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THESIS APPROVAL PAGE FOR MASTER OF SCIENCE IN ORAL BIOLOGY

“Non-thermal gas plasma cleaning of diamond coated dental burs”

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All work has been completed to the satisfaction of the research committee.

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Non-thermal gas plasma cleaning of diamond coated dental burs

by

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Thesis submitted to the Faculty of the
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ABSTRACT

Non-thermal gas plasma cleaning of diamond coated dental burs

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CLINICAL RELEVANCE STATEMENT

This study provides information on the use of sterilization protocols for multi-use diamond dental burs using the manufacture's recommendations and an experimental protocol involving non-thermal gas plasma to potentially improve upon those recommendations.

ABSTRACT

INTRODUCTION: Manufacturers continue to produce multi-use burs, despite concerns with infection control. These burs have FDA clearance to re-use if re-processed according to manufacturer's instructions

OBJECTIVES: This project compared the bioburden on soiled diamond burs after re-processing using manufacturer's instructions and after adding non-thermal plasma gas cleaning to the manufacturer's protocols.

METHODS: Fine and coarse diamond burs from Alpen Diamond Instruments (Coltene/Whaledent, Altstatten, Switzerland), with FDA 510(k) clearance for reprocessing, were soiled on sterilized extracted human teeth using a high speed hand piece with water. The burs were divided based on the coarseness and then again by cleaning protocol: manufacturer's instructions or the manufacturer's instructions with a 15 minute cycle in a plasma cleaner (Herrick Plasma, Ithica, NY). Following sterilization in an autoclave, all samples were stained with Crystal Violet stain, rinsed and the Crystal Violet released by a 10% acetic acid mix. The resulting released stain was pipetted into a culture plate and read by a BioTek Synergy 2microplate reader (BioTek Industries, Winooksi, VT) at an absorbance reading of 450 nm to determine the bioburden remaining on the burs after treatment. A two-way ANOVA analysis of the four datasets was completed and Sidak's multiple comparisons test was used for post-hoc testing of ANOVA results.

RESULTS: Two-way ANOVA analysis of the four datasets showed a highly significant interaction ($p=0.0002$). There was no effect for the material roughness ($p=0.14$), but a highly significant effect for treatment ($p=0.010$). A comparison of all group means showed significant differences between the manufacturer and plasma cleaning for the fine bur (0.0106 versus 0.0248, $p=0.0001$). For comparisons within the coarse bur group, there was no significant difference between the two cleaning methods ($p=0.58$).

CONCLUSIONS: All burs retained some biomaterial. The fine burs cleaned by the manufacturer's method retained the least biomaterial. Overall, plasma cleaning gave the most consistent results, although not significantly better than the manufacturer's method.

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CHAPTER 1: INTRODUCTION

STATEMENT OF THE PROBLEM

Transmission of disease through inadequate sterilization techniques is a concern that permeates the health care industry. Due to the surgical nature of many dental procedures, ideal methods for sterilization of dental instruments and appropriate use of such are a topic that deserves further evaluation. The vast majority of dental procedures involve the removal of tooth structure through mechanical means via a dental bur. Dental burs have varying surface configurations to affect cutting efficiency and remaining tooth surface texture. Manufacturers currently offer both multiple-use and single-use dental burs to dental professionals. Assuming proper processing and sterilization, a dental health care provider can reuse the multiple-use bur repeatedly until it could no longer cut efficiently. Unfortunately, there is no definitive guidance regarding the efficacy of this practice. The Center for Disease Control (CDC) is the body that the American Dental Association looks to when determining best practice for infection control. Currently, the CDC does not have an official guideline regarding the reuse of burs in a dental office, but recommends that dental health care personnel should refer to manufacturer instructions regarding validated reprocessing instructions. As dental burs are considered a medical device, the Food and Drug Agency (FDA) regulates the approval of single and multiple- use burs available in the marketplace in the United States. Manufacturers are required to submit reprocessing instructions to the FDA, via a 510(k) premarket notification, for the bur to be cleared and marketed as a multiple use medical device.

Since many multi-use diamond burs have received FDA clearance, the CDC does not currently have an official guideline regarding the use of multiple-use dental burs other than referring to the manufacturers' 510(k) for reprocessing. Regardless of FDA clearance, the CDC does offer recommendations on certain devices that may be hard to clean due to the

manner of the items construction to include: all burs, endodontic files and broaches. It is based on this suggestion that the US Army Dental Directorate has determined all burs used in practice should be pre-sterile, one-time use burs, regardless of the FDA approval of a manufacturer's submitted and approved 501(k) reprocessing status.

Due to surface irregularity, burs (diamond burs in particular) cannot be completely cleansed of all debris with our current levels of cleaning and sterilization. Accumulated debris may reduce cutting efficiency and has the potential to harbor prions and other proteins that could potentially cause disease and are not readily killed through current steam autoclave sterilization. A preliminary review of the literature reveals many studies have shown the ability of gas plasma to sterilize surfaces, but few studies that have looked at surface debris and bio-burden reduction through plasma treatment of the irregular surface of multiuse diamond dental burs.

SIGNIFICANCE

As large organizations, US Army Dental Activities (DENTACs) spend a significant portion of its supply budget on single use, pre-sterilized burs in an attempt to follow the CDC's suggestion. The cost for dental burs can range upwards of $30-40 a patient, depending on the procedure and the number of burs used. Many times a bur is used sparingly during a procedure and must be disposed of, regardless of remaining cutting efficiency, based on US Army Dental Directorate standard operating procedure. In times of fiscal constraint, managing a budget becomes more difficult when overhead directed to dental burs increases significantly. If plasma gas treatment could be shown to remove the debris left on a diamond bur (specifically on those that are approved as multi-use burs), it could potentially save an individual US Army DENTAC tens of thousands of dollars annually. When extrapolated to

the entire Dental Corp, this could save hundreds of thousands of dollars. Moreover, it could also provide cost savings in the private sector if bur life could be extended through the confident reuse over multiple patients.

CHAPTER 2: REVIEW OF THE LITERATURE

INFECTION CONTROL IN HEALTHCARE

The transmission of disease from one patient to another via surgical instruments is an issue of concern for all parties involved in healthcare: providers, assistants, administrators, and patients. The Center for Disease Control, a division of the U.S. Department of Health & Human Services, is the regulating body in the United States regarding the guidelines and required implementation of procedures and practices to reduce the risk of disease being passed from one patient to another. The guidelines produced by the CDC cover a variety of topics including Disinfection and Sterilization, Environmental Infection Control, hand hygiene, Infection Control in Healthcare Personnel, and Dentistry Setting-specific guidelines among others.[1]

Healthcare facilities have long been associated with illness and death. As early as 1847, the ideas that would lead to modern day infection control began to develop. Ignaz Semmelweis, an early advocate for antiseptic procedure, discovered a relationship between reducing the incidence of puerperal fever among newborns and hand washing. At the time, Semmelweis could not scientifically prove the root cause of his findings and many of his medical colleagues rejected his theory.[2] However, Semmelweis was ultimately proved correct

in his hypothesis, as scientists such as Louis Pasteur were able to prove germ theory of disease and subsequent physicians, namely Joseph Lister, advocated for sterile surgery.[3].

The sterile surgical technique (to include sterilization of surgical instruments) is now considered the only standard in healthcare settings, where breaches of infection control are highly publicized. Perez et al highlighted this issue by reporting that 33 outbreaks of viral hepatitis, causing 448 recognized cases of HBV or HCV occurred in a 10-year period.[4] These outbreaks were in a variety of non-hospital settings – outpatient clinics, dialysis centers and long-term care facilities.

To standardize the disinfection and sterilization procedures in health care settings, the CDC published their Guideline for Disinfection and Sterilization in Healthcare Facilities. The latest edition was published in 2008, and was last updated in 15 February, 2017.[5] This publication sets forth the basic steps required to prevent the spread of disease and serves as a framework for healthcare institutions to develop their own standard operating procedures when implementing standard precautions to prevent the spread of disease in their facilities.

PROTOCOLS IN DENTISTRY

Dentistry is a healthcare subspecialty where surgical procedures are performed on a near hourly basis. In 2003, the CDC recognized the need to set a standard for disinfection and sterilization in the dental field and published *Guidelines for Infection Control in Dental Health-Care Settings* in the December issue of the Morbidity and Mortality Weekly Report. The report discussed many topics for preventing the spread of disease in a dental setting, to include instrument processing (cleaning and decontamination) and sterilization.[6] It is from

this document that the recommendation for all burs be considered single use due to their physical construction making them difficult to clean.[6]

Standard practice when reusing a dental bur is to refer to the manufacturer's guidelines for reprocessing. These instructions typically indicate that the bur should be immediately rinsed and hand scrubbed, with a potential additional step to be placed in an ultrasonic cleanser. A visual inspection, ideally under magnification, should be performed after decontamination of the bur surface. It is at this point that the clinician must make a determination if the bur can be sterilized or if it should be disposed of properly. A study comparing multiple decontamination methods used for dental burs indicated that no method tested (manual scrubbing, hot air oven, glass bead sterilizer, ultrasonic cleaner and autoclave) was "absolutely efficacious in decontamination of dental burs".[7] The authors noted that the autoclave was the best of all the methods tested, however they did not visually evaluate the surface of the burs to determine remaining bio-burden or loss of cutting efficiency.

Recognizing the complexity of the surface of some dental instruments, Morrison and Conrod investigated the effectiveness of sterilization techniques used in dental practices on burs and endodontic files and compared the findings against those from the manufacturer's sterile packaging.[8] These techniques included hand scrubbing, ultrasonic cleaning, heat sterilization and steam sterilization, all of which varied by office. They concluded that current sterilization techniques for the removal of pathogens were effective on new instruments, but were ineffective on used instruments.

A case-control study by Al-Jandan et al studied the rate of bacterial contamination of both reused and new burs after high-pressure and low-pressure steam pressure autoclaving.[9] This study included a presoak in an alkaline detergent, followed by ultrasonic bath with

enzymatic detergent of both new and used burs. After treatment, bacterial growth was present in both groups and the authors concluded that decontamination and sterilization was not 100% effective.

A study utilizing gas vapor sterilization of bone cutting burs in a hospital-based oral surgery setting was conducted in 2005 by Hogg and Morrison.[10] Used burs were hand-debrided and processed through an ultrasonic cleaner for 3 minutes. They were rinsed, dried, packaged, and subjected to a gas vapor (10% ethylene oxide and 90% carbon dioxide) sterilization cycle. When incubated and cultured, 100% of used fissure burs and 45% of round burs demonstrated bacterial growth. The authors concluded that consideration of burs as a single use device might be warranted if sterilization cannot be guaranteed.

The bacterial contamination of dental burs after their use varies throughout the literature, based on the types of pre-treatment and final sterilization method used. Not discussed in the literature is the use of gas plasma as a disinfection or sterilization agent. Additionally, the overall health risk posed to a patient who received treatment with a previously used dental bur has not been evaluated, nor is any published research available of the documented transfer of infection from a reused, sterilized dental bur.

GAS PLASMA AND ITS USE IN DENTISTRY

Matter typical exists in one of three states – solid, liquid, or gas. Plasma can be considered the fourth state of matter. It is an ionized gas, which forms when a substance in a gaseous state is heated or energized to a sufficient level. The energy absorbed by a gas causes atoms to collide with each other and "knock" electrons off of the atoms, giving rise to plasma. Irving Langmuir was the first to name this substance plasma. [11] Plasma is utilized in many ways to include fluorescent lights, plasma TV displays, semiconductors and fusion reaction

for power.[12] Cha (2014) states that it is not clear when plasma was first used in dentistry. He postulated that it might have been derived from sterilizing instruments, but indicates that Eva Stoffels was the first to investigate its therapeutic use in medicine and dentistry.[13] Plasma has been used in dentistry to improve the osseous integration of implants through surface treatment, enhancing bonding strengths, increasing polymerization rates, cleaning and sterilization of instruments, root canal disinfection during endodontic therapy, and even in office tooth bleaching. [13-16] The cleaning of metallic surfaces through gas plasma treatment is of particular interest in dentistry as it can be used to not only disinfect, but also clean and sterilize. Plasma treatment has considerable advantages over traditional wet cleaning methods, namely reduction or elimination of potentially hazardous liquid waste and residual residue remaining on surfaces.[17]

APPLICATION IN STERILIZATION AND DISINFECTION

Reduction of surface debris and the inactivation of bacteria and bacterial spores is the goal of sterilization in the infection control process in dentistry. In fact, CDC guidelines for the monitoring of dental sterilizers include a weekly challenge of the sterilizer's ability to inactivate a highly resistant microorganism (typically a *Bacillus* species) through a biologic indicator.[6] Non-thermal atmospheric pressure plasmas are an alternative way to reprocess instruments and meet the CDC's recommendation and guidelines. A study by Tseng et al investigated the use of gas discharge plasmas to inactivate *Bacillus* and *Clostridium* spores, which can be used to evaluate a sterilization system's effectiveness.[18] They found that exposure of these spores to helium gas plasma for a period of 2-8 minutes resulted in an inactivation rate that was similar to other sterilization methods.

Nelson and Berger studied the inactivation of bacteria when exposed to oxygen gas plasma.[19] Using varying power settings and differing lengths of exposure, they found that oxygen gas plasma treatment may be an effective method of sterilization. Building on their work Lerouge et al studied the effect of various gas combinations and the ability of these combinations to inactivate bacteria.[20] They concluded that a mixture of oxygen and tetrafluoromethane was most efficacious, but cautioned that the mixture had the negative effect of ablating and oxidizing the treated surface.

A comparison of plasma treatment sterilization and UV exposure sterilization on dental burs and polyvinylsiloxane dental impression material was investigated by Sung et al. with *E. coli* and *B. subtilis* as the indicating species.[21] Using differing lengths of exposure times, they found that plasma effectively sterilized E. coli after 60 and 90 seconds, while it took 120 and 180 seconds to be effective against B. subtilis. They concluded that plasma treatment was more effective than the UV sterilizer against both species.

Endodontic files are similar to diamond dental burs in that it is difficult to remove residual bio-burden from their surfaces with traditional sterilization. In reviewing the literature, two articles were identified which were of particular interest. Smith et al evaluated residual protein levels on reprocessed endodontic files through visual inspection after traditional sterilization processing.[22] In their study, they obtained samples from general dental practices after they had been processed according to the practices' individual sterilization procedures. They found that 98% of files had residual visual debris and that all the files demonstrated residual protein contamination.

Another study was conducted by Whittaker et al in which they examined endodontic files by scanning electron microscopy (SEM) after plasma sterilization.[23] A sample of

endodontic files was visually inspected after use and reprocessing utilizing traditional sterilization protocols. After initial inspection, the files were subjected to plasma treatment and SEM evaluation at 20kV to provide resolution of better than 5nm. They found almost total absence of previous debris patches and that the remaining debris was either artifacts of the microscope or inorganic remnants from dentin. They found no nitrogen remaining, which would have been indicative of remaining proteins.

It is well established that gas plasmas can meet the sterilization requirements set forth by the CDC. However, there is little research that has been completed that visually evaluates the surface of diamond dental burs after treatment with gas plasma. Current reprocessing of multi-use burs with irregular surfaces may be improved upon if an additional step is implemented in the sterilization process, namely the treatment of burs by non-thermal gas plasma.

CHAPTER 3: MATERIALS AND METHODS

There were two objectives for this investigation: (1) Can treatment with non-thermal gas plasma improve upon a bur manufacturer's instructions for reprocessing a multi-use dental bur when compare to current industry practices and (2) Will there be a significant difference on material retained on the dental bur when compared by coarseness - fine and coarse samples. The independent variables, based on the objectives, were (1) the type of treatment applied to the burs and (2) the type of bur used to generate the sample. The outcome measured for both independent variables was the optical density (OD) of a Crystal Violet solution to be read at a wavelength of 450 nm.

Sample Size Determination

A power analysis was preformed using an online power calculator[24] designed for use with a two-way ANOVA. Using the following assumptions (α of 0.05, with a power of 0.8) gave a total n of 16. This value was divided by the 4 groups and resulted in a sample size of 4 per sample. For ease of ordering, sample size of each group was increased to 11. This allowed for additional burs to be used for negative and positive control groups.

Sample Preparation

Extracted human teeth were obtained from the Periodontics Department at Tingay Dental Clinic, Fort Gordon, GA. Teeth were sterilized and stored in a 1:10 solution of sodium hypochlorite for a period of 24 hours. The teeth were transferred into a container containing 0.9% saline solution at room temperature until they were used. Bur samples were created using fine and coarse diamond burs from Alpen Diamond Instruments (Coltene/Whaledent, Altstatten, Switzerland). These burs have 510(k) clearance from the FDA to be used multiple times, if the manufacturer's instructions[25] for reprocessing are followed.

Samples were divided into 6 groups: positive control (n=3), negative control (n=3), fine diamond bur with manufacturer's protocol for reprocessing (n=11), coarse diamond bur with manufacturer's protocol for reprocessing (n=11), fine diamond bur with non-thermal gas plasma protocol (n=11), and course diamond bur with non-thermal gas plasma protocol for reprocessing (n=11).

All burs, except for the negative controls, were soiled by running the burs against the sterile extracted teeth for a 30 second time period in which 45 strokes against the teeth were completed using a high speed hand instrument (Star 4330 SWL Torque Fiber-Optic

Handpiece, Star Dental Supply, Compton, CA) using sterile water as an irrigant. After soiling, the negative and positive control samples were packaged for sterilization in Kerr Peelvue Red pouches (Kerr Dental, Brea, CA) with class 5 indicator strips (Comply SteriGage, 3M Health Care, St. Paul, MN) for 30 minutes at a maximum of 270 degrees F under 29psi of pressure. The manufacturers protocol groups were treated as prescribed by the manufacturer's instructions as follows: (1) placed in an ultrasonic bath treated with VibraKleen E^2 enzymatic cleaning tablets (Kulzer, South Bend, IN) for 10 minutes, (2) bur surfaces were scrubbed with a nylon bristled brush for 2 minutes, and then (3) rinsed with cold water for two minutes. Following cleaning, burs were autoclaved in the same manner as the control burs. The non-thermal gas plasma groups were treated in the same manner initially as the manufacturer's protocol group. However, prior to sterilization through the autoclave, each bur was subjected to treatment in a plasma cleaning unit (PDC-001-HP with PDC-FMG flow meter, Herrick Plasma, Ithaca, NY) for 15 minutes using a argon/oxygen gas mixture of 9% oxygen and 94% argon at a pressure of 450 mtorr. Following treatment in the plasma cleaner, the burs were sterilized by autoclave in the same manner as all other groups.

All samples were then stained using Crystal Violet stain for 3 minutes. After this period the burs were rinsed with tap water. The Crystal Violet stained burs were placed in 300 µL of 10% acetic acid solution to allow the bound Crystal Violet stain to solubilize for 24 hours. 200 µL of the resulting solution from each sample was pipetted into a 96 well culture plate and read with a BioTek Synergy2 microplate reader (BioTek Instruments, Inc., Winooski, VT, USA), measuring the optical density of the solution at a wavelength of 450 nm. Results from the microplate reader are found in Tables 1-1 to 1-4 (fine) and Tables 2-1 to 2-4 (coarse). To account for the OD of the acetic acid in a blank well, twenty-three wells filled

with 200 µL 10% acetic acid were read (no lid) to provide a blank value for the plate reader. The value was subtracted from all raw test OD values to correct for any absorption in the wavelength due to the microplate. In addition, statistical analyses was completed using the negative control corrected values. Two different tests were used to test for the normality of the distribution of the values within each treatment group: the D'Agostino & Pearson omnibus normality test and the Shapiro-Wilk test. Skewness and kurtosis were also measured. For datasets with too few values for distribution analysis, normality was assumed. Sidak's multiple comparisons test was used for post-hoc testing of two-way ANOVA results with an Alpha 0.05.

CHAPTER 4: RESULTS

The negative control values were significantly different from zero for both the fine and coarse burs (one-sample t-test, p=0.020 and 0.002 respectively). That is, the negative controls both showed a significant level of OD 450 material being removed from the burs by 10% acetic acid. Therefore, two tests of the experimental results were performed: one with values corrected by subtraction of the acetic acid blank value (0.0425) and one by subtraction of the relevant negative control values from the raw values.

ACETIC ACID CORRECTED RESULTS

The acetic acid blank corrected mean positive control values were 0.1935 ± 0.0244 (Standard Error of the Mean, SEM) for the fine bur samples and 0.2105 ± 0.0346 (SEM) for the coarse bur samples. These values were not significantly different (t-test, p=0.67).

The acetic acid blank corrected mean negative control values were 0.0168±0.0024 (SEM) for the fine burs and 0.0142 ± 0.0007 (SEM) for the coarse burs. These were not significantly different (t-test with Welch's correction for unequal SDs, p=0.14).

The acetic acid blank corrected mean experimental values for the manufacturer protocol treated burs were 0.0106±0.0010 (SEM) for the fine burs and 0.0222±0.0023 (SEM) for the coarse burs. The acetic acid blank corrected mean experimental values for the plasma-treated burs were 0.0248±0.0028 (SEM) for the fine burs and 0.0194±0.0018 (SEM) for the coarse burs. (Table 3-1)

Two-way ANOVA analysis of the four datasets and treatment showed a highly significant interaction (p=0.0002). There was no effect for the material roughness (p=0.14), but a highly significant effect for treatment (p=0.010). A comparison of all group means showed significant differences between the manufacturer and plasma cleaning for the fine bur (0.0106 versus 0.0248, p=0.0001), between the fine and the coarse burs cleaned by the manufacturer's method (0.0106 versus 0.0222, p=0.002), and between the fine burs cleaned by the manufacturer's method and the coarse burs cleaned by Plasma (0.0106 versus 0.0194, p=0.030). All other comparisons did not show a significant difference ($p \geq 0.39$). For comparisons within the coarse bur group, there was no significant difference between the two cleaning methods (p=0.58), but there was a difference in the fine bur group between the methods ($p < 0.0001$), with plasma cleaning giving a higher OD 450, consistent with an increased retention of bioburden on the bur surface.

For comparisons within methods, there was not a significant difference in OD 450 between fine and coarse burs with plasma cleaning (p=0.15). However, the manufacturer

cleaning protocol showed a significant difference in material retained on the coarse burs when compared to using the same technique on the fine burs ($p=0.0007$).

Collectively, these results indicate that the manufacturer's method for cleaning fine burs lead to significantly less material retained on the bur surface when compared to plasma cleaning of the same bur roughness, and for either cleaning method with coarse burs. (Graph 1-1)

NEGATIVE CONTROL CORRECTED RESULTS

The control corrected mean experimental values for the manufacturer protocol treated burs were -0.0062 ± 0.0010 (SEM) for the fine burs and 0.0081 ± 0.0023 (SEM) for the course burs. The control corrected mean experimental values for the plasma-treated burs were 0.0079 ± 0.0028 (SEM) for the fine burs and 0.0052 ± 0.0018 (SEM) for the coarse burs. (Table 3-2)

All four treatment groups gave mean values significantly different from zero ($p \leq 0.019$). However, for the fine bur cleaned by the manufacturer's method, the value (-0.0062) was less than zero ($p=0.0001$). This may be the result of the lower n value for the control group ($n=3$) giving a slight overestimate of the mean value. This would suggest that the manufacturer's method is more efficient than plasma cleaning for fine burs (0.0079 ± 0.0028), where significantly more material was retained than with acetic acid alone ($p=0.019$).

A two-way ANOVA of the control corrected values gave a different result when compared to the acetic acid corrected values. There was a significant interaction ($p=0.0002$) and the treatment gave a significant effect ($p=0.010$), but now the roughness also showed a significant effect ($p=0.009$). For the fine bur, there was a significant difference in the effect of cleaning method, with the plasma cleaning protocol retaining significantly more material

on the bur surface when compared with the manufacturer's method (p=0.0001). When compared to fine burs, the manufacturer's protocol with coarse burs showed a significant difference of material retained, with coarse retaining more debris (p=0.00010). Similarly, the coarse burs cleaned with the plasma protocol showed more debris retained when compared with the fine bur cleaned using the manufacturer's method (p=0.002). No other comparisons showed a significant difference ($p \geq 0.92$). (Graph 1-2, Graph 1-3 shows all data points for negative control corrected data).

Using the negative control corrected results, the percent of bioburden removed for each method was calculated using the following formula: % removal = 100*(mean positive-mean experimental)/mean positive. Subtracting the mean fine negative (0.059333333) from the mean fine positive (0.236) gives 0.177. The fine manufacturer and fine plasma groups were -0.0062 and 0.00794 respectively, giving cleaning of 102.7% and 96.6%.

Subtracting the mean coarse negative (0.056666667) from the mean coarse positive (0.252) gives 0.196. The coarse manufacturer and coarse plasma groups were 0.008061 and 0.005242 respectively, giving cleaning of 96.8% and 97.9%. (Table 3.2)

CHAPTER 5: DISCUSSION

Infection control processes in the medical and dental field continues to be a major area of focus, especially in the Military Healthcare System (MHS). This area of focus is important due to the potential transferring of communicable disease in a health care setting. Dental providers, assistants, and patients are always at risk of transferring disease during treatment if extreme care is not taken. In the MHS, lapses in infection control are tracked and reported as sentinel events. In a period from 2013 to 2016, dentistry accounted for 23.4% of total MHS

Sentinel Events (SE). Within all SEs, product or device events accounted for 10%.[26] This percentage includes lapses in infection control protocol.

Infection control lapses are not only found in military healthcare systems. In 2016, the Tomah VA Medical Center discovered a breach in infection control protocol which potentially exposed nearly 600 veterans who received dental care from the facility. The risks from this breach was the potential inoculation with various viruses that could have included Hepatitis B, Hepatitis C and HIV. Not only does this erode public confidence in the institution, but it would incur a cost associated with personnel having to review records and track down patients who could have been infected. It also consumes clinic time with appointments for screening for these diseases, when others could be treated in that same appointment time.[27]

Unlike public institutions, where sterilization practices are usually an entire section of a department, private practice providers are responsible for their own sterilization protocol adherence. While not as potentially high volume as a public institution, these private practices can also have infection control lapses in the same manner with the same outcomes. In 2019, two clinics in the Seattle, Washington area had safety breaches. These breaches affected roughly 1,300 school age children.[28] In this case, the dental instruments were only being wiped with a disinfection agent and did not undergo steam autoclave sterilization, but the financial, time, and psychological effects were the same as the VA breach.

With sterilization of dental instruments being an important piece of the treatment puzzle, there is one questions that must be asked, especially within the scope of this study: Can dental instruments be truly decontaminated? Currently, there is no literature that shows the direct infection of a patient with disease from a previously used dental instrument that has

undergone sterilization.[29] This lack of evidence does not mean that it cannot happen, but that the literature is sparse on the issue.

This current study has come to some of the same conclusions as prior studies on the issue of the ability to clean dental burs of all contaminants, specifically diamond burs. Sajjanshetty et at[7] found that, regardless of the method used to clean diamond dental burs, bacteria remained on the surface of the dental bur when cultured.[7] Their study found that autoclaving the burs alone reduced bacterial load by 80%, but they did not follow any manufacturer protocols and only scrubbed their samples with detergent under running water prior to autoclaving. In a related systematic review, Bidra et al reviewed literature regarding the ability to sterilize and reuse implant abutments and cover screws.[30] They found that there was limited literature discussing the issue. Within the literature that was met their inclusion criteria, some studies that indicated that current methods of sterilization did not remove all contaminants and others found that adding adjuncts to the cleaning process could result in adequate decontamination. The overall recommendation from that systematic review was to adhere to a single use policy until the body of evidence was stronger. The current study combined ultrasonication in enzymatic solution with autoclave sterilization, and might show an improved reduction in bacterial load, however this hypothesis was not tested.

With regard to the issue of virus inactivation, Leotinous et al found that using chlorine containing compounds to treat dental burs did inactivate the hepatitis B virus.[31] Other disinfectants tests required an additional step of ultrasonication to be 100 % effective. Following the manufacturer's method for reprocessing dental burs with an approved enzymatic cleaner/disinfectant seems to be effective in eliminating viruses, but this was not tested in the current study.

All experimental groups in this study, regardless of cleaning method, retained some degree of bioburden, with exception of the fine diamond bur groups cleaned utilizing the manufacturer's protocol. This is evident in the fine manufacturer and fine plasma groups cleaned at a percentage of 102.7% and 96.6%, respectively, while the coarse manufacturer and coarse plasma samples cleaned at a percentage of 96.8% and 97.9%, respectively.

As discussed in the results section, there was an outlier in the fine plasma group when the data set was analyzed for skewness. This data point was included in the ANOVA due to the nature of the statistical analysis that assumes all samples are within the normal population. Increasing the sample sizes for the negative controls as well as the experimental groups could add to the power of the analysis, even though a power analysis was completed. However, this did not seem to affect the coarse bur samples. The samples were prepared by the same operator over a period of two days. Ideally the samples would have been prepared in one day with the same conditions, however, as the samples were prepared by hand, variations of pressure applied to the extracted tooth by the operator could still vary.

Inherent in the gas plasma process is the risk of etching the surface of the object being treated. Although this was taken into consideration with the gases chosen and the ratio of the gases used, there is the possibility that the plasma treatment etched the metallic surface of the fine burs and changed the topographical nature of the bur. If this did occur, the fine bur samples treated by the gas plasma might have retained more of the Crystal Violet. It was shown in the negative control samples of all bur types that some Crystal Violet was captured and not completely removed by the water rinse prior to addition of the 10% acetic acid solution. A follow on examination using a scanning electron microscope comparing the surface of the diamond burs before and after plasma treatment might elucidate this further.

CHAPTER 6: CONCLUSION

For the acetic acid blank corrected values, the fine burs cleaned by the manufacturer's method showed the lowest retention of bio-burden after treatment. When the control corrected values were tested, the fine bur cleaned by the manufacturer's method retained less material than with the acetic acid negative control alone. Overall, plasma cleaning gave the most consistent results, although not significantly better than the manufacturer's method for coarse burs. Based on these findings, the null hypothsis was accepted. Regardless of method used to treat the burs, all burs retained some amount of dentin bioburden and may lend credence to the single use policy adopted by various institutional programs. More studies are needed to determine if there is harm to the patient in the re-use of dental burs. Additionally, more studies may be indicated for the use of non-thermal gas plasma in the sterilzation process of dental burs as the current study used a pressure on the lower end of the spectrum of available pressures used with the plasma cleaning unit and all samples were cleaned for a set time which may be altered to increase the effectiveness of the non-thermal gas in contact with the surface of the bur.

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TABLES

Table 1-1. *Fine Sample – positive control.*

Positive Control	Value
+C1F	0.245
+C2F	0.19
+C3F	0.273
AVG	0.236

Table 1-2. *Fine Sample – negative control.*

Negative Control	Value
-C1F	0.064
-C2F	0.056
-C3F	0.058
AVG	0.059333

Table 1-3. *Manufacturer's Protocol Samples – Fine.*

Manufacturer Processed	Value
M1F	0.049
M2F	0.051
M3F	0.05
M4F	0.054
M5F	0.053
M6F	0.053
M7F	0.056
M8F	0.061
M9F	0.055
M10F	0.052
M11F	0.05
AVG	0.053091

Table 1-4. *Plasma Protocol Samples – Fine.*

Plasma Processed	Value
P1F	0.072
P2F	0.058
P3F	0.091
P4F	0.069
P5F	0.063
P6F	0.062
P7F	0.065
P8F	0.063
P9F	0.061
P10F	0.075
P11F	0.061
AVG	0.067273

Table 2-1. *Coarse Sample – positive control.*

Positive Control	Value
+C1C	0.215
+C2C	0.222
+C3C	0.322
AVG	0.253

Table 2-2. *Coarse Sample – negative control.*

Negative Control	Value
-C1C	0.056
-C2C	0.056
-C3C	0.058
AVG	0.056667

Table 2-3. *Manufacturer's Protocol Samples – Coarse.*

Manufacturer Processed	Value
M1C	0.064
M2C	0.071
M3C	0.059
M4C	0.073
M5C	0.049
M6C	0.065
M7C	0.059
M8C	0.059
M9C	0.072
M10C	0.072
M11C	0.069
AVG	0.064727

Table 2-4. *Plasma Protocol Samples – Coarse.*

Plasma Processed	Value
P1C	0.063
P2C	0.064
P3C	0.062
P4C	0.059
P5C	0.053
P6C	0.066
P7C	0.069
P8C	0.057
P9C	0.06
P10C	0.073
P11C	0.055
AVG	0.061909

Table 3-1. *Statistical analyses for acetic acid solution correction.*

Sample Group	AVG OD ± SEM[1]
Acetic acid solution OD	0.0425 ± 0.0013[2]
Blank corrected positive control (fine)	0.1935 ± 0.0244
Blank corrected positive control (coarse)	0.2105 ± 0.0346
Blank corrected negative control (fine)	0.0168 ± 0.0024
Blank corrected negative control (coarse)	0.0142 ± 0.0007
Manufacturer treated (fine)	0.0106 ± 0.0010
Manufacturer treated (coarse)	0.0222 ± 0.0023
Plasma-treated (fine)*	0.0248 ± 0.0028
Plasma-treated (coarse)	0.0194 ± 0.0018

[1] Standard error of the mean
[2] Range is Standard deviation
*outlier identified using ROUT test. Retained for ANOVA analyses

Table 3-2. *Statistical analyses for negative control correction.*

Sample Group	AVG OD ± SEM[1]
Control corrected manufacturer treated (fine)	-0.0062 ± 0.0010
Control corrected manufacturer treated (coarse)	0.0081 ± 0.0028
Control corrected plasma-treated (fine)*	0.0079 ± 0.0028
Control corrected plasma-treated (coarse)	0.0052 ± 0.0018

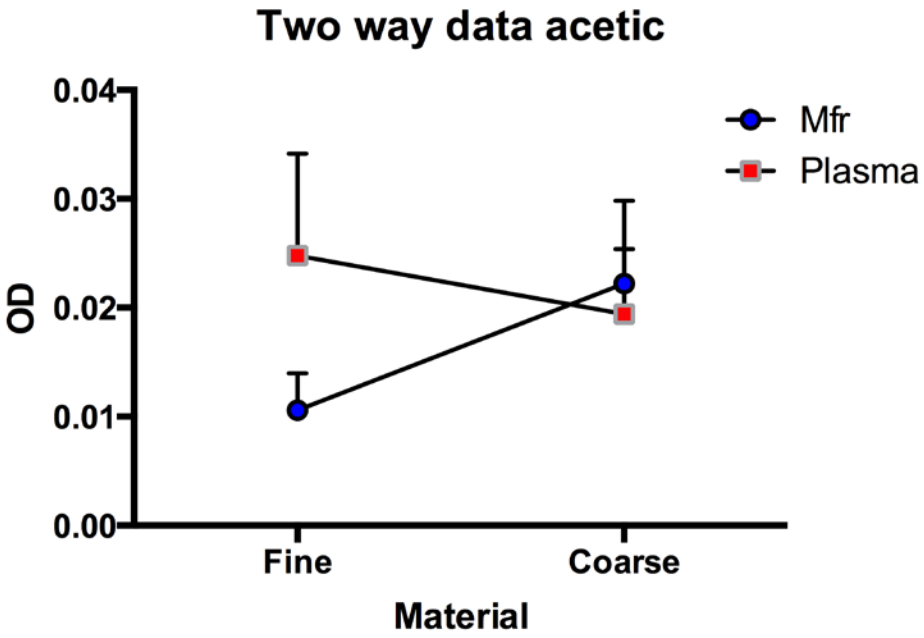
[1] Standard error of the mean
*outlier identified using ROUT test. Retained for ANOVA analyses

Table 3-2. *Percent of debris removed with negative control correction average.*

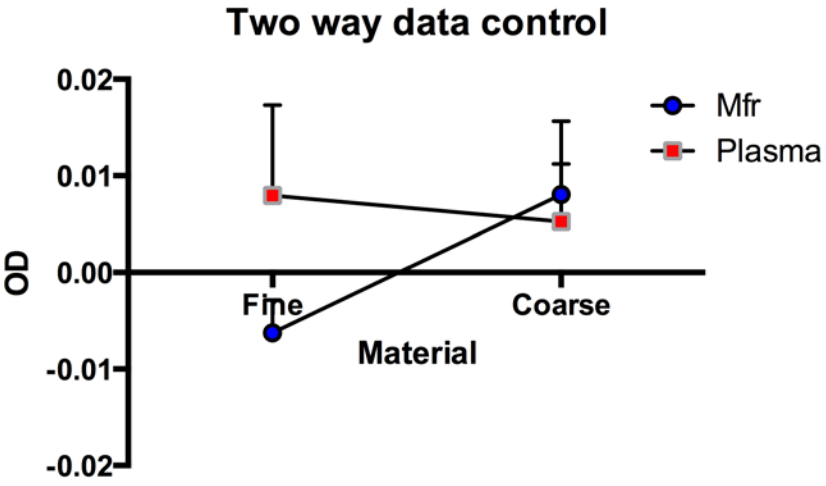
Sample Group	Percent Removal
Manufacturer fine	102.70%
Plasma fine	96.60%
Manufacturer coarse	96.80%
Plasma coarse	97.90%

GRAPHS

Graph 1-1. *Results of ANOVA analysis for acetic acid corrected results*

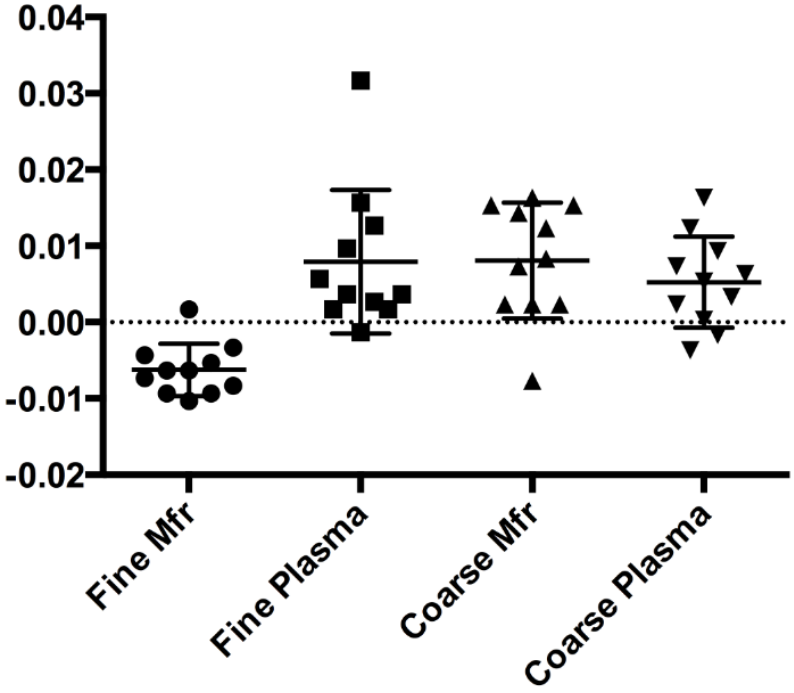


Graph 1-2. *Results of ANOVA analysis for negative control corrected results*



Graph 1-3. *Scatter plot of data after negative control correction*

Fine and Coarse data control corrected



FIGURES

Figure 1-2. *Representative image of positive control coarse bur sample (8x magnification).*

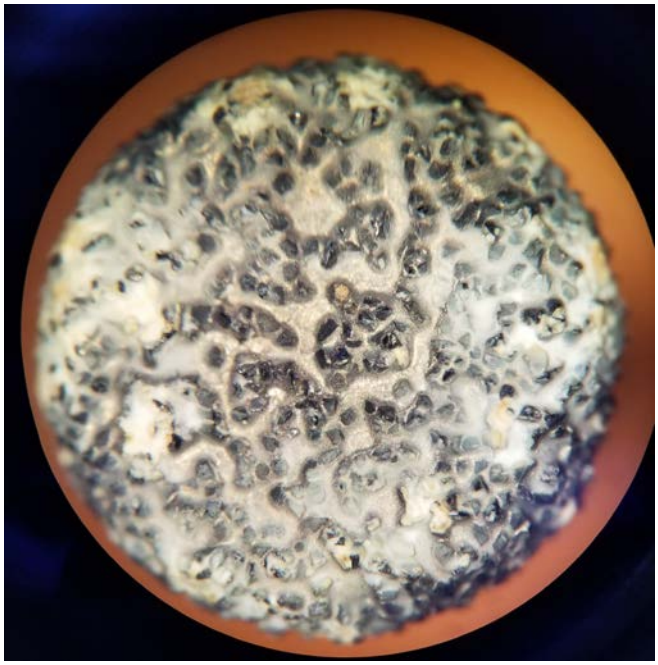


Figure 1-2. *Sample holder in chamber of Plasma Cleaner.*

Figure 1-3. *Plasma Cleaner unit in function.*

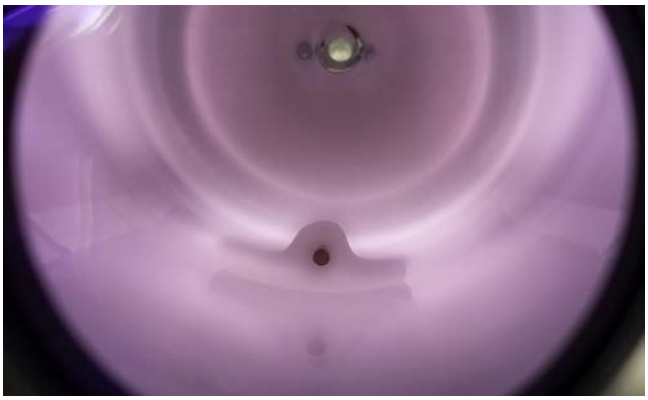


Figure 1-4. *Harrick Plasma PlasmaFlo unit (top) with Plasma Cleaner (bottom).*

Figure 1-5. BioTek Synergy 2 microplate reader used.

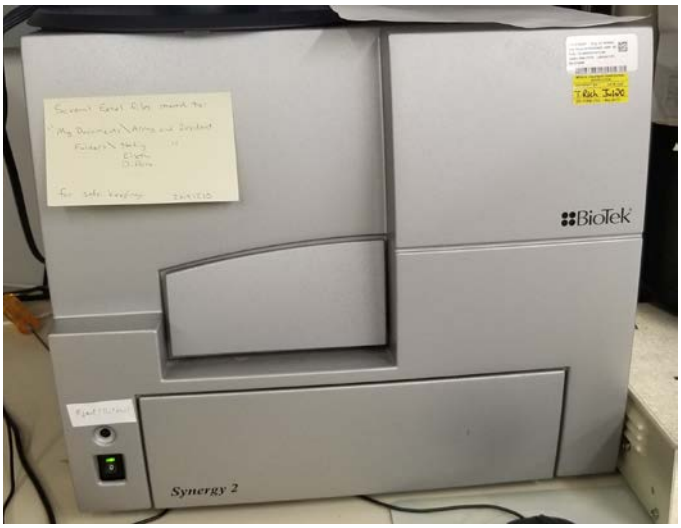


Figure 1-6. *Microplate used after pipetting sample solution.*