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CHAIRSIDE STERILIZATION OF ENDODONTIC FILES AND GUTTA-PERCHA CONES USING 8.25% SODIUM HYPOCHLORITE

by

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CERTIFICATE OF APPROVAL

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MASTER'S THESIS

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This is to certify that the Master's thesis of

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NAVAL POSTGRADUATE DENTAL SCHOOL
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ABSTRACT

CHAIRSIDE STERILIZATION OF ENDODONTIC FILES AND GUTTA-PERCHA CONES USING 8.25% SODIUM HYPOCHLORITE

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D.D.S., ENDODONTICS, 2020

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Introduction: Sodium hypochlorite is now available in a higher concentration of 8.25%. No research has been published examining this higher concentration's effectiveness in sterilizing endodontic files and gutta-percha cones. **Objective**: The purpose of this *in-vitro* study was two-fold: to determine the percentage of bacterial contamination of files and cones taken directly from their packaging and to determine if files and cones inoculated with *Staphylococcus epidermidis* could be sterilized by immersion in 8.25% sodium hypochlorite. **Methods:** Part 1: 100 files and 100 cones were transferred from original packaging into individual sterile tubes containing 10 ml of sterile broth, incubated for 72 hours and examined for turbidity. Part 2: 300 files and 300 cones were inoculated with *Staphylococcus epidermidis* followed by immersion in 8.25% sodium hypochlorite. Files were immersed for 1, 2, or 5 minutes and cones for 30, 45, and 60 seconds. Files and cones were transferred, incubated, and examined as described in part 1. Fisher's exact test compared frequencies of turbidity by immersion time and post-hoc comparisons were completed to determine significance in turbidity by brand (alpha<0.05). **Results:** Three out of 100 files and 3 out of 100 cones produced turbidity when tested directly from the manufacturer's packaging. File turbidity ranged from 3.0% after immersion for 2 minutes to 12.0% after immersion for 5 minutes with significant difference by immersion time

when combining brands (P=0.046). Cone turbidity ranged from 0% after immersion for 45 sec to 9.0% after immersion for 60 sec with a significant difference in turbidity by immersion times when combining brands (P<0.001). **Conclusions:** Immersion in 8.25% sodium hypochlorite did not guarantee sterility at any time tested for either files or gutta-percha cones.

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LIST OF ABBREVIATIONS

NaOCl	sodium hypochlorite
NPDS	Naval Postgraduate Dental School
NSRCT	non-surgical root canal treatment

Chapter 1: Introduction

The link between bacteria and the development of pulpal and periapical disease has been well established (1). The goal of clinicians is to eliminate these microbes by debriding, sterilizing, and obturating the canal system. To do this effectively and prevent iatrogenic infection, endodontic files and gutta-percha cones should be sterile prior to introduction into the root canal. Unfortunately, multiple studies have shown that both endodontic files and gutta-percha cones taken directly from the manufacturer's packaging are not sterile and are often contaminated by bacteria, organic debris, and metal shavings (2-5).

Previous studies have investigated the efficacy of chairside sterilization of both files and gutta-percha cones. Roth et al showed that immersion of contaminated files in 5.25% sodium hypochlorite (NaOCl) for five minutes achieved sterilization (5). Gnau et al, however, evaluated the efficacy of 6% NaOCl and its effectiveness in disinfecting endodontic rotary files chairside and found it was not effective in completely eliminating bacterial presence after a five minute exposure (6). Siqueira et al showed in 1999 that only 5.25% NaOCl was able to eliminate bacillus subtilis spores from gutta-percha cones after immersion for one minute (7). Gomes et al replicated these results and demonstrated the effectiveness of NaOCl in the disinfection of gutta-percha cones was positively related to its concentration (4). Again, the highest concentration tested by Gomes et al was 5.25% and showed that after one minute it effectively eliminated all vegetative bacteria and spores from tested gutta-percha cones.

The merits of using NaOCl in root canal therapy include its superior tissue dissolution, ability to disinfect and disrupt the bacterial biofilm, and improved effectiveness at higher concentrations (8-10). NaOCl is now commercially available in a higher concentration of 8.25% which has been shown to be significantly better at dissolving pulp tissue compared to 6% NaOCl

(11). To date, no research has been completed to test this higher concentration's effectiveness in sterilizing both gutta-percha cones and endodontic files. The tested files and gutta-percha cones will be inoculated with *Staphylococcus epidermidis* as it is the most common opportunistic pathogen isolated from human epithelium and it is frequently found on contaminated endodontic instruments (12, 13). If 8.25% NaOCl is able to sterilize an endodontic file chairside in less than five minutes or a gutta-percha cone in less than one minute, it would prove clinically useful to the clinician.

Chapter II: Objective

The purpose of this in-vitro study is two-fold: To determine the percentage of commonly used endodontic files and gutta-percha cones taken directly from their packaging that are contaminated by bacteria and to determine if endodontic files and gutta-percha cones inoculated with *Staphylococcus epidermidis* can be sterilized by immersion in 8.25% NaOCl.

Chapter III: Materials and Methods

The objective of Part 1 of this *in vitro* study was to determine the percentage of commonly used endodontic files and gutta-percha cones that were contaminated by bacteria straight from the manufacturer's packaging. One hundred new hand and rotary endodontic files of four brands [25 ProTaper Gold (size F3), 25 Vortex Blue (0.04 taper, size 30), 25 ProFile (0.04 taper, size 30), and 25 FlexoFile (0.02 taper, size 30, Dentsply Sirona, York, PA)] were transferred directly from their manufacturer's packaging via aseptic technique and placed individually into sterile tubes containing 10 ml thioglycolate broth. One hundred new gutta-percha cones of two brands [50 ProTaper (size F3, Dentsply Sirona, York, PA) and 50 DiaDent (0.04 taper, size 30, DiaDent, Burnaby, BC)] were taken directly from their manufacturer's packaging via aseptic technique and placed individually into sterile tubes containing 10 ml thioglycolate broth. Both files and gutta-percha cones were then incubated for 72 hours at 37° C. A blinded examiner evaluated each tube for turbidity by comparing against two sterile thioglycolate blanks included in the same run. The percentage of contaminated files and gutta-percha cones were analyzed by brand.

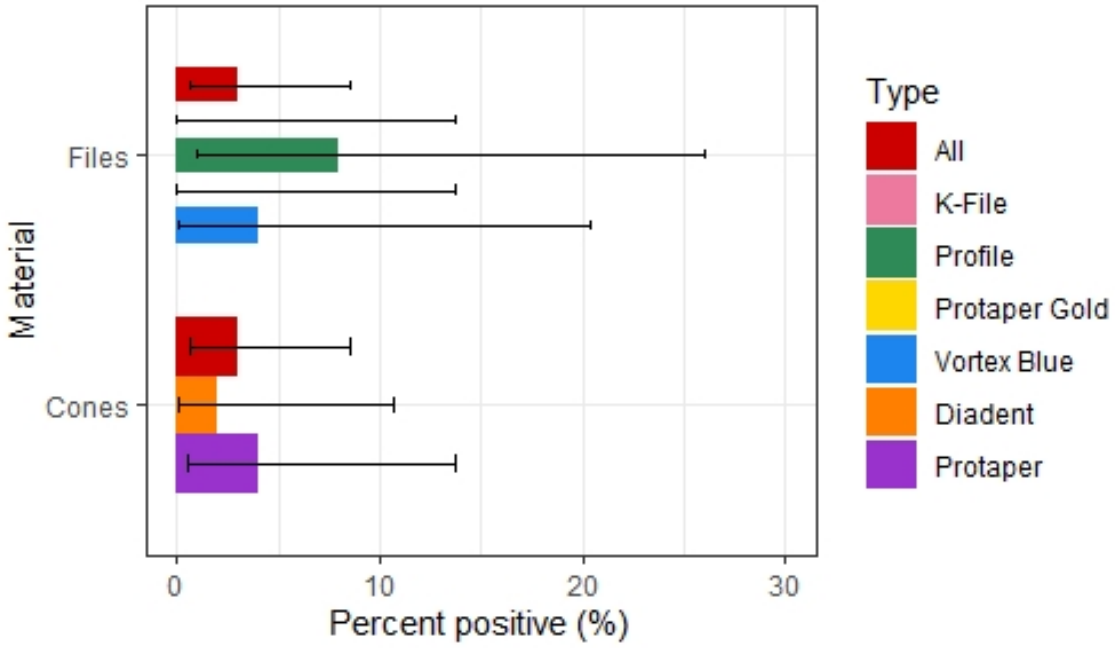
Staphylococcus epidermidis (American Type Culture Collection, Manassas, VA, USA) was streaked onto a Brain Heart Infusion (BHI) (Hardy Dynamics, VWR Scientific, Radnor, PA, USA) agar plate and incubated at 37° C for 24 hours. Isolated colonies were then transferred into 5 milliliters of sterile BHI broth and propagated at 37° C for 24 hours. A ten-fold dilution was prepared by adding 0.5 milliliters of the culture to 4.5 milliliters of sterile BHI broth and incubated at 37° C for 5 hours (log phase). Bacterial growth was monitored using spectrophotometry (Genesys 10S UV-VIS, Thermo Fisher, Waltham, MA, USA) until an optical density of 0.5 at 600nm was obtained and yielded an inoculum concentration of 1×10^8 colony forming units (CFU) per milliliter.

The objective of Part 2 of this study was to determine the amount of time required to sterilize endodontic files and gutta-percha cones by immersion in 8.25% NaOCl after first inoculating them with *Staphylococcus epidermidis*. Three hundred new files [75 ProTaper Gold (size F3), 75 Vortex Blue (0.04 taper, size 30), 75 ProFile (0.04 taper, size 30), and 75 FlexoFile (0.03 taper, size 30)] were inoculated by placing each file into 3 ml of the prepared *Staphylococcus epidermidis* culture and incubating at 37° C for 1 hour. The files were then dried in the 37° C incubator for 24 hours. Twenty-five of each file type were immersed in 8.25% NaOCl for 1, 2, or 5 minutes. Individual files were then transferred into a sterile tube containing 10 ml of sterile thioglycolate broth and incubated and analyzed as described in Part 1. Three hundred new gutta-percha cones [150 ProTaper (size F3) and 150 DiaDent (0.04 taper, size 30)] were also inoculated with *Staphylococcus epidermidis* in the same manner as the files. Fifty gutta-percha cones from both brands were then immersed in 8.25% NaOCl for 30 sec, 45 sec, or 1 minute and then transferred into a sterile tube containing 10 ml of sterile thioglycolate broth. The cones were incubated and analyzed as described in Part 1.

Eight file/eight gutta-percha cone positive controls (2 of each file type and 4 of each cone type, both inoculated with *Staphylococcus epidermidis* as described above) and 8 file/8 gutta-percha cone negative controls (files and cones were first autoclaved to ensure sterility) were incubated in the same manner as the experimental groups. Fisher's exact was used to compare groups (alpha = 0.05) using 'R' software.

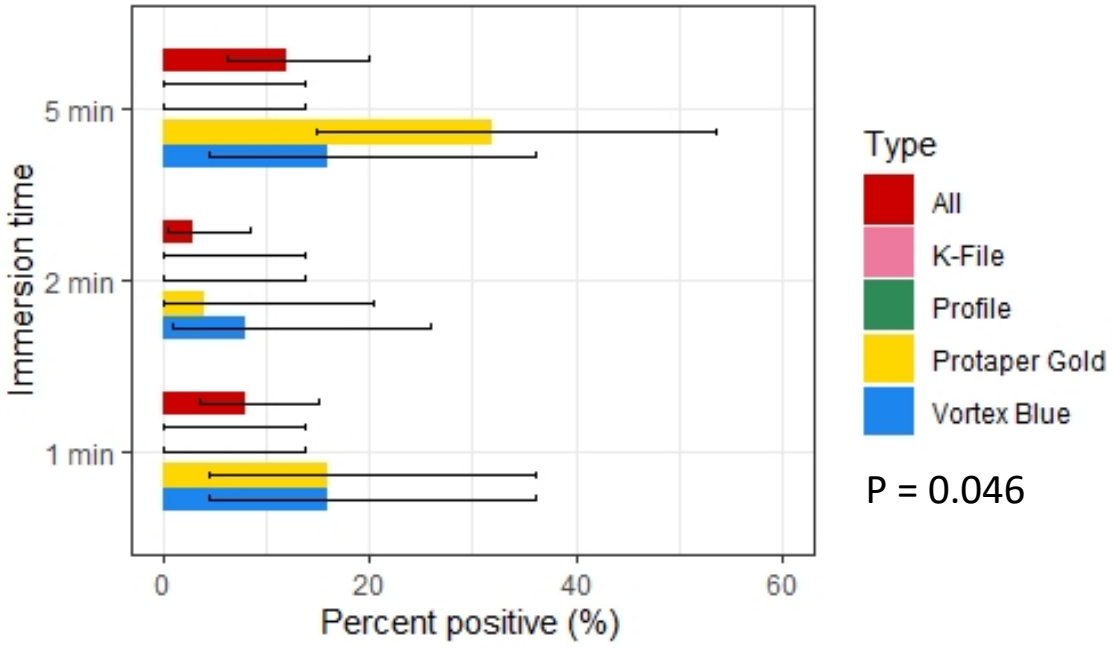
Chapter IV: Results

Graph 1: Part 1 – Turbidity straight from packaging



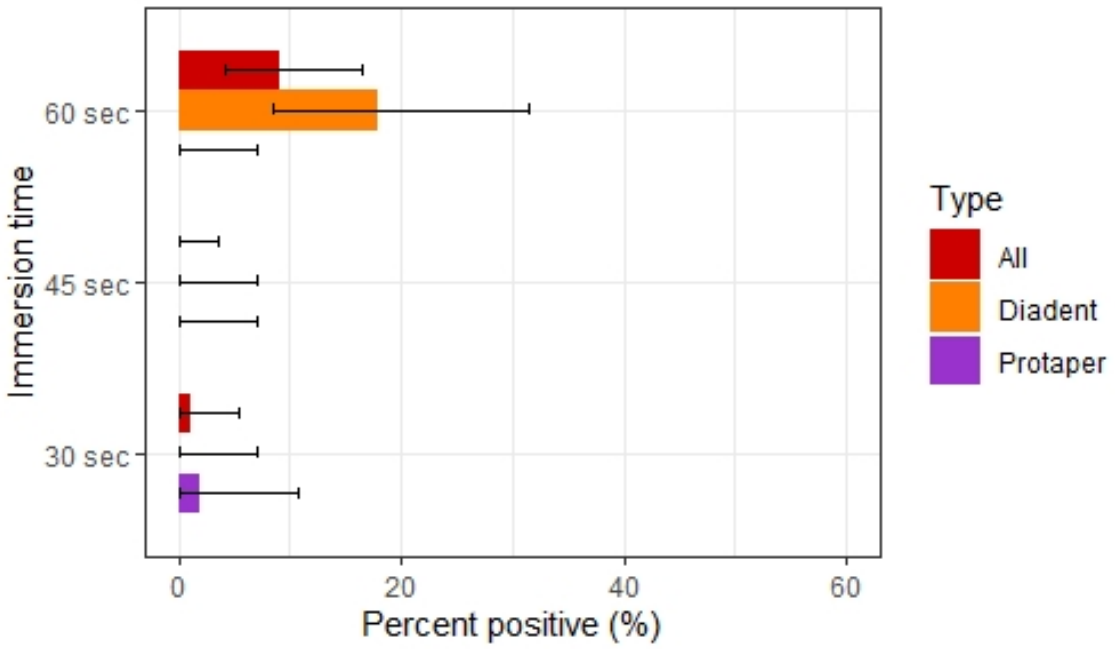
Graph 1 displays results from Part 1 of the study with a plot of samples that were labeled turbid, or positive, by the blinded examiner. The X axis lists the percentage of tubes that were observed to be turbid, or positive, and the Y axis lists different tested brands of files and cones. As noted in the legend on the right-hand side, each file and cone is identified by a distinct color. Lack of a colored bar indicates the absence of turbidity for that brand. The red color, labelled "All" in the legend, represents all brands combined. The error bars, shown as black horizontal lines, represent 95% confidence intervals (CI's) among both files and cones. Overall results from Part 1 indicate three out of one hundred endodontic files and three of out of one hundred gutta-percha cones (3.0%; 95% CI: 0.6-8.5%) were positive when tested directly from the manufacturer's packaging.

Graph 2: Part 2 – Turbidity with files after 8.25% NaOCl



In Part 2, files inoculated with *Staphylococcus epidermidis* were immersed for 1, 2, or 5 minutes in 8.25% NaOCl and then grown in sterile broth for 3 days. The hypothesis that turbidity would vary by immersion time when combining all file brands was evaluated. As noted in Graph 2, the proportion of turbid samples ranged from 3.0% (3 of 100) after immersion for 2 minutes to 12.0% (12 of 100) after immersion for 5 minutes. There was a significant difference in the proportion of turbid samples by immersion time when combining brands (P=0.046). However, the longest immersion time had the highest frequency of turbid samples, contrary to what would be expected. Of note, this increase was seen only in the ProTaper Gold and Vortex Blue file systems, with no turbidity noted in the K-File or Profile groups through any immersion time. This result led to an additional hypothesis that turbidity would not vary by brand. Fisher's exact test was used to compare frequencies of turbid samples by immersion time across all file brands. The difference in turbidity by brand was significant, P< 0.001.

Graph 3: Part 2 – Turbidity with cones after 8.25% NaOCl



Finally, Gutta-percha cones inoculated with *Staphylococcus epidermidis* were immersed for 30, 45, or 60 seconds in 8.25% NaOCl and then grown in sterile broth for 3 days. As noted in Graph 3, the proportion turbid ranged from 0% after immersion for 45 sec to 9.0% (9 of 100) after immersion for 60 sec. There was a significant difference in the proportions of turbid samples by immersion times when combining brands ($P<0.001$). However, the longest immersion time, 60 seconds, had the highest number of turbid samples which was unexpected. In a separate, post-hoc comparison, there was also a significant difference in turbidity by brand ($P=0.02$) as all but one of the contaminated gutta-percha cones were manufactured by DiaDent. As expected, turbidity was seen in the positive control group and no turbidity was observed in the negative control group.

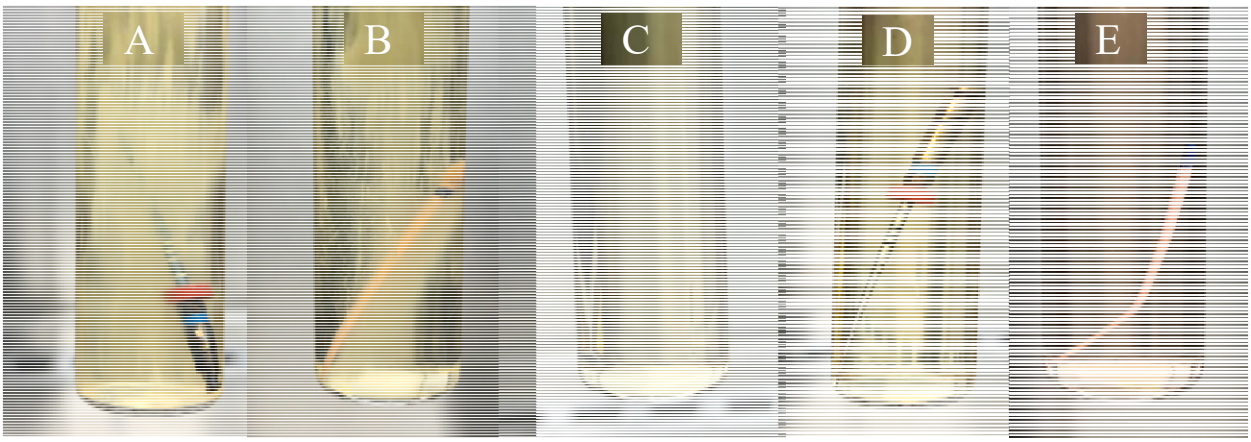


Figure 1: Positive and negative controls

Figure 1 shows examples of the positive control (A,B) and negative control (D,E) groups when viewed in comparison with a sterile broth blank (C) in the center. Turbidity is seen in the positive controls as a cloudy haze, however, the term turbidity was used to describe any inclusion found in the broth tubes after the 72 hours growth period.

Figure 2 shows more examples of observed turbidity. A positive control (F) and sterile

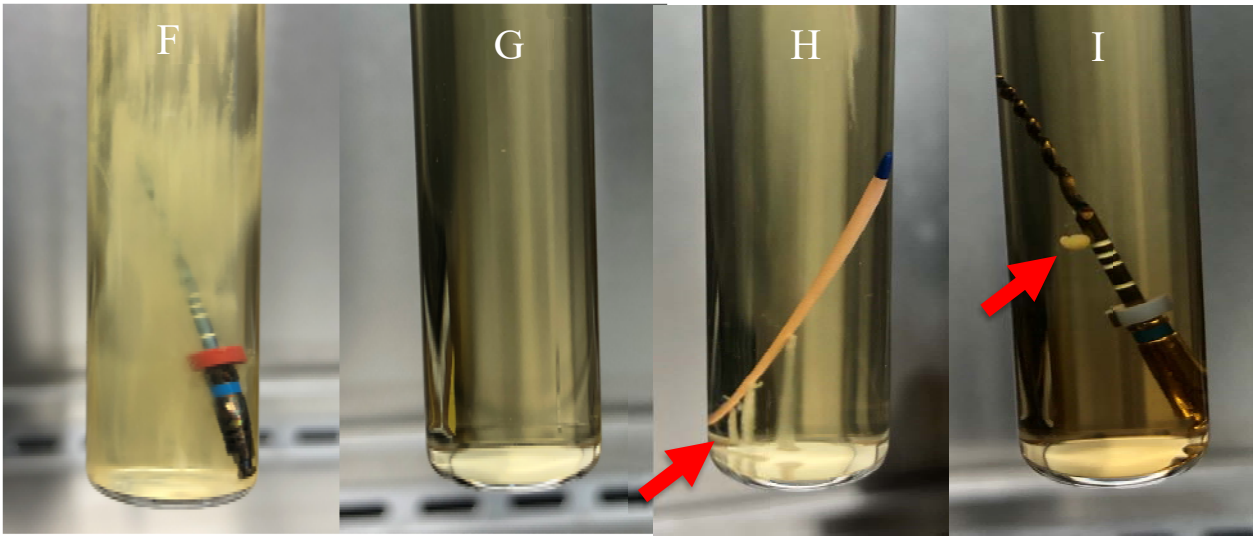


Figure 2: Examples of turbidity

blank (G) are shown for ease of comparison. In the first turbid sample (H, red arrow), tendril-like growth is evident on the gutta-percha cone and is similar in appearance to the positive control

growth pattern. However, a second turbid sample observed (I, red arrow) displays a unique, white inclusion that was found only in the Vortex Blue and ProTaper Gold groups. This growth is notably different than the presentation found in the positive control.

Chapter V: Discussion

Part 1 of this project analyzed turbidity from files and cones taken directly from their packaging. The results demonstrated a 3% contamination of both files and cones. This is in keeping with previous research by Gnau et al (6) which showed 6% contamination of files and with Gomes et al (4) who demonstrated a 5.5% contamination of gutta-percha cones. Unfortunately, the results obtained from Part 2, testing the efficacy of 8.25% NaOCl in sterilizing files and cones after inoculation with *Staphylococcus epidermidis* were inconclusive. The data revealed 12% of tested files exhibited turbidity following five minute immersion in 8.25% NaOCl. This is in contrast with Roth's study (5) that found after five minutes, all files were sterilized by immersion in a lower 5.25% solution. Gnau et al (6), however, demonstrated 2% file turbidity even after immersion in 6% NaOCl for five minutes. Of note, the files in the Gnau et al study were taken directly from the manufacturer's packaging and not inoculated with bacteria as was done in this study. Even without inoculation, Gnau et al showed that immersion in 6% NaOCl did not guarantee sterility.

The results of Part 2 regarding turbidity observed in inoculated gutta-percha cones showed a 9% overall turbidity even after immersion in 8.25% NaOCl for one minute. This is in contrast with the results of Siqueira and Gomes (4, 7) who showed that gutta-percha cones could be sterilized after a one minute immersion in 5.25% NaOCl. Instead of seeing a lower percentage of turbidity with longer immersion the opposite trend was observed in this study.

While immersed in NaOCl, it was noted that the non-cutting portion of the file that inserts into the rotary handpiece was blackening in only the Vortex Blue and ProTaper Gold file systems. Due to the different pattern of turbidity only seen in these two file systems (Figure 2, I), it is possible that this unique presentation is a product of corrosion vice bacterial contamination.

To further investigate these unexpected results, samples of broth from all twenty-three of the turbid file tubes throughout the three tested time periods were streaked and grown on agar plates for 72 hours at 37º C. After three days, only two of the twenty-three tested actually grew bacterial colonies on the plates which lends weight to the theory that this observed turbidity was potentially not bacteria-related. Finally, the only two samples of broth that grew plated bacteria were from the 30 second (shortest observed) immersion time. Regardless, due to the design and limitations of this study where turbidity was assessed as a binary outcome, either "yes or no" by the blinded observer, the ability to definitively determine if observed inclusions were bacteria or corrosive byproducts is limited.

Other explanations could elucidate why observed turbidity increased in both the file and cone groups with longer immersion time. A brand new, unopened bottle of NaOCl was used for of this study. However, while the concentration was labeled as 8.25%, no verification of actual concentration was established prior to beginning the experiment. A lower concentration of NaOCl could possibly delay or prevent sterility in the time periods tested.

Figure 3: Aggregation of files during immersion

Finally, while immersed in the NaOCl, files and cones tended to aggregate together as shown in Figure 3, possibly preventing the solution from completely covering the full surface area of each file or cone. Of note, the files and cones were simply immersed and not agitated or shaken in any way. Viable bacteria could survive. Perhaps if each file or cone were immersed individually vice as a larger group, results may have been different.

Chapter VI: Conclusion

Based on the results of this *in vitro* study, chairside immersion in 8.25% NaOCl did not guarantee sterility at any time tested for endodontic files or gutta-percha cones. While there is high likelihood that observed turbidity seen in the ProTaper Gold and Vortex Blue groups was corrosion vice bacterial contamination, a definitive conclusion cannot be made within the scope of this study. As evidenced in the negative controls, autoclaving remains a reliable and readily accessible way to ensure sterility of files. Finally, additional research is needed to control variables such as verifying actual NaOCl concentration and immersing each file and cone individually to ensure adequate coverage of the NaOCl.

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