
Cyrous Ardalan

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

by

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Abstract

A COMPARATIVE STUDY OF INTRARADICULAR ENTEROCOCCUS FAECALIS BIOFILM REMOVAL WITH THREE ROOT CANAL TREATMENT SYSTEMS: A SCANNING ELECTRON MICROSCOPY STUDY

By Cyrous Ardalan, DMD

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

Virginia Commonwealth University, 2017

Director: Garry L. Myers, DDS
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The objective of this study was to evaluate the biofilm removal efficacy of three root canal treatment systems: ProUltra® PiezoFlow™, traditional needle irrigation, and the GentleWave® system in an ex-vivo benchtop study. Twenty-four extracted maxillary and mandibular molars were selected. Teeth were all instrumented to a master apical file size #25 with 4% taper. Teeth were then randomly divided into four experimental groups and two control groups. The root canals were inoculated with a culture of Enterococcus faecalis and incubated for five weeks to form a biofilm. Each group was then treated with one of the different root canal treatment systems using 6% sodium hypochlorite (NaOCl) as per the respective manufacturer’s recommendation followed by a rinse with water. Following treatment, teeth were decoronated and roots were sectioned longitudinally. Three scanning electron microscope images were taken at the apical level per root half at 5000x magnification. Images were scored by four calibrated examiners blind to group membership using a four-point scoring system (<5% coverage, 5-33%, 34-66%, and >66%). Results were analyzed using mixed model ANOVA. All the experimental
groups were significantly better than the positive control group in removing biofilm. Among the experimental groups, the GentleWave® 15/04 group was significantly better than the other groups. There was no significant difference between the GentleWave® and the ProUltra® PiezoFlow™. Traditional needle irrigation scored the worst in reducing *E. faecalis* biofilm. The GentleWave™ system was as effective at intracanal biofilm removal as the ProUltra® PiezoFlow™ and better than traditional needle irrigation using 6% NaOCl as an irrigant.
Introduction

The elimination of bacteria in the root canal system and the treatment or prevention of apical periodontitis is the primary aim of endodontic therapy (1). Bacteria have been shown to play a major role in both the persistence and emergence of apical periodontitis after root canal therapy has been performed (2). The microflora that exist in apical periodontitis are predominantly facultative anaerobic bacteria. These bacteria are less susceptible to antimicrobial treatment and are more likely to persist following unsuccessful root canal therapy.

The most frequently isolated persistent bacteria in apical periodontitis is *Enterococci faecalis*, and its prevalence has been reported in 32% of teeth with previous root canal therapy and persistent apical periodontitis (3). For bacteria to be found in post endodontic treatment samples, they must resist disinfection procedures and adapt to a strikingly different environment (2). *E. faecalis* appears to be highly resistant to the medications used during root canal therapy and is one of few organisms that is resistant to the antibacterial effects of calcium hydroxide (4). It has also been postulated that a virulence factor of *E. faecalis* in failed endodontically treated teeth may be related to its ability to invade dentinal tubules and adhere to collagen in the presence of human serum (5).

One significant method of resistance *E. faecalis* may undergo is formation of a biofilm. Biofilms, or plaque, are colonies of bacteria embedded in a polysaccharide matrix (5). The ability of *E. faecalis* to form biofilms in root canals of teeth has been shown. These biofilms can enable bacteria to become 1000 times more resistant to phagocytosis, antibodies, and
antimicrobials than non-biofilm producing organisms. Some mechanisms of resistance are an impaired physical penetration of the biofilm matrix, cells within the biofilm antagonizing the effect of antimicrobials, and cells that may change phenotypically (6). Svensater and Bergenholtz proposed a hypothesis for biofilm formation in 2004. They described biofilm formation is likely initiated after organisms invade the pulp chamber. The inflammatory lesion front provides the fluid vehicle for organisms to then travel and attach to the root canal wall (7).

Ricucci et al. were able to identify a high incidence of intraradicular biofilm in both treated and untreated root canals. Biofilms were also significantly associated with periapical lesions and a small percentage of extraradicular biofilms were noted in their study as well (8). A case report by Carr et al. examined a retreatment failure 10 years after endodontic retreatment. They showed microbial communities that existed within the endodontic space for decades after treatment could thrive and prosper with no apparent nutrient source and consist of diverse, multispecies biofilms (9).

Thus, in an infected tooth with apical periodontitis, bacteria may be organized as a biofilm. The persistence of intra-radicular biofilms may be an important factor in post-treatment apical periodontitis. The reduction of this microflora can only be achieved by mechanical dislocation of the biofilm using hand and rotary instrumentation, washing away of the organisms and debris by irrigating with antimicrobial solutions and the use of an intracanal dressing (10).

The main purpose of instrumentation is the mechanical debridement and shaping of the root canal system to create space for delivery of antimicrobial substances (11). However, even with current nickel titanium rotary instrumentation, more than 35% of root canal surface areas can be left untouched (12). Canal walls that are instrumented also produce a smear layer, which can be penetrated by bacteria, and may offer protection to biofilms adhering to root canal walls.
Further, mechanical instrumentation with a chemically inert irrigating solution cannot reduce microorganisms in the root canal successfully (13).

The ideal properties of an irrigant is first and foremost to clean and disinfect the root canal system. The irrigant should not have any adverse effects on dentin or healing ability of the periapical tissues. Ultimately, the irrigant should have the ability to dissolve vital pulp tissue and inactivate endotoxins. In addition, it should be inexpensive, relatively easy to apply and not cause tooth discoloration. The complexity of canal anatomy, presence of a number of dentinal tubules within the roots, invasion of the tubules by microorganisms and formation of a smear layer are all obstacles in removing irritants from the root canal system (13).

Various types of irrigants have been advocated for use in root canal therapy. Sodium hypochlorite appears to be the most ideal root canal irrigant as it has antibacterial properties and the ability to dissolve necrotic tissue and the organic components of the smear layer. Chelators such as EDTA have shown efficiency in removing the smear layer, and in addition may detach biofilms adhering to root canal walls (14). Clegg et al. showed that 6% NaOCl was the only irrigant able to physically remove artificial biofilm and kill bacteria. The higher concentrations of NaOCl were more antibacterial (15). Del Carpio showed that dissolution of biofilms was not only related to the irrigant used, but also dependent on contact time (16).

Syringe irrigation has been used in endodontics since its establishment. The size of the syringe can affect the tactile force needed to irrigate at a certain flow rate. When a clinician depresses the plunger, the pressure inside the syringe barrel is higher than the pressure around the tip of the needle. This is known as a positive pressure technique (17). Sedgely et al. concluded that the mechanical efficacy of an irrigant in reducing intracanal bacteria was
significantly greater when delivered 1mm from the working length compared with 5mm from the working length (18).

Ultimately, syringe irrigation techniques rely on adequate canal enlargement, placement of a fine needle close to working length, and a relatively high flow rate. However, if the needle is placed too close to the end of the root, it is possible to express irrigant into periapical tissues which could result in post operative pain as well as the potential to experience a sodium hypochlorite accident. Clinical studies have shown that syringe irrigation may be unable to remove debris and soft tissue remnants from certain isthmuses and ramifications of molar teeth (19). Recently published randomized control trials using a syringe irrigation technique resulted in a similar radiographic success than a group with ultrasonic activation of irrigation (20). This study suggests that the increased cleaning efficacy of lateral canals and isthmuses may not result in a better treatment outcome.

Ultrasonic irrigation incorporates cavitation and acoustic streaming of the irrigant within a canal to aid in the debridement of the root canal system. Metzler and Montgomery found that passive ultrasonic irrigation (PUI) using NaOCl as an irrigant significantly improved the cleanliness of the isthmuses of mesial roots of mandibular molars in vitro at 1mm and 3mm levels from the apex (21). Some initial difficulties with PUI was that the irrigant needed constant replenishment and this added heavily to clinical treatment time. Thus, new products were created to provide continuous ultrasonic irrigation (CUI).

The Pro Ultra® PiezoFlow™ (Dentsply, Inc, York, PA) is an ultrasonic needle used to facilitate irrigation. The needle is connected to the ultrasonic unit and has a connecting tube, which carries the irrigant from the syringe to the needle. A study by Castelo Baz et al. reported that CUI with the Piezoflow® was more effective than PUI in getting irrigant into lateral canals
However, Howard et al. reported no differences in debris removal with the Piezoflow® over conventional needle irrigation (23).

Recently, a novel device, the GentleWave™ System (Sonendo, Inc, Laguna Hills, CA) has been developed to clean the root canal system. The system is composed of a console and handpiece that delivers treatment solution from the tip of the handpiece into the pulp chamber while excess fluid is simultaneously removed by a vented suction through the handpiece. The treatment protocol uses both sodium hypochlorite and EDTA to create a strong cavitation cloud, which generates a broad spectrum of sound waves within the degassed fluid inside the tooth.

Haapasalo et al. have shown that the tissue dissolution effect of the GentleWave™ system was 8 times greater than that of conventional irrigation systems including the Endovac® and needle irrigation using a simulated tooth model (24). Another study concluded that the GentleWave™ system showed a greater cleaning capacity and reduction in residual debris of mandibular and maxillary molars than those cleaned conventionally (25). A micro-CT study showed complete removal of calcium hydroxide in mandibular molar canals within 90 seconds without the use of any instruments in the canals using the GentleWave™ system (26). A recent prospective clinical study using the GentleWave™ system reported high levels of success after a 12-month follow up (27).

Previous studies have used various irrigation systems to evaluate E. faecalis biofilm dissolution and removal. Estrela described a model of biofilm inoculation using single canaled teeth with 60 days of incubation for effective SEM visualization (31). Bhuva showed that the use of both conventional syringe irrigation and passive ultrasonic irrigation with sodium hypochlorite were effective at removing intraradicular E. faecalis biofilms in extracted maxillary anterior teeth (28). This study evaluated biofilm by scanning electron microscopy. Schaudinn
discussed the use of SEM as an invaluable tool for describing biofilms because of its ability to provide a detailed view of surfaces with high resolution and magnification. However, there are limitations as biofilm bacteria can lose their characteristic size and shape as well as being difficult to visualize if they are embedded in a matrix (29). In another study, *E. faecalis* biofilms were grown in root canal systems and treated using both positive and negative pressure irrigation and evaluated by scanning electron microscopy. This study concluded that positive and negative pressure irrigation protocols using 2.5% NaOCl showed a similar capacity to reduce *E. faecalis* in infected root canals, but biofilm was still noted in all image samples (30).

Most biofilm studies have focused on using dentinal slices or extracted single rooted teeth as their biofilm model (16,28). This study will use mesial and distal roots of maxillary and mandibular molars, which can be more difficult to treat because of the curvatures and complex anatomy found in these canal systems. No study to date has compared the GentleWave™ multisonic system to other irrigation systems using this extracted tooth model. The purpose of this study was to compare the effectiveness of the GentleWave™ multisonic system, ProUltra® PiezoFlow®, and conventional needle irrigation on reduction of intraradicular *Enterococcus faecalis* biofilms in extracted molar human teeth using sodium hypochlorite as an irrigant.
Materials and Methods

Twenty-six freshly extracted maxillary and mandibular molar teeth were obtained and stored in phosphate buffered saline until use. Any teeth with decay or fractures below the cemento-enamel junction, internal or external resorption, open apices, or previous root canal therapy were excluded. For all treatment groups, the roots of teeth were firmly secured within a water saturated porous medium to simulate blood-saturated periradicular tissue.

Canal Preparation

Endodontic access was achieved per standard practice of patency, confirmed utilizing a #10K hand file visually extending past the root apex. Working length was defined as 1mm from the clinical apex. The root canals were prepared with #15/.04 and #25/.04 Endosequence rotary files (Brassler USA, Savannah, GA) to the full working length using a step back approach. After completion of canal preparation all samples were treated with the GentleWave system using 3% NaOCl for 5 minutes to provide a baseline for all samples to be free of any tissue remnants. The samples were then submerged in 10mL of PBS and autoclaved for sterility at 121° C for 25 minutes.

Inoculation

Using an inoculation loop, a stock culture of *E. faecalis* (ATCC #19433) was streaked onto a Brain Heart Infusion (BHI) agar plate (Teknova, Hollister, CA). The plate was then incubated at 37 °C for 24 hours. Fifty mL of sterile BHI broth (Teknova, Hollister, CA) was then inoculated with 5 colonies from the 24-hour stock culture plate. The inoculated BHI
broth was then incubated at 37 °C for 4 hours. The inoculated BHI broth was placed in a vortex mixer for 10 seconds to resuspend bacteria within the solution. Using sterile tweezers, the autoclaved molar samples were then placed in 15mL sterile centrifuge tubes (McMaster-Carr, USA).

Using a sterile 30 G Max-i-Probe needle (Dentsply, York, PA) and a 3cc syringe (Medtronic, Minneapolis, MN), the root canals were injected with the bacterial suspension in BHI broth until the canals were filled (approximately 150 µL). The samples were then centrifuged for 5 minutes each at 3500 rpm and this step was repeated twice. The coronal portion of the molar samples were then filled with the bacterial suspension and centrifuged for 5 minutes at 3500 rpm. The media was replenished every 3 days as needed using sterile BHI broth. The incubation period of the samples was five weeks. Care was taken that all samples were handled with utmost sterility.

**Testing Procedures**

Following incubation of a mature biofilm, the samples were divided randomly into four treatment groups and two control groups listed below:

A. GentleWave™ system (n=6)

B. ProUltra PiezoFlow® Active Ultrasonic (n=6)

C. Conventional Needle Irrigation (n=6)

D. GentleWave™ system 15/04 (n=2)

E. Positive control (Biofilm inoculation, no treatment) (n=3)

F. Negative control (No Biofilm, no treatment) (n=3)
A single operator performed all endodontic treatment procedures. So as to standardize the treatments, care was taken to ensure that the sequence, concentration, exposure of treatment fluids for each tooth would be the same across all treatment groups.

A. **GentleWave™ System:**
The teeth were treated using the GentleWave system following the manufacturer’s recommended procedure. The GentleWave system was used with 3% NaOCl for 5 minutes per tooth followed by sterile water for 15 seconds. The canals were then dried with paper points.

B. **ProUltra PiezoFlow® Ultrasonic System:**
Irrigation was performed with the ProUltra PiezoFlow system. The ultrasonic irrigation needle was attached to the ultrasonic handpiece. Then, a syringe pump (New Era Pump Systems, Farmingdale, NY) was connected to a 0.5L vial of sodium hypochlorite which was then attached to the Luer-lock connection on the ultrasonic irrigation needle. The flow rate was set to 15 ml per minute.

After completing canal preparation, the needle was trial-fit into the canal and the length at which the needle began to bind against the canal walls was determined. The needle was pulled back approximately 1 mm and the silicon stopper was set to this depth. The power level was set to 5 and 6% NaOCl was used for 1 minute per canal, as per the manufacturer’s recommendation. Afterwards, each canal was rinsed with water for 20 seconds using a 30 gauge max-i-probe needle. Small paper points were used to dry the canal.

C. **Conventional Needle Irrigation**
A 30-gauge needle was placed 2mm short of WL, without binding and moved in an up and down motion while irrigating. The needle was attached to a syringe pump set at 5ml per minute. Six percent NaOCL was delivered for 1 minute per canal followed by 30 seconds of water in
sequence. Paper points were used to dry the canal.

D. GentleWave™ System 15/.04:
The teeth were treated using the GentleWave™ system. The GentleWave™ system was used with 3% NaOCl for 5 minutes per tooth, and sterile water for 15 seconds.

E. Positive control

Treatment teeth in this group were inoculated with biofilm but no irrigation protocol was completed.

F. Negative Control

Teeth in the negative control group did not receive any inoculation or treatment.

After each tooth had been subjected to its allocated irrigation protocol (Table 1), the crown was removed from the tooth using a diamond disc. The roots were then sectioned and split along their long axis using a diamond disc and blade. Next, they were prepared for scanning electron microscopy.
Table 1. Random assignment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chart-Tooth</th>
</tr>
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<tbody>
<tr>
<td>GentleWave xx179-3</td>
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<tr>
<td>GentleWave xx340-18</td>
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<td>GentleWave xx463-3</td>
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<tr>
<td>GentleWave xx805-30</td>
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</tr>
<tr>
<td>GentleWave 15 0.04 xx804-32</td>
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<tr>
<td>GentleWave 15 0.04 xx952-15</td>
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<tr>
<td>ProUltra/ Piezo Flow xx052-14</td>
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</table>

Scanning Electron Microscopy Preparation

The root samples were dehydrated by serial dehydration in graded ethanol with the following dilutions in distilled water: 50% ethanol, 70% ethanol, 80% ethanol, and 100% ethanol. The roots were then sputter coated with gold palladium prior to imaging. All roots were imaged with a Hitachi TM3030 scanning electron microscope. All images were acquired in the apical 1.5-2.5mm of the root at 50x, 200x and 5000x. Three images of each root half were taken.
at 5000x unless it was not feasible due to splitting or excessive splitting debris. SEM and image selection were performed in a blinded fashion.

**Image preparation**

After roots were sectioned, each root had aspects labeled as “Side 1” and “Side 2”. An SEM image at 5000x magnification was taken at three locations labeled “X1”, “X2”, or “X3”. Table 2 shows the number of usable images available for each tooth and root. Note that not all roots produced the planned 6 images. In addition, note that two roots were randomly chosen to be assessed multiple times—these correspond to the count=4 in the table. These replicate samples were chosen to assess intra-rater repeatability.

**Table 2. Number of images per tooth and root**

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</tr>
<tr>
<td>xx965-2</td>
<td>MB</td>
</tr>
</tbody>
</table>
Data Acquisition

Four examiners were calibrated using a series of images obtained from a database created during preliminary studies. A number of different images representing each score were used to ensure that the examiners were exposed to slight variations in the appearance of the biofilm. A calibration test was completed by the examiners to confirm a high level of agreement.

Counting the 60 replicate images, there were 348 images available for assessment. In order to reduce the cognitive load on the raters, it was decided that each rater would evaluate one-third of the images on three separate occasions. The images were randomly assigned to one of three blocks in a manner to equalize the number of treatment groups, sides, and x-aspects (see Table 3). The replicate images were also assigned so that an SEM was repeated, twice in one block and repeated in each of the other two blocks.

Table 3. Assignment of images to one of three blocks

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Random Block</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1  2  3</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
</tr>
<tr>
<td>GentleWave</td>
<td>28 26 27</td>
</tr>
<tr>
<td>GentleWave 15 0.04</td>
<td>11 11 11</td>
</tr>
<tr>
<td>Needle Irrigation</td>
<td>30 32 28</td>
</tr>
<tr>
<td>ProUltra/ Piezo Flow</td>
<td>28 26 24</td>
</tr>
<tr>
<td>Negative Control</td>
<td>11 12 13</td>
</tr>
<tr>
<td>Positive Control</td>
<td>9  11 10</td>
</tr>
<tr>
<td>Side</td>
<td></td>
</tr>
<tr>
<td>Side 1</td>
<td>68 66 59</td>
</tr>
<tr>
<td>Side 2</td>
<td>49 52 54</td>
</tr>
<tr>
<td>X</td>
<td></td>
</tr>
<tr>
<td>X1</td>
<td>38 25 49</td>
</tr>
<tr>
<td>X2</td>
<td>38 57 22</td>
</tr>
<tr>
<td>X3</td>
<td>41 36 42</td>
</tr>
<tr>
<td>Total</td>
<td>117 118 113</td>
</tr>
</tbody>
</table>

The images were cropped to exclude the image data: after which they were numbered from 1 to
348. Using a random number sequence generator, all of the images in a block were randomly ordered, with the exception that in each block the first two images were of controls—one negative control and one positive control.

A single SEM image was shown via a REDCap® survey and evaluators were asked to score the image using a 4 point scoring system adapted from Bhuva (28). For example, see Figure 1.
Figure 1. Recap Survey Question

Statistical Analysis

Differences between the five groups were assessed using a repeated-measures mixed-model ANOVA that took into account the random assignment of images into blocks for assessment purposes, the rater and position differences. Inter and intra-examiner agreements were assessed by the chance-corrected Kappa index of agreement.
Results

Each of four raters scored the 348 images independently. Table 4 shows the number ratings for each of the four rating categories and summarized the average rating using the mean and standard deviation of the ratings of the respective images. Nominally, the GentleWave 15/0.04 had the lowest average rating and the needle irrigation had the highest average rating. It is comforting to observe that the raters largely rated the Negative Control images as 0 and the Positive Control images as 3, although these were not the only rating produced in these controls. It is also apparent that the four raters had different average ratings. Raters A and C more commonly gave low ratings and raters B and D gave high ratings more often.

Table 4. Overall rating summary

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rating</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 1 2 3 total</td>
<td>Mean Std Dev</td>
</tr>
<tr>
<td>GentleWave</td>
<td>214 54 22 34 324</td>
<td>0.617 1.002</td>
</tr>
<tr>
<td>GentleWave 15 0.04</td>
<td>103 10 4 15 132</td>
<td>0.477 1.000</td>
</tr>
<tr>
<td>ProUltra/ Piezo Flow</td>
<td>174 73 26 39 312</td>
<td>0.776 1.046</td>
</tr>
<tr>
<td>Needle Irrigation</td>
<td>143 45 42 130 360</td>
<td>1.442 1.330</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative Control</td>
<td>104 31 5 4 144</td>
<td>0.368 0.687</td>
</tr>
<tr>
<td>Positive Control</td>
<td>3 7 41 69 120</td>
<td>2.467 0.721</td>
</tr>
<tr>
<td>Rater</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>249 33 10 56 348</td>
<td>0.635 1.122</td>
</tr>
<tr>
<td>B</td>
<td>117 72 51 108 348</td>
<td>1.431 1.242</td>
</tr>
<tr>
<td>C</td>
<td>282 6 25 35 348</td>
<td>0.463 0.999</td>
</tr>
<tr>
<td>D</td>
<td>93 109 54 92 348</td>
<td>1.417 1.145</td>
</tr>
</tbody>
</table>

The control images were constructed so that the “correct” rating for a Negative Control image was 0. The raters were trained to be calibrated to these standards but there remains evidence of rater differences (Table 5). Rater A appears to match best with the expectations. Rater B often
gave non-zero scores to the negative control images and gave a rating of 3 to the positive control images somewhat more than half the time. Rater C got all the negative control images correct but was clearly reticent to assign ratings of 3, even to the positive control images. Rater D rated the negative control images correctly less than half the time but was more nearly correct on the positive images. All this indicates a potential bias between the raters.

**Table 5. Rater differences on the control images**

<table>
<thead>
<tr>
<th>Rating</th>
<th>Score</th>
<th>Mean</th>
<th>Std Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rater</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>34  2  0  0  36</td>
<td>0.056</td>
<td>0.232</td>
</tr>
<tr>
<td>B</td>
<td>21 13 0  2  36</td>
<td>0.528</td>
<td>0.774</td>
</tr>
<tr>
<td>C</td>
<td>36 0  0  0  36</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>D</td>
<td>13 16 5  2  36</td>
<td>0.889</td>
<td>0.854</td>
</tr>
<tr>
<td></td>
<td>Negative Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1  1  3  25  30</td>
<td>2.733</td>
<td>0.691</td>
</tr>
<tr>
<td>B</td>
<td>1  3 10 16  30</td>
<td>2.367</td>
<td>0.809</td>
</tr>
<tr>
<td>C</td>
<td>1  1 19  9  30</td>
<td>2.200</td>
<td>0.664</td>
</tr>
<tr>
<td>D</td>
<td>0  2  9 19  30</td>
<td>2.567</td>
<td>0.626</td>
</tr>
<tr>
<td></td>
<td>Positive Control</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All four raters assessed every image, and so it’s possible to compare the agreement of the ratings. The full description of each pair of the four raters is given in the Appendix, Table 6, and the level of agreement is summarized in Table 6. All of the Kappa agreement scores were significantly above chance agreement (P < .001). Raters A and C had the highest level of agreement—81% exact agreement and a chance-corrected Kappa agreement of 53%. Raters C and D had the lowest level of agreement.
Table 6. Summary of between rater agreements

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>Exact</th>
<th>Kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>A v. B</td>
<td>46.6%</td>
<td>22.0%</td>
</tr>
<tr>
<td>A v. C</td>
<td>81.0%</td>
<td>52.6%</td>
</tr>
<tr>
<td>A v. D</td>
<td>44.5%</td>
<td>24.2%</td>
</tr>
<tr>
<td>B v. C</td>
<td>45.7%</td>
<td>20.4%</td>
</tr>
<tr>
<td>B v. D</td>
<td>53.4%</td>
<td>37.1%</td>
</tr>
<tr>
<td>C v. D</td>
<td>39.1%</td>
<td>17.7%</td>
</tr>
</tbody>
</table>

There were 4 positive control images and 4 negative control images that were repeatedly assessed—there were four occasions where these duplicate images were assessed. Accordingly, the within-rater agreement may be described by counting the number of times the same image was rated with an identical rating. Table 8 in the Appendix shows each rater’s assessment of these duplicate images. The results are summarized as follows: Rater A and C gave identical ratings on all 8 images 88% of the time, and rater B gave identical ratings 75% of the time. For each of the 8 duplicate images, rater D never gave the same rating across the four occasions where he was shown the same image. The negative control images seemed somewhat easier to assess identically, as this occurred in 69% of these images whereas an identical assessment occurred in 56% of the positive control images.

In summary, the raters each exhibited a different mean level and varied somewhat in their agreement with the other raters and with themselves. The possibility of these differences were anticipated and were taken into account in the analysis.

Analysis of the primary aim

The primary aim of the study was to compare the rating of biofilm coverage between the 4 treatment groups. The analysis of this aim was accomplished using a repeated-measures mixed-model ANOVA. The following factors were included in the model: Treatment, Random block (1, 2, or 3), Position of the image (Side 1 or 2, and X1-X3), and Rater (A, B, C, or D). Each Chart-
Tooth-Root was treated as independent and the six positions of the image were treated as a repeated (correlated) assessment. Table 7 shows that, after adjusting for Rater, Block, and Position differences, there were significant differences between the Treatment groups. The least-squares mean rating is shown with its 95% confidence interval. Differences between the means were identified by Tukey’s LSD multiple comparison procedure. Those LS Means sharing the same letter were not significantly different. The superscript letters for the Treatment effect show that Needle Irrigation is significantly better than the Positive Control and worse than all other groups. The ProUltra® Piezoflow™ and GentleWave™ groups were not different from one another. The Negative control is significantly better than all other treatments except the GentleWave™15/0.04.
Table 7. Group differences

<table>
<thead>
<tr>
<th>Effect</th>
<th>LS Mean</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>&lt;.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive Control</td>
<td>2.56a</td>
<td>(2.41 to 2.71)</td>
<td></td>
</tr>
<tr>
<td>Needle Irrigation</td>
<td>1.55b</td>
<td>(1.47 to 1.64)</td>
<td></td>
</tr>
<tr>
<td>ProUltra/ Piezo Flow</td>
<td>0.77c</td>
<td>(0.67 to 0.86)</td>
<td></td>
</tr>
<tr>
<td>GentleWave</td>
<td>0.70c</td>
<td>(0.61 to 0.79)</td>
<td></td>
</tr>
<tr>
<td>GentleWave 15 0.04</td>
<td>0.65cd</td>
<td>(0.50 to 0.80)</td>
<td></td>
</tr>
<tr>
<td>Negative Control</td>
<td>0.44d</td>
<td>(0.30 to 0.57)</td>
<td></td>
</tr>
<tr>
<td>Rater</td>
<td>&lt;.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1.56a</td>
<td>(1.47 to 1.64)</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>1.54a</td>
<td>(1.45 to 1.63)</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.76b</td>
<td>(0.67 to 0.85)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.59c</td>
<td>(0.50 to 0.67)</td>
<td></td>
</tr>
<tr>
<td>Random block</td>
<td>&lt;.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.30a</td>
<td>(1.23 to 1.38)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.03b</td>
<td>(0.95 to 1.11)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.00b</td>
<td>(0.92 to 1.08)</td>
<td></td>
</tr>
<tr>
<td>Position</td>
<td>0.0381</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Side 2 X1</td>
<td>1.25a</td>
<td>(1.14 to 1.37)</td>
<td></td>
</tr>
<tr>
<td>Side 2 X3</td>
<td>1.15ab</td>
<td>(1.04 to 1.25)</td>
<td></td>
</tr>
<tr>
<td>Side 2 X2</td>
<td>1.11ab</td>
<td>(0.99 to 1.23)</td>
<td></td>
</tr>
<tr>
<td>Side 1 X3</td>
<td>1.08ab</td>
<td>(0.98 to 1.19)</td>
<td></td>
</tr>
<tr>
<td>Side 1 X2</td>
<td>1.08ab</td>
<td>(0.98 to 1.18)</td>
<td></td>
</tr>
<tr>
<td>Side 1 X1</td>
<td>1.00b</td>
<td>(0.89 to 1.10)</td>
<td></td>
</tr>
</tbody>
</table>

* Significant differences identified by Tukey’s LSD multiple comparison procedure. Groups sharing the same superscript letter are not significantly different (P < .05)
Figure 2. Treatment group differences
Discussion

This was an ex-vivo study that examined the efficacy of biofilm removal in extracted teeth using 3 different irrigation systems: standard syringe irrigation using a 30g needle, continuous ultrasonic irrigation (CUI) with the ProUltra® Piezoflow™ and a new irrigation system, the GentleWave™. The syringe irrigation and CUI both used 6% NaOCl and the GentleWave™ system used 3% NaOCl as the choice of irrigant. The study used the mesial and distal roots of extracted molars with E. faecalis biofilm formation on the root walls. The teeth were treated with each of the irrigation systems and then the remaining biofilm was evaluated in the apical third of the roots. Scanning electron microscopy (SEM) was used to evaluate the biofilm formation and effects of the irrigation systems similar to studies by Clegg and Bhuva (15,28). Four raters then scored the percentage of biofilm for 348 SEM images using a REDCap® survey.

This study design used extracted human molar teeth for the study model. According to Estrela, an adequate human root canal with variations and complexity reflects the best environment for a biofilm study (31). Previous studies have used 3D models, membrane filters, dentinal slices, and single rooted teeth as vectors for biofilm formation (5,10,15,16,28). The use of mesial and distal roots of maxillary and mandibular molars increases the difficulty for both biofilm formations ex-vivo as well as effective irrigation due to the anatomical complexities of molar teeth. In addition, this study design focused only on the apical third of these teeth, where ramifications and lateral canals are mostly located (1,11).

Table 4 details the raters overall scoring summary. Overall, the Gentlewave™ system had 83% of images with less than 33% biofilm coverage, the ProUltra® Piezoflow™ had 79% with
less than 33% biofilm coverage, and needle irrigation had 52% with less than 33% biofilm coverage. Raters A and C tended to rate lower scores for biofilm coverage. These raters were also more experienced with viewing SEM images and may have been better able to delineate biofilm versus dentin or debris. Raters B and D tended to have higher scores, which may have been due to less experience in viewing SEM images.

Table 5 shows rater differences on control sample images. Ultimately, if biofilm inoculation was covering a majority of the walls of the positive controls, raters should have scored a “3” for these images. However, from the table we can gather that only 58% of positive controls were scored a “3” and 34% were scored as a “2”. Thus, raters established that 92% of positive controls had greater than 33% biofilm coverage but, only 58% had greater than 66% biofilm coverage. From this data we can infer that biofilm inoculation was more challenging to form on walls in the apical third of molar teeth.

Previous biofilm studies have shown that coronal and middle thirds of canals were covered more heavily with biofilm and more easily removed after irrigation (28,31). Several studies have shown that it takes a considerable amount of time for biofilm formation to occur. This study inoculated molars with biofilm for a period of 5 weeks (35 days). Estrela et al. suggested that mature *E. faecalis* biofilm on human root dentin may need 60 days to develop under low oxygen and nutrient rich environment. They believed it would allow for a satisfactory colonization time and viable for antimicrobial strategies (31). It is likely that a longer biofilm incubation period may have given more scores of “3” for positive controls. Greater wall coverage could have resulted in more accurate scoring of the SEM images and better rater agreement.
This study used scanning electron microscopy (SEM) for evaluation of biofilm in the apical third in molar teeth. Schaudinn discussed that SEM is a useful imaging approach for identifying bacterial biofilms in their dental habitat. However, he stated that most biofilms are embedded in a thick layer of extracellular matrix that prevents a clear identification of individual bacteria by SEM. Biofilms can also lose their characteristic shape which can make them difficult to visualize (29). In addition, splitting of teeth results in the accumulation of dentinal debris. This debris could possibly be interpreted as biofilm in certain images. Although raters were shown multiple images to be able to differ between the two, it is possible that identification could be challenging. SEM images were taken at 5000x magnification. A lower magnification may have given a better sense of biofilm coverage in the apical third. Another visualization approach is the use of confocal laser microscopy in combination with fluorescence which can enable the visualization of matrix embedded biofilms (29).

Some of the SEM images showed atypical dentin structure. Possible reasons for this may be dehydration occurring during instrumentation. All teeth were stored in PBS, but they were not in any solution when teeth were instrumented and possibly long term handling may have led to changes within the dentinal structure. It is also not known if any of the SEM preparation could have disturbed biofilm on the root walls after teeth were split. Any changes to the root structure could have influenced SEM scoring.

In this study, negative controls without biofilm inoculation should have a “correct” rating of 0. However, Table 1 shows that 39/144 negative control images were scored higher than 0. A possible reason for this may be that the raters were not equally calibrated. The calibration exercise consisted of a presentation that showed SEM images with a range of no coverage to complete dentinal wall coverage. This was followed by scorers rating 15 images on their own.
For any images where there was a discrepancy, those images were reviewed and discussed in terms of what a reasonable score for the image would be. The calibration may not have adequately prepared raters for the scoring of 348 images. Rater fatigue was to be helped by separating the surveys into 3 blocks, but if a rater took all 3 blocks simultaneously then fatigue could be an issue.

One solution for better calibration could have been to have raters complete a single survey block and evaluate the agreement between raters. Then, all raters could have discussed their scoring protocol and been re-calibrated as a group to have a better consistent agreement. After re-calibration raters would have completed additional survey blocks.

Table 6 shows the data for between rater agreements. The two raters who had the greatest agreement, A and C, also had the most experience in viewing SEM images of biofilm. This shows that experience of viewing many of these images was important in rating more consistently with one another. Table 9 in the appendix describes the within rater agreements on duplicate images. One aspect of this study was that only control images were used as duplicate images, thus, raters should have a more consistent agreement for negative and positive controls.

Table 9 reveals that when raters were shown images more than once, generally most raters agreed with themselves. But, if they were to score differently, the score was usually within 1 level of scoring. For instance, if a positive control was scored a “3” on one occasion, it may be scored a “2” on another occasion. This may infer that if biofilm coverage was near 66% then it may be difficult for all raters to agree whether that score was a 2 or a 3. It is of note that rater D was shown a negative control 4 times and gave it 3 different scores when repeated. This shows a poor intra-rater agreement on the part of rater D. It is also of interest that rater D was
least experienced in rating biofilm images. Rater D also may have scored the images more quickly and this could have led to a poorer agreement.

Within the limitations of this study, it was shown that the GentleWave™ system was as effective as the ProUltra® Piezoflow™ ultrasonic irrigation system in removing intracanal \textit{E. faecalis} biofilm in maxillary and mandibular molar teeth using 3\% NaOCl and 6\% NaOCl respectively. Syringe irrigation was less effective at removing biofilm using 6\% NaOCl. No system was able to completely remove all biofilm according to the raters. This study differs from the findings of Bhuva et al. that concluded conventional syringe irrigation and passive sonic irrigation with NaOCl was completely effective at removing \textit{E. faecalis} biofilm (28). The Bhuva study only used single rooted maxillary anterior teeth which do not possess the same anatomical challenges in cleaning. Their study also evaluated the root apices at coronal, middle, and apical areas.

\textit{Enterococcus faecalis} can adapt to environmental changes after endodontic therapy and remain a pathogen in the root canal system, making elimination difficult. In the present study, none of the irrigation systems completely eradicated \textit{E. faecalis} biofilms from the root canals (30). These findings are in accordance with previous studies, which showed microbial persistence after the use of irrigants in infected root canals (18,30,32).

Unlike other studies, this study incorporated more challenging root canal systems of molars where efficacy of biofilm removal may be more difficult. Adding additional irrigants such as EDTA or higher concentrations of NaOCl may also have proven to be more effective at biofilm removal. Additional calibration of raters may have also led to a greater agreement between raters. The use of additional microscopy or possibly software that could identify biofilm may have been useful to account for human error and bias. Further studies should be undertaken...
using different evaluation measures to investigate biofilm removal and how it may effect the success of root canal treatment.

In conclusion, the GentleWave\textsuperscript{TM} system and ProUltra\textsuperscript{®} Piezoflow\textsuperscript{TM} ultrasonic irrigation systems were most effective in removing intracanal \textit{E. faecalis} biofilm in this model. Syringe irrigation was much less effective in biofilm removal. No system was able to completely remove all biofilm according to the raters.
List of References


Appendices

Appendix A: REDCap survey, block 1
First block of Images

Please complete the survey below.

Thank you!

Identify rater

1) Who is rating these images?

- Garry Myers
- Vaughn Mayo
- Todd Kitten
- Cyrus Ardalan
2) **image001**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
3) image002

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
4) image003

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
5) image004

0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
6) image005

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
7) image006

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
8) image007

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
9) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
   2: Biofilm coverage between 34-66% of the root canal wall
   3: Biofilm coverage between 67-100% of the root canal wall
10) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
   2: Biofilm coverage between 34-66% of the root canal wall
   3: Biofilm coverage between 67-100% of the root canal wall
11) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
   2: Biofilm coverage between 34-66% of the root canal wall
   3: Biofilm coverage between 67-100% of the root canal wall
12) image011

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
13) **image012**

- **0**: Less than 5% biofilm coverage of the root canal wall
- **1**: Biofilm coverage between 5-33% of the root canal wall
- **2**: Biofilm coverage between 34-66% of the root canal wall
- **3**: Biofilm coverage between 67-100% of the root canal wall
14) **image013**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
15) image014

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
16) \( \text{image015} \)

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
17) image016

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
18) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
   2: Biofilm coverage between 34-66% of the root canal wall
   3: Biofilm coverage between 67-100% of the root canal wall
19) Image018

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
20) image019

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
21) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
   2: Biofilm coverage between 34-66% of the root canal wall
   3: Biofilm coverage between 67-100% of the root canal wall
O 0: Less than 5% biofilm coverage of the root canal wall
O 1: Biofilm coverage between 5-33% of the root canal wall
O 2: Biofilm coverage between 34-66% of the root canal wall
O 3: Biofilm coverage between 67-100% of the root canal wall
23) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
   2: Biofilm coverage between 34-66% of the root canal wall
   3: Biofilm coverage between 67-100% of the root canal wall
24) image023

○ 0: Less than 5% biofilm coverage of the root canal wall
○ 1: Biofilm coverage between 5-33% of the root canal wall
○ 2: Biofilm coverage between 34-66% of the root canal wall
○ 3: Biofilm coverage between 67-100% of the root canal wall
25) image024

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
26) 0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
27) **image026**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
28) 0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
30) 0: Less than 5% biofilm coverage of the root canal wall
○ 1: Biofilm coverage between 5-33% of the root canal wall
○ 2: Biofilm coverage between 34-66% of the root canal wall
○ 3: Biofilm coverage between 67-100% of the root canal wall
31) ○ 0: Less than 5% biofilm coverage of the root canal wall
○ 1: Biofilm coverage between 5-33% of the root canal wall
○ 2: Biofilm coverage between 34-66% of the root canal wall
○ 3: Biofilm coverage between 67-100% of the root canal wall
32) Image 031

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
33) 0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
34) image033

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
35) Select the appropriate level of biofilm coverage:

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
37) image036

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
40) image039

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
41) 0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
43) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
   2: Biofilm coverage between 34-66% of the root canal wall
   3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
45) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
   2: Biofilm coverage between 34-66% of the root canal wall
   3: Biofilm coverage between 67-100% of the root canal wall
46) image045

○ 0: Less than 5% biofilm coverage of the root canal wall
○ 1: Biofilm coverage between 5-33% of the root canal wall
○ 2: Biofilm coverage between 34-66% of the root canal wall
○ 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
48) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
   2: Biofilm coverage between 34-66% of the root canal wall
   3: Biofilm coverage between 67-100% of the root canal wall
49) 0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
50) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
   2: Biofilm coverage between 34-66% of the root canal wall
   3: Biofilm coverage between 67-100% of the root canal wall
51) 0: Less than 5% biofilm coverage of the root canal wall  
1: Biofilm coverage between 5-33% of the root canal wall  
2: Biofilm coverage between 34-66% of the root canal wall  
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
54) **image053**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
57) **image056**

- ○ 0: Less than 5% biofilm coverage of the root canal wall
- ○ 1: Biofilm coverage between 5-33% of the root canal wall
- ○ 2: Biofilm coverage between 34-66% of the root canal wall
- ○ 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
61) 0: Less than 5% biofilm coverage of the root canal wall
    1: Biofilm coverage between 5-33% of the root canal wall
    2: Biofilm coverage between 34-66% of the root canal wall
    3: Biofilm coverage between 67-100% of the root canal wall
62) image061

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
63) image062

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
64) circle 0: Less than 5% biofilm coverage of the root canal wall
   circle 1: Biofilm coverage between 5-33% of the root canal wall
   circle 2: Biofilm coverage between 34-66% of the root canal wall
   circle 3: Biofilm coverage between 67-100% of the root canal wall
65) image064

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
67) Image066

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
69) image068

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
71) image070

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
73) **image072**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
80) image079

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
82) o 0: Less than 5% biofilm coverage of the root canal wall
o 1: Biofilm coverage between 5-33% of the root canal wall
o 2: Biofilm coverage between 34-66% of the root canal wall
o 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
84) image083

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
86) **image085**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
87) Image 086

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
88) image087

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
90) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
   2: Biofilm coverage between 34-66% of the root canal wall
   3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
92) **image091**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
96) **image095**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
101) image100

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
102) image101

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
103) image102

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
104) Image 103

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
105) image104

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
106) image105

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
107) image106

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
109) image108

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
110) image109

☐ 0: Less than 5% biofilm coverage of the root canal wall
☐ 1: Biofilm coverage between 5-33% of the root canal wall
☐ 2: Biofilm coverage between 34-66% of the root canal wall
☐ 3: Biofilm coverage between 67-100% of the root canal wall
111) image110

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
112) image111

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall  
1: Biofilm coverage between 5-33% of the root canal wall  
2: Biofilm coverage between 34-66% of the root canal wall  
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
118) Image 117

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
Appendix B: REDCap survey, block 2
Block 2

Please complete the survey below.

Thank you!

Identify rater

1) Who is rating these images?

- Garry Myers
- Vaughan Mayo
- Todd Kitten
- Cyrus Ardalan
2) 0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
3) image119

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
4) Less than 5% biofilm coverage of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
5) 0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
6) image122

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
7) image123

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
8) 0: Less than 5% biofilm coverage of the root canal wall
○ 1: Biofilm coverage between 5-33% of the root canal wall
○ 2: Biofilm coverage between 34-66% of the root canal wall
○ 3: Biofilm coverage between 67-100% of the root canal wall
9) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
   2: Biofilm coverage between 34-66% of the root canal wall
   3: Biofilm coverage between 67-100% of the root canal wall
10) **image126**

- **0**: Less than 5% biofilm coverage of the root canal wall
- **1**: Biofilm coverage between 5-33% of the root canal wall
- **2**: Biofilm coverage between 34-66% of the root canal wall
- **3**: Biofilm coverage between 67-100% of the root canal wall
11) image127

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
12) **image128**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
13) **image129**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
14) Image 130

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
15) Image131

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
16) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
   2: Biofilm coverage between 34-66% of the root canal wall
   3: Biofilm coverage between 67-100% of the root canal wall
17) image133

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
18) **image134**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
19) image135

○ 0: Less than 5% biofilm coverage of the root canal wall
○ 1: Biofilm coverage between 5-33% of the root canal wall
○ 2: Biofilm coverage between 34-66% of the root canal wall
○ 3: Biofilm coverage between 67-100% of the root canal wall
20) **image136**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
21) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
   2: Biofilm coverage between 34-66% of the root canal wall
   3: Biofilm coverage between 67-100% of the root canal wall
22) **image138**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
23) 0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
24) **image140**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
26) A microscopic image of the root canal wall shows a biofilm coverage. The categories are:

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
27) image143

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
28) 0: Less than 5% biofilm coverage of the root canal wall
   ○ 1: Biofilm coverage between 5-33% of the root canal wall
   ○ 2: Biofilm coverage between 34-66% of the root canal wall
   ○ 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
30) **image146**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
31) **image147**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
32) 0: Less than 5% biofilm coverage of the root canal wall  
1: Biofilm coverage between 5-33% of the root canal wall  
2: Biofilm coverage between 34-66% of the root canal wall  
3: Biofilm coverage between 67-100% of the root canal wall
33) 0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
34) 0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
35) **image151**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
36) image152

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
37) 0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
38) **image154**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
41) image157

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
42) image158

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
43) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
   2: Biofilm coverage between 34-66% of the root canal wall
   3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
45) image161

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
- **0**: Less than 5% biofilm coverage of the root canal wall
- **1**: Biofilm coverage between 5-33% of the root canal wall
- **2**: Biofilm coverage between 34-66% of the root canal wall
- **3**: Biofilm coverage between 67-100% of the root canal wall
48) 0: Less than 5% biofilm coverage of the root canal wall
○ 1: Biofilm coverage between 5-33% of the root canal wall
○ 2: Biofilm coverage between 34-66% of the root canal wall
○ 3: Biofilm coverage between 67-100% of the root canal wall
49) **image165**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
51) **image167**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
53) 0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
56) **image172**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
58) **image174**

- ○ 0: Less than 5% biofilm coverage of the root canal wall
- ○ 1: Biofilm coverage between 5-33% of the root canal wall
- ○ 2: Biofilm coverage between 34-66% of the root canal wall
- ○ 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
60) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
   2: Biofilm coverage between 34-66% of the root canal wall
   3: Biofilm coverage between 67-100% of the root canal wall
61) image177

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
62) image178

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
63) 0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
64) 0: Less than 5% biofilm coverage of the root canal wall
○ 1: Biofilm coverage between 5-33% of the root canal wall
○ 2: Biofilm coverage between 34-66% of the root canal wall
○ 3: Biofilm coverage between 67-100% of the root canal wall
65) **image181**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
66) image182

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
67) image183

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
68) image184

- ○ 0: Less than 5% biofilm coverage of the root canal wall
- ○ 1: Biofilm coverage between 5-33% of the root canal wall
- ○ 2: Biofilm coverage between 34-66% of the root canal wall
- ○ 3: Biofilm coverage between 67-100% of the root canal wall
69) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
   2: Biofilm coverage between 34-66% of the root canal wall
   3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
72) Image

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
73) Image 189

- **0**: Less than 5% biofilm coverage of the root canal wall
- **1**: Biofilm coverage between 5-33% of the root canal wall
- **2**: Biofilm coverage between 34-66% of the root canal wall
- **3**: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
78) **image194**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
80) **image196**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
81) image197

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
82) image198

0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
97) 0: Less than 5% biofilm coverage of the root canal wall  
1: Biofilm coverage between 5-33% of the root canal wall  
2: Biofilm coverage between 34-66% of the root canal wall  
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
99) image215

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
100) image216

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
101) image217

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
102) image218

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
104) image220

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
105) 0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
108) image224

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
109) image225

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
110) image226

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
111) **image227**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
112) image228

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
113) image229

○ 0: Less than 5% biofilm coverage of the root canal wall
○ 1: Biofilm coverage between 5-33% of the root canal wall
○ 2: Biofilm coverage between 34-66% of the root canal wall
○ 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
115) image231

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
116) image232

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
117) image233

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
118) image234

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
Appendix C: REDCap survey, block 3
Please complete the survey below.

Thank you!

---

**Identify rater**

1) Who is rating these images?

- Garry Myers
- Vaughan Mayo
- Todd Kitten
- Cyrus Ardalan
2) image236

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
3) **image237**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
4) **image238**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
5) image239

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
6) image240

○ 0: Less than 5% biofilm coverage of the root canal wall
○ 1: Biofilm coverage between 5-33% of the root canal wall
○ 2: Biofilm coverage between 34-66% of the root canal wall
○ 3: Biofilm coverage between 67-100% of the root canal wall
7) **image241**

- **0**: Less than 5% biofilm coverage of the root canal wall
- **1**: Biofilm coverage between 5-33% of the root canal wall
- **2**: Biofilm coverage between 34-66% of the root canal wall
- **3**: Biofilm coverage between 67-100% of the root canal wall
8) **image242**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
9) **image243**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
10) image244

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
11) **image245**

- ○ 0: Less than 5% biofilm coverage of the root canal wall
- ○ 1: Biofilm coverage between 5-33% of the root canal wall
- ○ 2: Biofilm coverage between 34-66% of the root canal wall
- ○ 3: Biofilm coverage between 67-100% of the root canal wall
12) image246

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
13) image247

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
14) **image248**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
15)  0: Less than 5% biofilm coverage of the root canal wall
    1: Biofilm coverage between 5-33% of the root canal wall
    2: Biofilm coverage between 34-66% of the root canal wall
    3: Biofilm coverage between 67-100% of the root canal wall
16) 0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
17) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
   2: Biofilm coverage between 34-66% of the root canal wall
   3: Biofilm coverage between 67-100% of the root canal wall
18) image252

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
19) image253

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
20) image254

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
21) **image255**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
22) **image256**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
24) image258

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
25) 0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
26)  image260

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
27) image261

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
28) **image262**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
29) **image263**

- **0**: Less than 5% biofilm coverage of the root canal wall
- **1**: Biofilm coverage between 5-33% of the root canal wall
- **2**: Biofilm coverage between 34-66% of the root canal wall
- **3**: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
31) image265

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
3) image267

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
34) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
   2: Biofilm coverage between 34-66% of the root canal wall
   3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
36) **image270**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
37) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
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   3: Biofilm coverage between 67-100% of the root canal wall
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1: Biofilm coverage between 5-33% of the root canal wall
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3: Biofilm coverage between 67-100% of the root canal wall
40) 0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
41) image275

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
42) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
   2: Biofilm coverage between 34-66% of the root canal wall
   3: Biofilm coverage between 67-100% of the root canal wall
43) image277

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
44) image278

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
45)  $\mathbb{O}$ 0: Less than 5% biofilm coverage of the root canal wall
$\mathbb{O}$ 1: Biofilm coverage between 5-33% of the root canal wall
$\mathbb{O}$ 2: Biofilm coverage between 34-66% of the root canal wall
$\mathbb{O}$ 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
47)  image281

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
49) **image283**

- ☐ 0: Less than 5% biofilm coverage of the root canal wall
- ☐ 1: Biofilm coverage between 5-33% of the root canal wall
- ☐ 2: Biofilm coverage between 34-66% of the root canal wall
- ☐ 3: Biofilm coverage between 67-100% of the root canal wall
50) 0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
51) image285

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
52) image286

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
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1: Biofilm coverage between 5-33% of the root canal wall
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3: Biofilm coverage between 67-100% of the root canal wall
55) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
   2: Biofilm coverage between 34-66% of the root canal wall
   3: Biofilm coverage between 67-100% of the root canal wall
56) image290

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
57) 0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
58) image292

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
60) **image294**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
61) image295

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
62) image296

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
63) image297

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
64) image298

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
65) 0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
66) image300

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
67) image301

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
68) 0: Less than 5% biofilm coverage of the root canal wall
   1: Biofilm coverage between 5-33% of the root canal wall
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   3: Biofilm coverage between 67-100% of the root canal wall
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1: Biofilm coverage between 5-33% of the root canal wall
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3: Biofilm coverage between 67-100% of the root canal wall
71) image305

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
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1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
image307

○ 0: Less than 5% biofilm coverage of the root canal wall
○ 1: Biofilm coverage between 5-33% of the root canal wall
○ 2: Biofilm coverage between 34-66% of the root canal wall
○ 3: Biofilm coverage between 67-100% of the root canal wall
74) **image308**

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
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1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
Image 310

76) Image 310

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
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1: Biofilm coverage between 5-33% of the root canal wall
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3: Biofilm coverage between 67-100% of the root canal wall
83) image317

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
84) image318

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
85) image319

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
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88) image322

- 0: Less than 5% biofilm coverage of the root canal wall
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90) image324

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
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- 3: Biofilm coverage between 67-100% of the root canal wall
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3: Biofilm coverage between 67-100% of the root canal wall
94) image328

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
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98) image332

○ 0: Less than 5% biofilm coverage of the root canal wall
○ 1: Biofilm coverage between 5-33% of the root canal wall
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○ 3: Biofilm coverage between 67-100% of the root canal wall
0: Less than 5% biofilm coverage of the root canal wall
1: Biofilm coverage between 5-33% of the root canal wall
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3: Biofilm coverage between 67-100% of the root canal wall
100) image334

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
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- 3: Biofilm coverage between 67-100% of the root canal wall
101) image335

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
102) image336

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
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- 3: Biofilm coverage between 67-100% of the root canal wall
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3: Biofilm coverage between 67-100% of the root canal wall
104) image338

- 0: Less than 5% biofilm coverage of the root canal wall
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- 3: Biofilm coverage between 67-100% of the root canal wall
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1: Biofilm coverage between 5-33% of the root canal wall
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3: Biofilm coverage between 67-100% of the root canal wall
106) image340

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
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1: Biofilm coverage between 5-33% of the root canal wall
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3: Biofilm coverage between 67-100% of the root canal wall
109) image343

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
- 2: Biofilm coverage between 34-66% of the root canal wall
- 3: Biofilm coverage between 67-100% of the root canal wall
110) image344

- 0: Less than 5% biofilm coverage of the root canal wall
- 1: Biofilm coverage between 5-33% of the root canal wall
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1: Biofilm coverage between 5-33% of the root canal wall
2: Biofilm coverage between 34-66% of the root canal wall
3: Biofilm coverage between 67-100% of the root canal wall
Appendix D: Rater agreement

Table 8. Between rater agreement

<table>
<thead>
<tr>
<th>rater B</th>
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<th>3</th>
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<td>55</td>
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<td>33</td>
</tr>
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<td>2</td>
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Exact agreement = 46.6%. Kappa = 22.0%.

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Exact agreement = 81.0%. Kappa = 52.6%.

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Exact agreement = 44.5%. Kappa = 24.2%.

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Exact agreement = 45.7%. Kappa = 20.4%.
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Exact agreement = 53.4%. Kappa = 37.1%.

rater D

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Exact agreement = 39.1%. Kappa = 17.7%.
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Vita

Dr. Cyrous Ardalan was born on June 19th, 1981 in Panorama City, CA. Dr. Ardalan received his Bachelor of Science in Biology from the University of North Carolina at Chapel Hill in 2003 and Doctor of Dental Medicine from Tufts University School of Dental Medicine in 2007. Dr. Ardalan then completed a General Practice Residency at Cedars Sinai Medical Center in 2008. He then worked as a general dentist in private practice as well as a Chief Dental Officer of a Federally Qualified Health Center in New Orleans, Louisiana for 6 years. In 2015, he enrolled in the Advanced Dental Program in Endodontics at Virginia Commonwealth University School of Dentistry. Dr. Ardalan is a member of the American Association of Endodontists, American Dental Association, and Louisiana Dental Association. He will graduate with a Master of Science in Dentistry and a Certificate in Endodontics.