already being marketed as the first sporicidal disinfectant available on the market for use in the medical/dental realm for room and instrument sterilization. As was stated earlier, STERIPLEXTM HC has been shown in clinical trials to be non-toxic to the skin and non-oxidizing on materials such as stainless steel, plastics, and polymers. This disinfectant has applications not only in a hospital or dental clinic here in the United States, but also in developing countries for use during humanitarian outreach missions.

In conclusion, the following observations have been made. Both NaOCl and STERIPLEX[™] HC showed absolute kill at all concentrations above 1%. The cells treated with 0.1% NaOCl were found to be nonviable. However, at 0.1% STERIPLEX[™]



HC, the cells were viable and able to replicate, filling the void and returning to confluence.



References

- Chang Y, Huang F, Tai K, Chou M. The effect of sodium hypochlorite and chlorhexidine on cultured human periodontal ligament cells. Oral Surg Oral Med Oral Pathol 2001;92:446-50.
- 2. Zehnder M. Root canal irrigants. J Endod 2006;32:389-98.
- 3. Basrani BR, Manek S, Sodhi RNS, Fillery E, Manzur, A. Interaction between sodium hypochlorite and chlorhexidine gluconate. J Endod 2007;33:966-9.
- 4. Rasimick BJ, Nekich M, Hladek MM, Musikant BL, Deutsch AS. Interaction between chlorhexidine gluconate and EDTA. J Endod 2008;34:1521-3.
- Bui TB, Baumgartner JC, Mitchell JC. Evaluation of the interaction between sodium hypochlorite and chlorhexidine gluconate and its effect on root dentin. J Endod 2008;34:181-5.
- Walker A. Definite and dependable therapy for pulpless teeth. J Am Dent Assoc 1936;23:14-18.
- Rosenfeld EF, James GA, Burch BS. Vital pulp tissue response to sodium hypochlorite. J Endod 1978;4:140-46.
- Hand RE, Smith ML, Harrison JW. Analysis of the effect of dilution of the necrotic tissue dissolution property of sodium hypochlorite. J Endod 1978;4:60-4.
- Bystrom A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. Oral Surg Oral Med Oral Pathol 1983;55:307-12.



- Rutala WA, Weber DJ. Uses of inorganic hypochlorite (bleach) in health-care facilities. Clin Microbiol Rev 1997;10:597-610.
- 11. Lapenna D, Cuccurullo F. Hypochlorous acid and its pharmacological antagonism: an update picture. Gen Pharmacol 1996;27:1145-47
- 12. Dakin HD. On the use of certain antiseptic substances. Br Med J 1915;2:318-20
- Pashley EL, Birdsong NL, Bowman K, Pashley DH. Cytotoxic effects of NaOCl on vital tissue. J Endod 1985;11:525-28.
- Heggers JP et al. Bactericidal and wound healing properties of sodium hypochlorite solutions: The 1991 Lindberg Award. J Burn Care & Rehabil 1991;12:420-24.
- 15. Baumgartner JC, Ibay AC. The chemical reactions of irrigants used for root canal debridement. J Endod 1987;13:47-51.
- 16. Spanberg L, Engstrom B, Langeland K. Biologic effects of dental materials. III. Toxicity and antimicrobial effect of endodontic antiseptics *in vitro*. Oral Surg Oral Med Oral Path 1973;36:856-71.
- 17. Baumgartner JC, Cuenin PR. Efficacy of several concentrations of sodium hypochlorite for root canal irrigation. J Endod 1992;18:605-12.
- Hauman CH, Love RM. Biocompatibility of dental materials used in contemporary endodontic therapy: a review. Part 1. Intracanal drugs and substances. Int Endod J 2003;36:75-85.
- Hulsman M, Rodig T, Nordmeyer S. Complications during root canal irrigation. Endod Topics 2009;16:27-63.



- 20. Hulsmann M, Hahn W. Complications during root canal irrigation-literature review and case reports. Int Endod J 2000;33:186-93.
- Clarkson RM, Moule AJ. Sodium hypochlorite and its use as an endodontic irrigant. Aust Dent J 1998;43:250–56.
- 22. Witton R, Henthorne K, Ethunandan M, Harmer S, Brennan PA. Case Report-Neurological complications following extrusion of sodium hypochlorite solution during root canal treatment. Int Endod J 2005;38:843-48.
- 23. Gatot A, Arbelle J, Leibermann M, Yanai-Inbar I. Effects of sodium hypochlorite on soft tissues after its inadvertent injection beyond the root apex. J Endod 1991;17:573-74.
- 24. Department of Microbiology and Molecular Biology. Brigham Young University. STERIPLEX[™] HC White Paper. Differential response of bacillus anthracis and other spore species to sporicidal disinfectants.
- 25. Gaffney JS, Streit GE, Spall WD, Hall JH. Beyond acid rain. Do soluble oxidants and organic toxins interact with SO2 and NOx to increase ecosystem effects? Environ Sci Technol 1987;21:519-24.
- 26. National Library of Medicine. Hazardous Substances Data Bank.
- Masillamoni CRM, Kettering JD, Torabinejad M. The biocompatibility of some root canal medicaments and irrigants. Int Endod J 1981;14:115–20.
- Zhang W, Torabinejad M, Li Y. Evaluation of cytotoxicity of MTAD using the MTT-tetrazolium method. J Endod 2003;29:654-57.
- 29. Sagripanti JL, Bonifacino A. Bacterial spores survive treatment with commercial sterilants and disinfectants. Appl Environ Microbiol 1999; 65:4255-60.



- 30. U.S. General Accounting Office. 1990. Disinfectants, EPA lacks assurance they work. Federal document GAO/RCED-90-139. General Accounting Office, Washington, D.C.
- 31. Coudron J, Replogle K, Kitten T, Best A, The Antimicrobial Effect of STERIPLEXTM HC in comparison with Sodium Hypochlorite on *Enterococcus faecalis*. Currently submitted/Thesis topic Feb 2012. Virginia Commonwealth University.
- 32. Hidalgo E, Bartolome R, Dominguez C. Cytotoxicity mechanisms of sodium hypochlorite in cultured human dermal fibroblasts and its bactericidal effectiveness. Chem Bio Inter 2002;139:265-82.
- Sipes R, Binkely C. The use of formocresol in dentistry: a review of the literature. Quin Inter 1986;7:415-17.



Vita

Dr. Jesse Bluford Harris was born on May 4, 1978 in Norfolk, VA. He is currently a citizen of the United States of America. Dr. Harris received a Bachelor of Science in Biology from Virginia Tech in 2000 followed by a Doctor of Dental Surgery from Virginia Commonwealth University, School of Dentistry in 2005. Dr. Harris entered active duty in the United States Air Force following graduation and completed a one year Advanced Education in General Dentistry residency in 2006 at Wright Patterson Medical Center. While on active duty, Dr. Harris served as the OIC Support Element for the 354th Medical Operations Squadron Dental Flight, 354th Medical Group, Fighter Wing, Eielson AFB, Alaska. Following his honorable discharge from active duty in 2009, Dr. Harris practiced general dentistry for one year in Fairbanks, AK prior to enrolling in the Advanced Specialty Program in Endodontics at Virginia Commonwealth University School of Dentistry. Dr. Harris is a member of the AAE, ADA, and AGD. Dr. Harris will be starting his own private practice in Mechanicsville, VA upon graduation. He will graduate from VCU with a Master of Science in Dentistry and a Certificate in Endodontics in June of 2012.

