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Effectiveness of Plasma Sterilization for Dental Implant Drills

by

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Thesis submitted to the Faculty of the
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ABSTRACT

Evaluating the Use of Plasma Treatment in the Process of Sterilization of Dental Implant Drills

Daniel Bruin, MS, 2021

Thesis directed by: Robert W. Herold DDS, MS; COL, DC, USA, Assistant Director, Periodontics Residency

Objective: This study aims to evaluate the use of a plasma cleaner (Harrick Plasma) for the removal of bioburden from dental implant drills utilizing a manufacturer based and clinically reproducible decontamination protocol.

Materials and Methods: Four experimental groups of 15 burs each were used to evaluate reprocessing protocols (Figure 1). Group 1 was removed and evaluated straight from the package as a clean control. Group 2 was processed through the decontamination and sterilization protocol as a protocol control. Groups 3 and 4 were contaminated by drilling to depth in the inferior border of a pig mandible (Sus scrufa). After routine processing, Group 3 was treated in a plasma cleaner with a flow-meter. A combination of Oxygen and Argon (5% and 95% respectively) with RF excitation at a power level of 30W was used and a flow rate of approximately 70.8 ml/min at 10 psi. Group 4 was treated with the traditional decontamination and sterilization protocol. Two additional groups of three burs each were used as positive controls, Groups 5 and 6. Group 5 was treated with a fixative agent, stained and evaluated immediately after contamination. After contamination, Group 6 was debrided with a moist 2x2 before being treated with a fixative agent, stained and evaluated. Evaluation of all groups was done with a stereomicroscope at 50x magnification.

Results: The only group that demonstrated no contamination was Group 1. A single bur from the protocol control group, Group 2, demonstrated one area that appeared to be contaminated. This area was much smaller than areas seen on the burs from the contaminated groups but suggests cross contamination from the decontamination step. Elemental analysis was not performed to confirm composition of the contaminants. The treatment groups were not statistically significant as assessed by the Chi Square test ($P = 0.3059$). The Kruskall-Wallis test was used to determine if the median contamination values were different between the treatment groups. There was no difference in the median contamination scores between control, plasma, and autoclave treatment methods ($P = 0.3141$).

Conclusions: Our results were consistent with the findings of previous studies that demonstrated residual debris on previously used dental instruments. The results of this study were inconsistent with studies using plasma to remove organic debris from inert surfaces. It was evident, as others have noted, that the initial cleaning step to remove the gross amount of debris may be the most important step. Future research should focus on

altering wattage, time, and/or gas composition in order to utilize plasma cleaning as part of an instrument reprocessing protocol.

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CHAPTER 1: Introduction

Case reports in the 21st century United States have demonstrated the transmission of hepatitis B from dental treatment with no evidence of a breach of standard infection control protocols.[1,2] Current methods for decontamination and sterilization of dental instruments consistently result in incomplete removal of bioburden.[3-9] The presence of bioburden has brought into question the safety of reusing instruments, although microbial viability is generally not in question. Multiple studies suggest that the presence of bioburden doesn't affect the ability to sterilize equipment.[4,10-12] In response to concerns with bioburden transmission, the Department of Veterans Affairs (VA) and the Department of Defense (DOD) healthcare facilities have adopted a single use policy for dental implant drills citing the risk of transfer of blood borne pathogens and decreased exposure risk to personnel during handling and reprocessing of contaminated instruments.[13] Dental implant drills have been shown to be effective for as many as 100 uses without significant increases in heat generation.[14-18] The ability to safely reuse dental implant drills has significant financial and ecological implications

Plasma cleaning has been shown to remove organic debris from sensitive surfaces without significant heat generation above ambient temperature.[19-22] The aim of this study is to evaluate the use of plasma cleaning as part of a routine sterilization process to remove bioburden from dental implant drills. In addition to a conventional reprocessing protocol, in one group of burs, we added an Oxygen/Argon plasma cleaning step to evaluate its effectiveness on the removal of bioburden from dental implant drills.

CHAPTER 2: Materials and Methods

Four experimental groups of 15 burs each were used to evaluate reprocessing protocols. Group 1 was evaluated straight from the package as a clean control. Group 2 was processed through the decontamination and sterilization protocol as a protocol control (Ultrasonic, Washer-Disinfector, and Steam Autoclave). Groups 3 and 4 were contaminated by drilling to depth in the inferior border of a pig mandible (Sus scrufa) that was degloved with a #10 scalpel and a Prichard elevator. Group 3 was treated in a plasma cleaner with flow-meter (PDC-001-HP; Plasmaflo PDC-FMG; Harrick Plasma; Ithaca, NY) in addition to the traditional processing protocol (Ultrasonic, Washer-Disinfector, plasma cleaner, Steam Autoclave). Group 4 was treated with the standard decontamination and sterilization protocol (Ultrasonic, Washer-Disinfector, and Steam Autoclave).

Two groups of three burs each were used as positive controls, Groups 5 and 6. Group 5 was treated with a fixative agent, stained and evaluated immediately after contamination (Figure 1). After contamination, Group 6 was debrided with a moist 2x2 before being treated with fixative, stained and evaluated.

DRILLING PROTOCOL – TEST GROUPS (3 AND 4):

Groups 3 and 4 were contaminated by drilling into the inferior border of a porcine mandible. Drilling was completed using an ImplantMED surgical unit (W&H; Burmoos, Austria) using a 20:1 latch handpiece at 1500 RPM and copious sterile water irrigation. Each drill was grossly debrided with a fresh 2x2 gauze moistened with sterile saline and then placed in a beaker of 0.9% Sodium Chloride. The lumen of each drill was

individually flushed by attaching it to the irrigation tubing and the bur was placed in an Eppendorf tube. The tube was labeled by group number and bur number. Once in the Eppendorf tubes, the burs were Sprayed with PRE-Klenz Instrument transport gel (Steris Corporation; Mentor, OH) and the outside of Eppendorf tubes cleaned with Caviwipes (Metrex; Orange, CA) in preparation for transportation in a biohazard bag.

DRILLING PROTOCOL – POSITIVE CONTROLS:

Groups 5 and 6 were contaminated by drilling into the inferior border of the porcine mandible to act as positive controls. No irrigation was used and no attempt was made to flush the lumen in either group. Group 5 was immediately subjected to the staining protocol (See below). The burs of group 6 were wiped with fresh 2x2 woven gauze moistened with sterile saline to grossly debride the bur and then subjected to the staining protocol.

DECONTAMINATION:

Groups 2, 3, and 4 were all subjected to the same decontamination protocol. Group 2 was removed from the package and immediately placed in the ultrasonic washer. The burs of Groups 3 and 4 were removed from the Eppendorf tubes and individually rinsed with tap water to remove the PreKlenz solution. The burs were then placed in the ultrasonic cleaner (Maxisweep S3100; Henry Schein; Mellville, NY) with fresh solution (Prosonic General Purpose; Sultan Healthcare; York, PA) for 15 minutes in a basket. The Groups were run individually and the solution was made fresh for every group to avoid dilution of the enzyme and maximize efficacy.

The function of the ultrasonic cleaner was verified by Sonocheck ultrasonic cleaner monitors (Healthmark; Fraser, MI) prior to and subsequent to use. The baskets were then rinsed with tap water and placed in the automated washer-disinfector (Steris Amsco; Model #5052; Mentor, OH) using enzymatic cleaner, non-silicone lubricant, and neutral detergent chemicals (Steris; Mentor, OH). The automated Washer-Disinfector function was verified by TOSI test (Healthmark; Fraser, MI) before and after use. After washing, the burs of Group 2 (Processing control) and Group 4 (Autoclave Control) were placed individually in pill packs labeled with group number and bur number with heat indicating tape and a class 5 indicator (3M; St. Paul, MN). Groups 2 and 4 were then submitted to traditional steam sterilization (Steris Amsco; Century V-120 Prevac Steam Sterilizer; Mentor, OH). Group 3 was placed in pill packs labeled with group number and bur number and heat indicating tape for transportation in a biohazard bag.

PLASMA TREATMENT:

Group 3 was subjected to plasma treatment for 30 minutes. Validation of the plasma sterilizer in this study was done by use of a fluorescent light bulb in the empty chamber, per manufacturer instructions. The bulb was placed in the empty chamber with gloved hands and plasma activated. When the machine is working, the bulb lights up.

After confirmation, the samples were removed from pill packs with sterile cotton pliers and placed in the reaction chamber of the plasma cleaner in groups of 7 or 8. The door was closed and the chamber evacuated. The processing gas (95% Argon, 5% Oxygen; 40mmHg:2mmHg on the flow meter) was bled into the chamber with the 3-way valve. Vacuum was regulated using the needle valve and was maintained between 690-

710 mTorr.[23] Plasma was activated with the radio frequency (RF) generator on "Hi" (29.6 Watts) for the 30 minute cycle. After the cycle, the process gas was turned off at the regulator. The vacuum pump was turned off and the 3-way valve opened to vent the chamber. Burs were removed from the chamber with sterile cotton pliers and placed in pill packs with a class 5 indicator and submitted to traditional steam sterilization. The traditional steam sterilization step is necessary for individually wrapping the burs for storage since the integrity of the pill-packs is compromised by the plasma cleaning and the plasma may not form as effectively inside of the packages.[19]

CHAPTER 3: Data Analysis

STAINING:

After steam sterilization, burs of Groups 2, 3, and 4 were individually removed from their packages with cotton pliers and placed in fixative (Citrate: Acetone: Formaldehyde; 25:65:8) in a fresh Eppendorf tube for at least 30 seconds. They were then transferred with cotton pliers and placed in 1% Crystal Violet in an Eppendorf tube for 3 minutes. After staining, they were rinsed with tap water for 15 seconds and placed in a fresh Eppendorf tube labeled with group and bur number and sealed for evaluation.

EVALUATION:

The burs were evaluated and photographed one at a time in five areas on each bur using a stereo microscope equipped with an integrated camera (Stemi 508; Axiocam ICc5; Axiovision; Carl Zeiss AG; Oberkochen, Germany). The 5 areas evaluated were: The tip, lumen, 11.5mm depth indicating groove and two random spots in the lands and/or grooves. Evaluation was done at 50x magnification. Areas of contamination were also photographed at lesser magnification, 12.5x, to document gross contamination. Each area of evaluation was given a score of "0" if no debris was visualized, or "1" if contamination was noted for a total of 5 points per bur or 75 points per group. The burs were also given a "0" or "1" score for the entire bur for a total of 15 points per group.

RESULTS (TABLE 1):

Group 1 (Figure 2) had no debris as detected by the Crystal Violet staining protocol. It was the only group with no debris. Group 2 (Figure 3) had one bur that demonstrated one relatively small area of stain, suggesting the possibility of cross contamination during the processing procedure. This area was smaller than areas of contamination seen in the other groups. This could be due to cross contamination from the decontamination steps or a defect in the surface of the bur resulting in diffuse reflection. SEM analysis of the site was not performed and therefore the composition can't be confirmed. Groups 3 (Figure 4) and 4 (Figure 5) demonstrated multiple areas of debris. There was no statistical significance in the quantity of debris between the treatment groups. Group 5 (Figure 6), stained directly after contamination, was so debris laden that there was no visualization of small anatomical features of the burs and in some areas debris was so thick that it flaked off en masse. Group 6 (Figure 7) was contaminated the same way Group 5 was, but was immediately wiped with moist gauze. Those burs demonstrated a very clean appearance, with the exception of the lumen which was consistently obturated.

STATISTICAL ANALYSIS:

Groups were compared using the Chi Square test and Kruskall-Wallis test. The Chi Square test was used to determine if the proportion of samples that were contaminated are different between the treatment groups. The negative control was not analyzed. The treatment groups were not statistically significant as assessed by the Chi Square test ($P = 0.3059$). The Kruskall-Wallis test was used to determine if the median contamination values were different between the treatment groups. This non-parametric

test was chosen as the data are categorical and not normally distributed. There was no difference in the median contamination scores between control, plasma, and autoclave treatment methods ($P = 0.3141$). Additionally, there was no difference when all 5 contamination sites were analyzed independently, with a total of 75 data points per group ($P = 0.1869$).

Chapter 4: Discussion

In Europe, prion transfer between patients is of great concern and research has demonstrated in animal models that dental tissues can harbor transmissible prion proteins. The studies showed that prion transmission is possible in animals from infected endodontic files, although those instruments were not subjected to reprocessing and sterilization.[24] According to the Centers for Disease Control and Prevention (CDC), studies have found no evidence for potential iatrogenic transmission of spongiform encephalopathies via dental procedures.[25] Still, the World Health Organization suggests that single use items be utilized when possible and that re-usable items contaminated with neurovascular tissue should be destroyed.[26]

Most studies evaluating the effectiveness of standard reprocessing and sterilization procedures use endodontic files and have demonstrated that endodontic files cannot be reliably debrided,[4,5,8,27,28] although some research suggests that under ideal circumstances reprocessing procedures are able to effectively remove debris from endodontic files.[29,30] The presence of bioburden after routine processing and sterilization has brought into question the safety of reusing instruments, although microbial viability is generally not in question.[4,10,12] Some studies have demonstrated microbial growth after traditional decontamination and sterilization protocols have been carried out,[6,31] though few studies have been directly carried out on implant drills.[7,10,11]

Using dental implant drills, Price did not question sterility but demonstrated the presence of foreign material via scanning electron microscopy.[10] The nature of the material was not investigated but it was speculated that what was seen was "contamination" spots from the lubricant additive from the washer disinfector. Proff and

colleagues evaluated the disinfection and sterilization of internally cooled implant drills contaminated with blood and E. faecium and determined that "after proper sterilization, internally cooled drills do not pose an infection risk".[11] Contrarily, Parnia and colleagues studied implant drills contaminated with TC99 labeled blood and determined that no cleaning protocols were effective in removal of contaminants and suggested a single use protocol.[7] This study utilized distilled water rather than an enzymatic detergent in the ultrasonic bath and did not utilize an automated washer-disinfector which has been shown to be more effective than hand scrubbing instruments.[9,32-35] The vast majority of studies utilized the traditional steam autoclave, however several studies have shown promising results for the use of plasma cleaning for the removal of bioburden from materials like stainless steel discs, dental burs, and endodontic files.[20-22,36,37]

Plasma is not a new concept. Irving Langmuir first used the term plasma to describe the ionized form of a gas in 1927.[28] When a gas is placed under low pressure and subjected to high frequency energy, accelerated ions in the gas collide with gas molecules, causing ionization and formation of plasma. The ionized particles in the plasma remove organic contaminants from surfaces yielding carbon dioxide or methane gases via ablation. The plasma can also modify the surface characteristics of a material by chemical reaction between the gas molecules and the treated surface. The nature of the plasma/surface interaction depends on intensity and frequency of the RF power used to generate the plasma, the type of gas used as well as the pressure and the flow rate of the gases in combination with the amount of time the object is exposed to the plasma.[19,39]

The application of plasma has a history of use for cleaning contaminants off of heat and chemical susceptible devices. In medicine, combinations of Oxygen, room air, Hydrogen Peroxide, Argon, other gases and combinations thereof are used for sterilization. Halfmann et al conducted sterilization experiments on glass slides and showed that plasma treatment of less than 60 seconds rendered the slides sterile and this principle was extended to the sterilization of heat-intolerant silicone implant material.[36] Testing various gas combinations, Hauser et al. demonstrated the ability of a low pressure plasma to remove spores from silicone implant material without harm to the material.[21] The machine in that project was a large plasma reactor operating at 1000 W input power and an operating pressure of 5 Pascals for a treatment period of only 10 seconds.

Plasma has been shown to vaporize prions, endotoxin and organic debris from different surfaces.[19-22,35,36] This research seeks to evaluate the use of plasma cleaning in addition to a routine sterilization process to remove bioburden from dental implant drills using a combination of Oxygen and Argon Plasmas. The cleaning process will maximize the use of automated techniques and chemicals compliant with the Nobel processing and sterilization guidelines.[40] The process must be consistent and reproducible by a trained sterilization technician. The environmental, economic and logistical implications of predictable decontamination and sterilization allowing re-use of dental implant drills is without question.

Lerouge outlined four factors effecting the sterilization of materials using plasma: 1) The quantity, geometry, and composition of the device(s), 2) The presence of organic residue, 3) Packaging of the subject, and 4) The nature and surface density of

microorganisms.[19] In this study, every attempt was made to reduce the density of organic residue and debris with each step before application of plasma – specifically chairside debridement, keeping the burs moist during transportation, use of an enzymatic detergent in the ultrasonic and use of the automated washer disinfector. The group of burs that were subjected to plasma cleaning were packaged in standard pill packs after the plasma step. In clinical practice, burs must be packaged in a manner that allows storage until their next use. The plasma can cause degradation of the pill pack and would limit access of the plasma to the bur surface.[19]

The composition of the gas used in a plasma cleaner alters the intensity and wavelength of the UV radiation emitted. The choice of 5% O_2 in the Ar/O_2 mixture was due to the demonstration that Oxygen Plasmas are more effective than Argon but that Argon enhances UV intensity.[19,35] Stapelmann demonstrated that the etching rate of spores had a ceiling effect at 5% O_2 in an Ar/O_2 mixture. It should be acknowledged, that they used a 200W machine in contrast to the 30W machine used in this study.

Studies have repeatedly underlined the importance of gross decontamination as an initial step.[6,19,31,34,41] In a study evaluating sequential steps in cleaning contaminated instruments, Evangelista demonstrated a decrease in microbial load at each additional stage of cleaning contaminated surgical instruments without sterilization.[33] Army dental treatment facilities (DTF) are required to used automated cleaning equipment to clean instruments prior to sterilization to improve cleaning effectiveness, consistency, and decrease worker exposure to blood and other potentially infectious materials.[13] Multiple studies have shown greater efficacy and repeatability with automated equipment and procedures over manual procedures with the majority of studies favoring the automated

washer disinfector over the ultrasonic.[9,31,33,34,42-44] Studies have shown the ultrasonic to be an effective instrument to reduce the debris on burs and instruments. This study used the ultrasonic prior to the automated washer-disinfector to maximize reproducibility and debris removal prior to the application of plasma. The ultrasonic washer works by forming microscopic vacuum bubbles which progressively implode, causing cavitation, creating suction which has been shown to effectively remove debris from instruments.[29,31,42,43] Bentley recommended cycle times of 3-5 minutes for instruments, and at least 12 minutes for cassettes. In this study, the boxes that contained the group of drills were placed in the ultrasonic for 15 minutes prior to rinsing with tap water and being washed in the automated washer-disinfector. The washer-disinfector may not be feasible in a small clinic or private practice setting but is standard in DOD and VA clinics unless precluded by the nature of the clinic.[13]

When processing equipment, the possibility of cross contamination is a realistic question. Overuse of the ultrasonic solution can lead to dilution of the enzyme in the detergent.[33] This was addressed in this study by preparing fresh solution for each group of burs and maximizing chairside debridement. It was noted that one bur not contaminated by drilling in the porcine mandible demonstrated apparent contamination in the shank area (Figure 3). This area was much smaller than those of the contaminated groups but highlights the possibility of cross contamination, discussed in other studies.[32] Evaluation of that spot and comparing those known to be contaminated by SEM with elemental analysis would be important in confirming the makeup of the contaminants.

The findings of this study have been affected by a multitude of confounding variables. In an effort to make this study clinically relevant, multiple burs were placed in

the chamber simultaneously, perpendicular to the long axis of the chamber. The presence of a greater number of subjects and their orientation in the chamber could affect their access to ions in the plasma.[19] Future studies could evaluate single burs and parallel placement. Placing a single bur at a time would not be a clinically relevant experience as dental implant surgery requires multiple drills. Our results were not consistent with some other studies that demonstrated effective removal of various organic substrates from different inert surfaces.[21,22,34,35] The unit used in the current study had a maximum power of 29.6 watts, where other studies have used machines in the order of 200 or even 1000 Watts.[21,35] Perhaps the greatest finding of this study is the importance of a gross chairside decontamination step. Group 6, which was decontaminated with a moist 2x2 and then fixed/stained/evaluated looked grossly like the unused burs. This would suggest that any instrument that is to be reused should be carefully debrided immediately after use. Another takeaway was the consistent location of the debris when burs were evaluated. It was the authors' hypothesis that there would be a concentration of debris at the tip, where the cutting work was being done, and at the lumen since external irrigation was used. Perakaki noted accumulation of debris at the tip when evaluating the cleaning of endodontic files.[43] What was found was that the debris was consistently located in the 11.5mm depth groove and the flutes. Out of 8 surfaces demonstrating debris, 3 sites of debris appeared to be residual contaminated water retained in the semilunar 11.5mm depth groove as seen in Figures 4 and 5. The remaining 5 sites were in the flutes/lands which consisted of more physical proteinaceous debris as seen in Figure 4. If the goal was to reuse equipment, the design could be optimize to minimize retention of debris by physical design or incorporation of a surface coating.

Future research should seek to establish a protocol that will effectively remove debris from a single bur before extrapolating those findings to simultaneous treatment of multiple burs. This can be done by changing power settings, exposure time, gas mixtures, and by changing the number of burs in the chamber at a time. The amount of protein present after processing could be evaluated with protein recovery kits for a more accurate comparison between the Groups. The sterilization/cleaning feature of the plasma machine could be verified with stainless steel disks of simpler geometry before treating subjects with complex geometries like dental implant drills.

Chapter 5: Conclusion

This study was designed to evaluate the use of a plasma cleaner as part of a routine processing procedure to remove bioburden from contaminated dental implant drills. This study did not test for sterility. Our results were consistent with the findings of previous studies of dental burs and files that demonstrated residual debris on previously used dental instruments. With the protocol, equipment, and parameters used, we were unable to match the results of other studies using plasma to remove organic debris from inert surfaces. It was evident based on the contaminated control groups that the initial cleaning step to remove the gross amount of debris may be the most important step in a reuse protocol. Any instrument whose design does not facilitate that initial cleaning step should be considered single use.

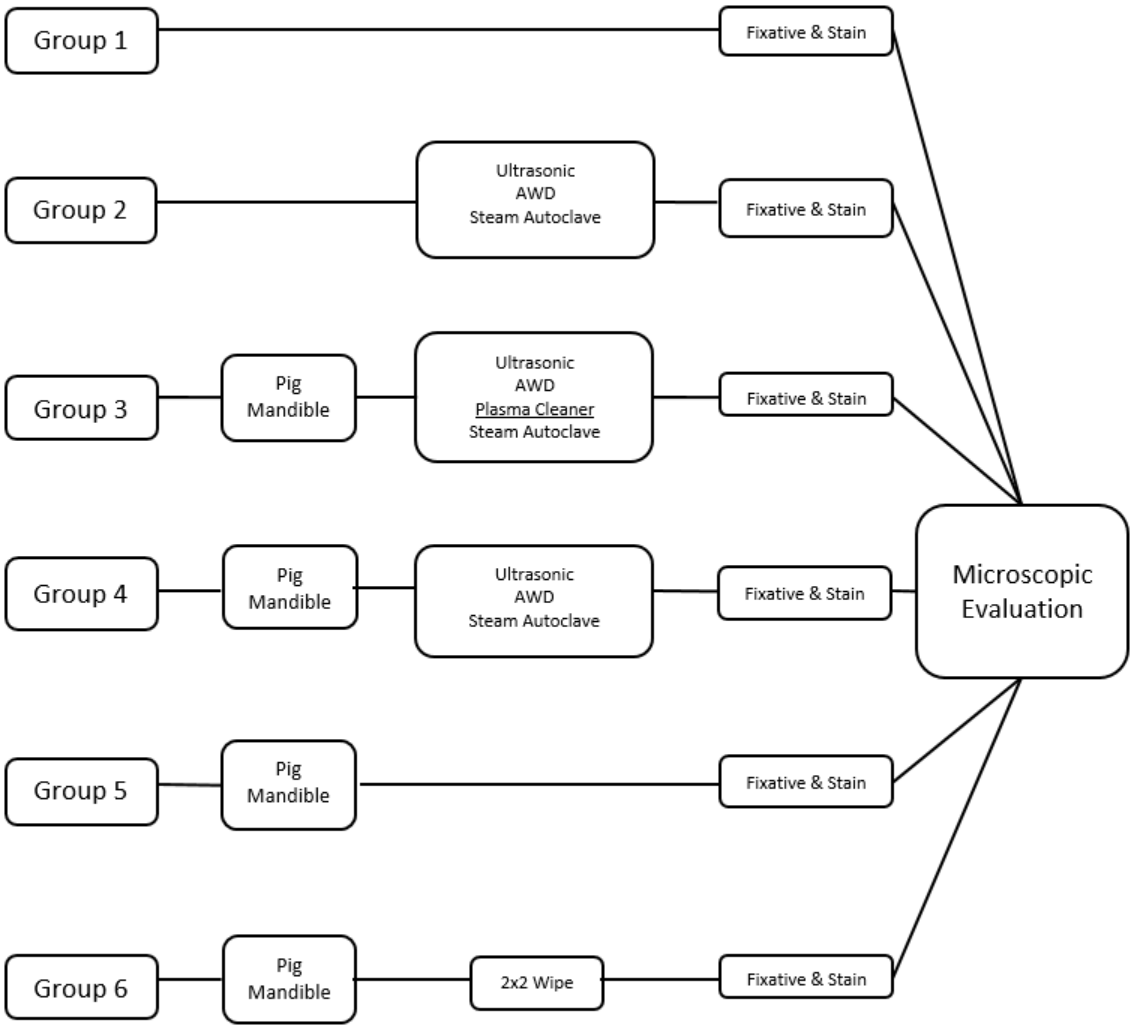

Figure 1: Flow chart of bur treatment Groups. (AWD: Automated Washer/Disinfector)

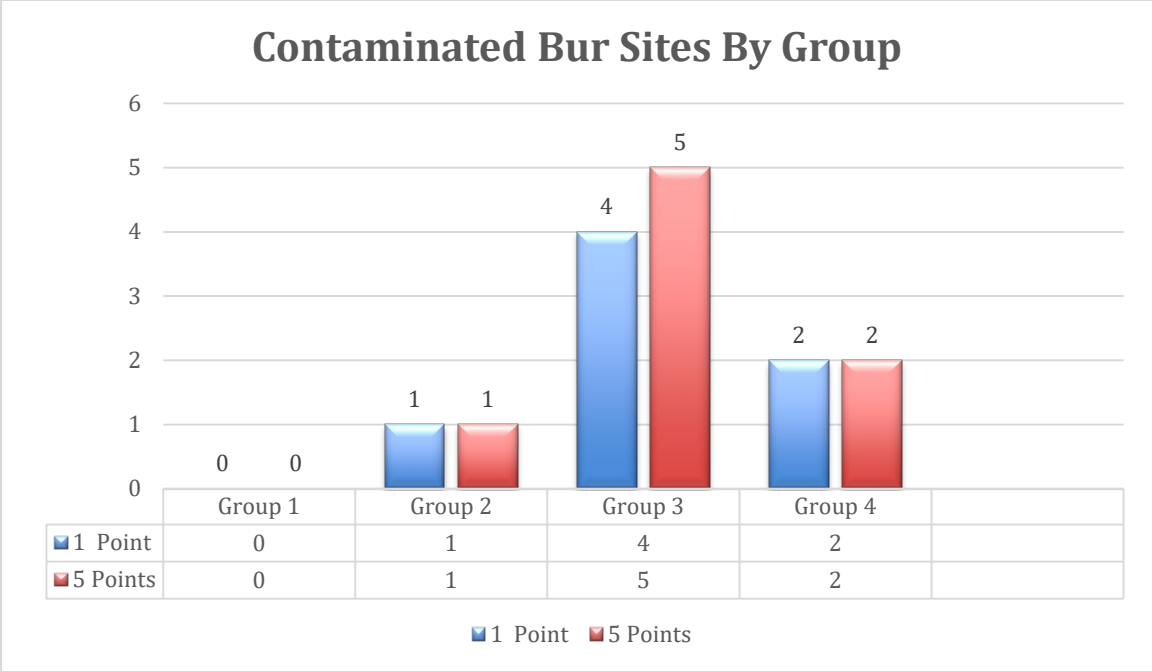

Figure 2: Graphical representation of contaminated burs when evaluated at the bur level (1 point per bur) or site level (5 sites per bur)

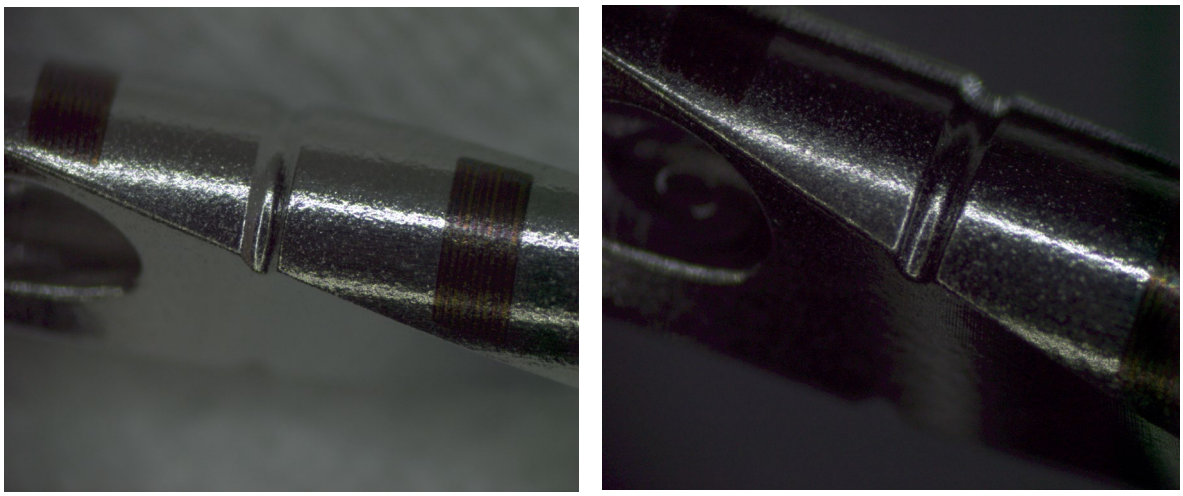

Figure 3: A representative photograph of Group 1 burs. Magnification 50x.

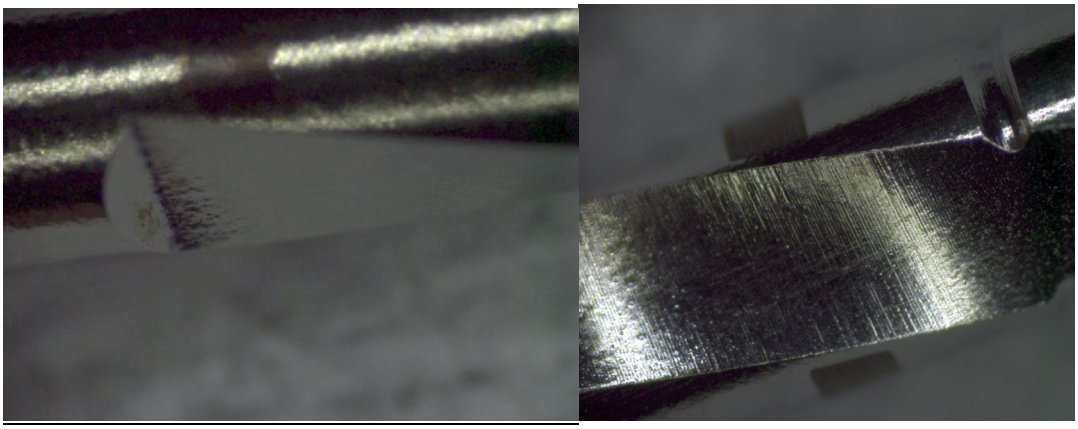

Figure 4: A representative photograph of Group 2 Burs. Magnification 50x.

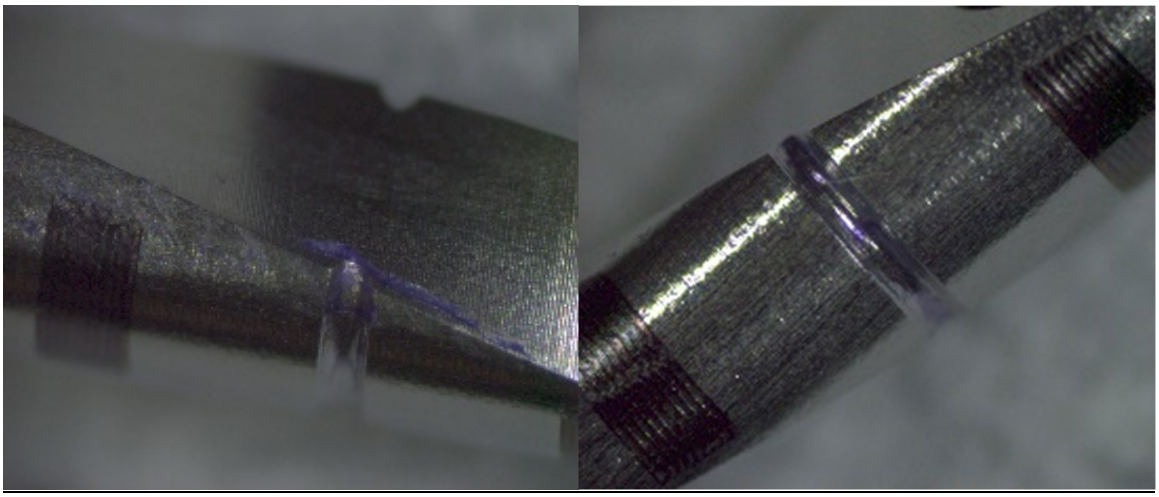

Figure 5: Representative photographs of Group 3 Burs. Magnification 50x.

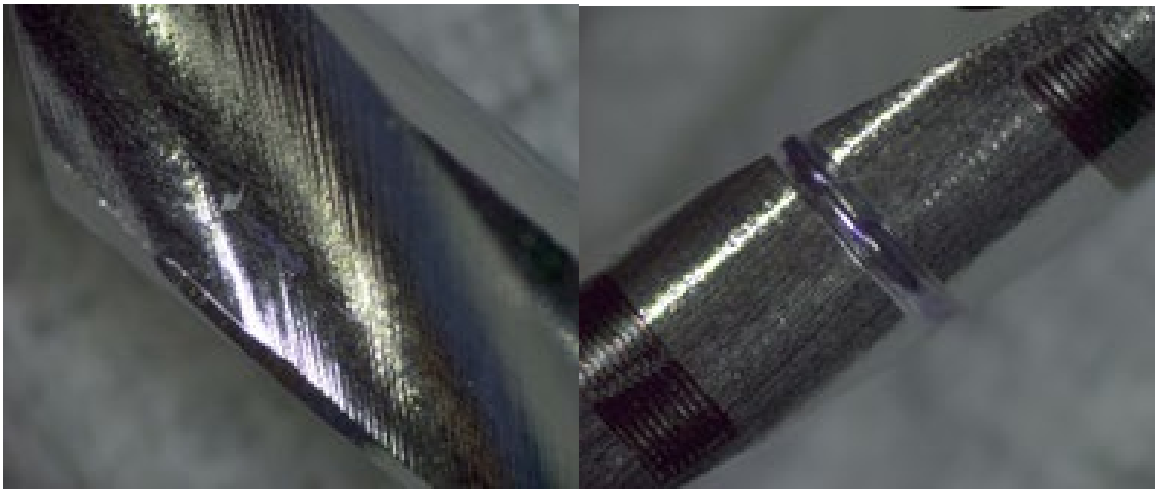

Figure 6: Representative photographs of Group 4 Burs. Magnification 50x.

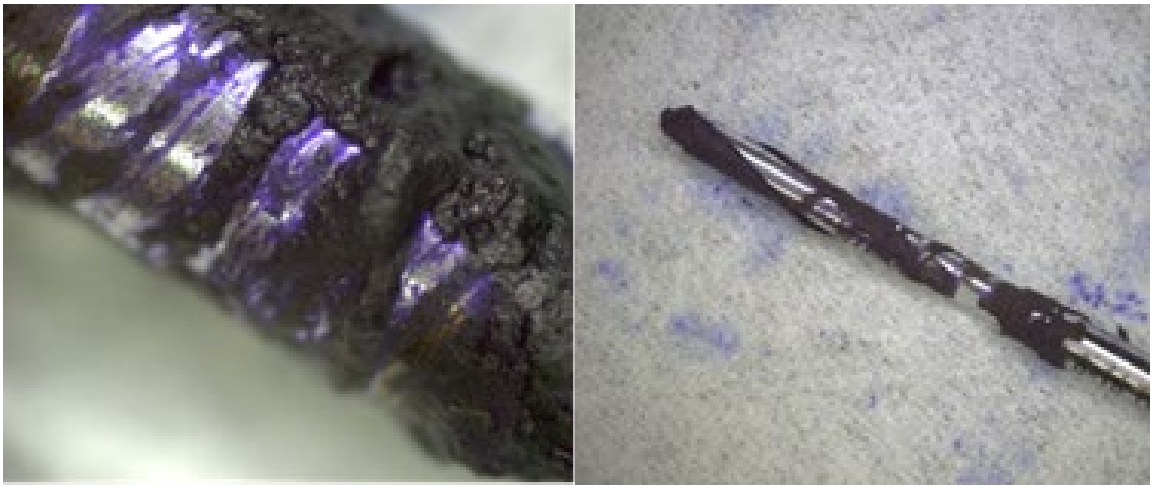

Figure 7: Representative photographs of Group 5 Burs. Magnification 50x.

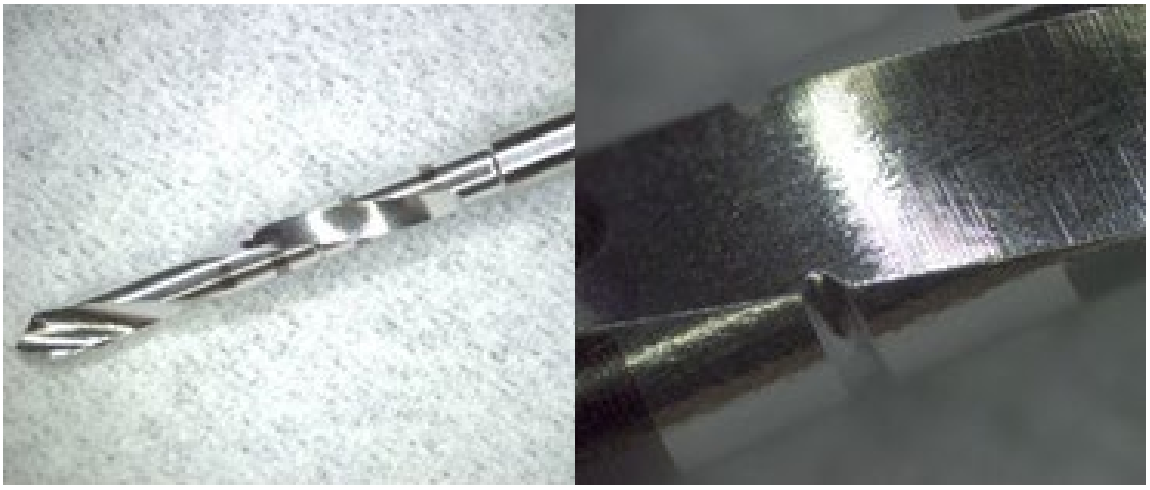

Figure 8: Representative photographs of Group 6 Burs. Magnification 50x.

Group	Contamination	Treatment	Outcome	
			1 Point (Of 15 total)	5 Point (Of 75 total)
1	None – direct from package	Autoclave Only	0	0
2	None – Negative Control	Conventional Processing + Autoclave	1	1
3	Pig Mandible	Conventional processing, 30 minutes of plasma treatment, Autoclave	4	5
4	Pig Mandible	Conventional Processing + Autoclave	2	2
5	Pig Mandible	None – straight from contamination to stain	n/a	n/a
6	Pig Mandible	Moist 2x2 debridement then stain	n/a	n/a

Table 1: Outcome assessment, evaluating debris at the bur and site level.

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